HANDBOOK OF ENVIRONMENTAL ENGINEERING™ VOLUME 10

Environmental Biotechnology

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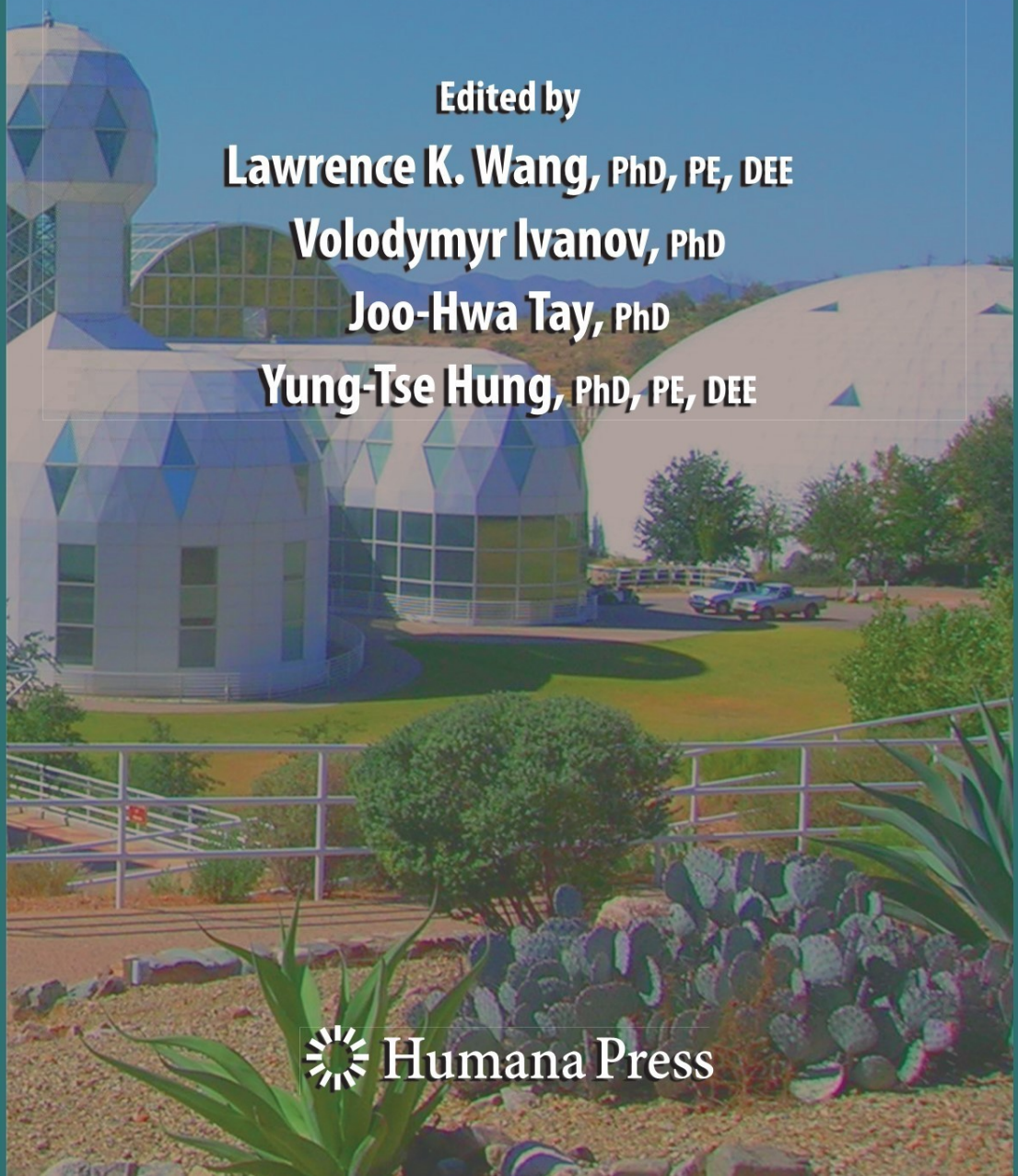

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Dedications

The Editors of the Handbook of Environmental Engineering series dedicate this volume to late Thomas L. Lanigan (1938–2006), the founder and former president of Humana Press, who encouraged and vigorously supported the editors and many contributors around the world to embark on this ambitious, life-long handbook project (1978 to present) for the sole purpose of protecting our environment, in turn, benefiting our entire mankind.

The Editors of this Handbook series also would like to dedicate this volume to Dr. Jao Fan Kao (1923–2008) of National Cheng Kung University (NCKU), Tainan, Taiwan, ROC. Dr. Kao was the founder and former Professor of the University's Department of Environmental Engineering. He educated over 1,500 environmental and civil engineers to serve the planet of earth. Both Dr. Lawrence K. Wang, Chief Editor, and Dr. Yung-Tse Hung, Co-editor, were Dr. Kao's students at National Cheng Kung University.

Preface

The past 30 years have seen the emergence of a growing desire worldwide that positive actions be taken to restore and protect the environment from the degrading effects of all forms of pollution – air, water, soil, and noise. Since pollution is a direct or indirect consequence of waste production, the seemingly idealistic demand for "zero discharge" can be construed as an unrealistic demand for zero waste. However, as long as waste continues to exist, we can only attempt to abate the subsequent pollution by converting it to a less noxious form. Three major questions usually arise when a particular type of pollution has been identified: (1) How serious is the pollution? (2) Is the technology to abate it available? and (3) Do the costs of abatement justify the degree of abatement achieved? This book is one of the volumes of the *Handbook of Environmental Engineering* series. The principal intention of this series is to help readers formulate answers to the last two questions above.

The traditional approach of applying tried-and-true solutions to specific pollution problems has been a major contributing factor to the success of environmental engineering, and has accounted in large measure for the establishment of a "methodology of pollution control." However, the realization of the ever-increasing complexity and interrelated nature of current environmental problems renders it imperative that intelligent planning of pollution abatement systems be undertaken. Prerequisite to such planning is an understanding of the performance, potential, and limitations of the various methods of pollution abatement available for environmental scientists and engineers. In this series of handbooks, we will review at a tutorial level a broad spectrum of engineering systems (processes, operations, and methods) currently being utilized, or of potential utility, for pollution abatement. We believe that the unified interdisciplinary approach presented in these handbooks is a logical step in the evolution of environmental engineering.

Treatment of the various engineering systems presented will show how an engineering formulation of the subject flows naturally from the fundamental principles and theories of chemistry, microbiology, physics, and mathematics. This emphasis on fundamental science recognizes that engineering practice has in recent years become more firmly based on scientific principles rather than on its earlier dependency on empirical accumulation of facts. It is not intended, though, to neglect empiricism where such data lead quickly to the most economic design; certain engineering systems are not readily amenable to fundamental scientific analysis, and in these instances we have resorted to less science in favor of more art and empiricism.

Since an environmental engineer must understand science within the context of application, we first present the development of the scientific basis of a particular subject, followed by exposition of the pertinent design concepts and operations, and detailed explanations of their applications to environmental quality control or remediation. Throughout the series, methods of practical design and calculation are illustrated by numerical examples. These examples clearly demonstrate how organized, analytical reasoning leads to the most direct and clear solutions. Wherever possible, pertinent cost data have been provided.

Our treatment of pollution-abatement engineering is offered in the belief that the trained engineer should more firmly understand fundamental principles, be more aware of the similarities and/or differences among many of the engineering systems, and exhibit greater flexibility and originality in the definition and innovative solution of environmental pollution problems. In short, the environmental engineer should by conviction and practice be more readily adaptable to change and progress.

Coverage of the unusually broad field of environmental engineering has demanded an expertise that could only be provided through multiple authorships. Each author (or group of authors) was permitted to employ, within reasonable limits, the customary personal style in organizing and presenting a particular subject area; consequently, it has been difficult to treat all subject material in a homogeneous manner. Moreover, owing to limitations of space, some of the authors' favored topics could not be treated in great detail, and many less important topics had to be merely mentioned or commented on briefly. All authors have provided an excellent list of references at the end of each chapter for the benefit of interested readers. As each chapter is meant to be self-contained, some mild repetition among the various texts was unavoidable. In each case, all omissions or repetitions are the responsibility of the editors and not the individual authors. With the current trend toward metrication, the question of using a consistent system of units has been a problem. Wherever possible, the authors have used the British system (fps) along with the metric equivalent (mks, cgs, or SIU) or vice versa. The editors sincerely hope that this duplicity of units' usage will prove to be useful rather than being disruptive to the readers.

The goals of the *Handbook of Environmental Engineering* series are: (1) to cover entire environmental fields, including air and noise pollution control, solid waste processing and resource recovery, physicochemical treatment processes, biological treatment processes, biosolids management, water resources, natural control processes, radioactive waste disposal, and thermal pollution control; and (2) to employ a multimedia approach to environmental pollution control since air, water, soil, and energy are all interrelated.

As can be seen from the above handbook coverage, no consideration is given to pollution by the type of industry, or to the abatement of specific pollutants. Rather, the organization of the handbook series has been based on the three basic forms in which pollutants and waste are manifested: gas, solid, and liquid. In addition, noise pollution control is included in the handbook series.

This particular book, Vol. 10, *Environmental Biotechnology*, mainly deals with theories and principles of biotechnologies, and is a sister book to Vol. 11, *Environmental Bioengineering*, which mainly deals with environmental applications of microbiological processes and technologies.

Specifically this book, Vol. 10, *Environmental Biotechnology*, introduces the mechanisms of environmental biotechnology processes, different microbiological classifications useful for environmental engineers, microbiology, metabolism, and microbial ecology of natural and environmental engineering systems, microbial ecology and bioengineering of isolated life support systems, classification and design of solid-state processes and reactors, value-added biotechnological products from organic wastes, design of anaerobic suspended bioprocesses and reactors, selection and design of membrane bioreactors, natural environmental

biotechnologies systems, aerobic and anoxic suspended-growth systems, aerobic and anaerobic attached-growth systems, and sequencing batch reactors.

This book's sister book, *Environmental Bioengineering*, Vol. 11, however, introduces various environmental applications, such as land disposal of biosolids, heavy metal removal by crops, pretreatment of sludge for sludge digestion, biotreatment of sludge, fermentaion of kitchen garbage, phytoremediation for sludge treatment, phyotoremediation for heavy metal removal from contaminated soils, vetiver grass bioremediatioon, wetland treatment, biosorption of heavy metals, rotating biological contactors (RBC) for carbon and nitrogen removal, anaerobic biofilm reactor, biological phosphorus removal, black and grey water treatment, milk wastewater treatment, tomato wastewater treatment, gelatine and animal glue production from skin wastes, fungal biomass protein production, algae harvest energy conversion, and living machine for wastewater treatment.

Both books together (Vols. 10 and 11) have been designed to serve as comprehensive biotechnology textbooks as well as wide-ranging reference books. We hope and expect they will prove of equal high value to advanced undergraduate and graduate students, to designers of water and wastewater treatment systems, and to scientists and researchers. The editors welcome comments from readers in all of these categories.

The editors are pleased to acknowledge the encouragement and support received from their colleagues and the publisher during the conceptual stages of this endeavor. We wish to thank the contributing authors for their time and effort, and for having patiently borne our reviews and numerous queries and comments. We are very grateful to our respective families for their patience and understanding during some rather trying times.

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Applications of Environmental Biotechnology

Volodymyr Ivanov and Yung-Tse Hung

CONTENTS

Abstract Environmental biotechnology is a system of scientific and engineering knowledge related to the use of microorganisms and their products in the prevention of environmental pollution through biotreatment of solid, liquid, and gaseous wastes, bioremediation of polluted environments, and biomonitoring of environment and treatment processes. The advantages of biotechnological treatment of wastes are as follows: biodegradation or detoxication of a wide spectrum of hazardous substances by natural microorganisms; availability of a wide range of biotechnological methods for complete destruction of hazardous wastes; and diversity of the conditions suitable for biodegradation. The main considerations for application of biotechnology in waste treatment are technically and economically reasonable rate of biodegradability or detoxication of substances during biotechnological treatment, big volume of treated wastes, and ability of natural microorganisms to degrade substances. Type of biotreatment is based on physiological type of applied microorganisms, such as fermenting anaerobic, anaerobically respiring (anoxic), microaerophilic, and aerobically respiring microorganisms. All types of biotechnological treatment of wastes can be enhanced using optimal environmental factors, better availability of contaminants and nutrients, or addition of selected strain(s) biomass. Bioaugmentation can accelerate start-up or biotreatment process in case microorganisms, which are necessary for hazardous waste treatment, are absent or their concentration is low in the waste; if the rate of bioremediation performed by indigenous microorganisms

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is not sufficient to achieve the treatment goal within the prescribed duration; when it is necessary to direct the biodegradation to the best pathway of many possible pathways; and to prevent growth and dispersion in waste treatment system of unwanted or nondetermined microbial strain which may be pathogenic or opportunistic one. Biosensors are essential tools in biomonitoring of environment and treatment processes. Combinations of biosensors in array can be used to measure concentration or toxicity of a set of hazardous substances. Microarrays for simultaneous qualitative or quantitative detection of different microorganisms or specific genes in the environmental sample are also useful in the monitoring of environment.

Key Words Environmental biotechnology • wastes • biotreatment • biodegradation • bioaugmentation • biosensors • biomonitoring.

1. INTRODUCTION

Environmental biotechnology is a system of sciences and engineering knowledge related to the use of microorganisms and their products in the prevention, treatment, and monitoring of environmental pollution through solid, liquid, and gaseous wastes biotreatment, bioremediation of polluted environments, and biomonitoring of environmental and treatment processes.

Biotechnological agents used in environmental biotechnology include Bacteria and Archaea, Fungi, Algae, and Protozoa. Bacteria and Archaea are prokaryotic microorganisms. Prokaryotes are the most active organisms participating in the biodegradation of organic matter and are used in all areas of environmental biotechnology. Fungi are eukaryotic organisms that assimilate organic substances. Fungi are important degraders of biopolymers and are used in solid waste treatment, especially in composting, or in soil bioremediation. Fungal biomass can also be used as an adsorbent of heavy metals. Algae are eukaryotic microorganisms that assimilate light energy and are used in environmental biotechnology for the removal of organic matter and nutrients from water exposed to light. Protozoa are unicellular animals that absorb and digest organic food. Protozoa play an important role in the treatment of industrial hazardous solid, liquid, and gas wastes by grazing on bacterial cells, thus maintaining adequate bacterial biomass levels in the treatment systems and helping to reduce cell concentrations in the waste effluents.

The main application of environmental biotechnology is the biodegradation of organic matter of municipal wastewater and biodegradation/detoxication of hazardous substances in industrial wastewater. It is known that approximately two-thirds of the hazardous substances of oil polluted soil and sludges, sulfur-containing wastes, paint sludges, halogenated organic solvents, non-halogenated organic solvents, galvanic wastes, salt sludges, pesticide-containing wastes, explosives, chemical industry wastewaters, and gas emissions can be treated by different biotechnological methods. Organic substances, synthesized in the chemical industry, are often difficult to biodegrade. Substances that are not produced naturally and are slowly/partially biodegradable are called xenobiotics. The biodegradability of xenobiotics can be characterized by biodegradability tests such as rate of CO_2 formation (mineralization rate), rate of oxygen consumption (respirometry test), ratio of BOD to COD (oxygen used for biological or chemical oxidation), and the spectrum of intermediate products of biodegradation.

Other applications of environmental biotechnology are the prevention of pollution and restoration of water quality in reservoirs, lakes and rivers, coastal area, in aquifers of groundwater, and treatment of potable water.

Areas of environmental biotechnology also include tests of toxicity and pathogenicity, biosensors, and biochips to monitor quality of environment, prevent hazardous waste production using biotechnological analogs, develop biodegradable materials for environmental sustainability, produce fuels from biomass and organic wastes, and reduce toxicity by bioimmobilization of hazardous substances.

2. COMPARISON OF BIOTECHNOLOGICAL TREATMENT AND OTHER METHODS

The pollution of water, soil, solid wastes, and air can be prevented or removed by physical, chemical, physicochemical, or biological (biotechnological) methods. The advantages of biotechnological treatment of wastes are as follows:

1. Biodegradation or detoxication of a wide spectrum of hazardous substances by natural microorganisms
2. Availability of a wide range of biotechnological methods for complete destruction of hazardous wastes
3. A diverse set of conditions that are suitable for biotechnological methods

However, there are also many disadvantages of biotechnological methods for the prevention of pollution and treatment of environment and wastes:

1. Nutrients and electron acceptors must be added to intensify the biotreatment
2. Optimal conditions must be maintained in the treatment system
3. There may be unexpected or negative effects of applied microorganisms, such as emission of cells, odors or toxic gases during the biotreatment, presence or release of pathogenic, toxigenic, opportunistic microorganisms into the environment
4. There may be unexpected problems in the management of the biotechnological system because of the complexity and high sensitivity of the biological processes

The main considerations for application of biotechnology in waste treatment are as follows:

1. Technically and economically reasonable rate of biodegradability or detoxication of waste substances during biotechnological treatment
2. Large volume of treated wastes
3. A low concentration of pollutant in water or waste is preferred
4. The ability of natural microorganisms to degrade waste substances
5. Better public acceptance of biotechnological treatment

The efficiency of actual biotechnological application depends on its design, process optimization, and cost minimization. Many failures have been reported on the way from bench laboratory scale to field full-scale biotechnological treatment because of the instability and diversity of both microbial properties and conditions in the treatment system (1).

In some cases, a combination of biotechnological and chemical treatments may be more efficient than one type of treatment (2, 3). Efficient pre-treatment schemes, used prior to biotechnological treatment, include homogenization of the particles of solid or undissolved

wastes in water, chemical oxidation of hydrocarbons by H_2O_2, ozone, or Fenton's reagent, photochemical oxidation, and preliminary washing of wastes using surfactants.

3. AEROBIC TREATMENT OF WASTES

Aerobic microorganisms require oxygen as a terminal acceptor of electrons donated by organic or inorganic substances. The transfer of electrons from donor to acceptor is a source of biologically available energy. Xenobiotics such as aliphatic hydrocarbons and derivatives, chlorinated aliphatic compounds (methyl-, ethyl, methylene and ethylene chlorides), aromatic hydrocarbons and derivatives (benzene, toluene, phthalate, ethylbenzene, xylenes and phenol), polycyclic aromatic hydrocarbons, halogenated aromatic compounds (chlorophenols, polychlorinated biphenyls, dioxins and relatives, DDT and relatives), AZO dyes, compounds with nitrogroups (explosive-contaminated waste and herbicides), and organophosphate wastes can be treated effectively by aerobic microorganisms.

3.1. Aerobic Treatment of Solid Wastes

Composting is the simplest way to treat solid waste aerobically. Composting converts biologically unstable organic matter into a more stable humus-like product that can be used as a soil conditioner or organic fertilizer. Additional benefits of composting of organic wastes include the prevention of odors from rotting wastes, destruction of pathogens and parasites (especially in thermophilic composting), and the retention of nutrients in the end products. There are three main types of composting technology: windrow system, static pile system, and in-vessel system. Composting in windrow systems involves mixing an organic waste with inexpensive bulking agents (wood chips, leaves, corncobs, bark, peanut, and rice husks) to create a structurally rigid matrix, to diminish heat transfer from the matrix to the ambient environment, to increase the treatment temperature and to increase the oxygen transfer rate. The mixed matter is stacked in rows 1–2 m high called windrows. The mixtures are turned over periodically (two to three times a week) by mechanical means to expose the organic matter to ambient oxygen. Aerobic and partially anaerobic microorganisms, which are present in the waste or were added from previously produced compost, will grow in the organic waste. Due to biooxidation and release of energy, the temperature in the pile will rise. This is accompanied by successive changes in the dominant microbial communities, from less thermoresistant to more thermophilic ones. This composting process ranges from 30 to 60 days in duration.

The static pile system is an intensive biotreatment because the pile of organic waste and bulking agent is intensively aerated using blowers and air diffusers. The pile is usually covered with compost to remove odors and to maintain high internal temperatures. The aerated static pile process typically takes 21 days, after which the compost is cured for another 30 days, dried, and screened to recycle the bulking agent.

In-vessel composting results in the most intensive biotransformation of organic wastes. In-vessel composting is performed in partially or completely enclosed containers in which moisture content, temperature, oxygen content in gas can be controlled. This process requires

little space and takes some days for treatment, but its cost is higher than that of open systems. To intensify the composting of solid waste, the following pre-treatments can be used:

1. Mechanical disintegration and separation or screening to improve bioavailability of substances
2. Thermal treatment
3. Washing of waste using water or solution of surfactants to diminish toxic substances in waste
4. Chemical pre-treatment by H_2O_2, ozone, or Fenton's reagent to oxidize and cleave aromatic rings of hydrocarbons

Soil bioremediation is used in or on the sites of post-accidental wastes. There are many options in the process design described in the literature (4–6). The main options tested in the field are as follows:

1. In situ bioremediation (in-place treatment of a contaminated site)
2. On-site bioremediation (the treatment of a percolating liquid or eliminated gas in reactors placed on the surface of the contaminated site). The reactors used for this treatment are suspended biomass stirred-tank bioreactors, plug-flow bioreactors, rotating-disk contactors, packed-bed fixed biofilm reactors (biofilter), fluidized bed reactors, diffused aeration tanks, airlift bioreactors, jet bioreactors, membrane bioreactors, and upflow bed reactors (7)
3. *Ex situ* bioremediation (the treatment of contaminated soil or water that is removed from a contaminated site)

The first option is used when the pollution is weak, treatment time is not a limiting factor, and there is no groundwater pollution. The second option is usually used when the pollution level is high and there is secondary pollution of groundwater. The third option is usually used when the pollution level is so high that it diminishes the biodegradation rate due to the toxicity of substances or a low mass transfer rate. Another reason for using this option might be that the conditions in situ or on site (pH, salinity, dense texture or high permeability of soil, high toxicity of substance, and safe distance from public place) are not favorable for biodegradation.

Preventing hazardous substances from dispersing from the accident site into the environment is an important task of environmental biotechnology. This goal can be achieved by creating physical barriers in the migration pathway with microorganisms capable of biotransformation of intercepted hazardous substances, e.g., in polysaccharide (slime) viscous barriers in the contaminated subsurface. Another approach, which can be used to immobilize heavy metals in soil after pollution accidents, is the creation of biogeochemical barriers. These biogeochemical barriers could comprise gradients of H_2S, H_2, or Fe^{2+} concentrations, created by anaerobic sulfate-reducing bacteria (in absence of oxygen and presence of sulfate and organic matter), fermenting bacteria (after addition of organic matter and in absence of oxygen), or iron-reducing bacteria (in presence of Fe(III) and organic matter), respectively. Other bacteria can form a geochemical barrier for the migration of heavy metals at the boundary between aerobic and anaerobic zones. For example, iron-oxidizing bacteria oxidize Fe^{2+} and its chelates with humic acids in this barrier and produce iron hydroxides that can diminish the penetration of ammonia, phosphate, organic acids, cyanides, phenols, heavy metals, and radionuclides through the barrier.

3.2. Aerobic Treatment of Liquid Wastes

Wastewater can be treated aerobically in suspended biomass stirred-tank bioreactors, plug-flow bioreactors, rotating-disk contactors, packed-bed fixed biofilm reactors (or biofilters), fluidized bed reactors, diffused aeration tanks, airlift bioreactors, jet bioreactors, membrane bioreactors, and upflow bed reactors (4, 7). Secondary wastes include polluted air and sediments produced in the bioreactor. Wastewater with low concentrations of hazardous substances may reasonably be treated using biotechnologies such as granular activated carbon (GAC) fluidized-bed reactors or co-metabolism. GAC or other adsorbents ensure sorption of hydrophobic hazardous substances on the surface of GAC or other adsorbent particles. Microbial biofilms can also be concentrated on the surface of these particles and can biodegrade hazardous substances with higher rates compared to situations when both substrate and microbial biomass are suspended in the wastewater.

Cometabolism refers to the simultaneous biodegradation of hazardous organic substances (which are not used as a source of energy) and stereochemically similar substrates, which serve as a source of carbon and energy for microbial cells. Biooxidation of the hazardous substance is performed by the microbial enzymes due to stereochemical similarity between the hazardous substance and the substrate. The best-known applications of cometabolism are the biodegradation/detoxication of chloromethanes, chloroethanes, chloromethylene, and chloroethylenes by enzyme systems of bacteria for the oxidization of methane or ammonia as a main source of energy. In practice, bioremediation is achieved by adding methane or ammonia, oxygen (air), and biomass of methanotrophic or nitrifying bacteria to soil and groundwater polluted by toxic chlorinated substances.

To intensify the biotreatment of liquid waste, the following pre-treatments can be used:

1. Mechanical disintegration/suspension of the particles and hydrophobic substances to improve the reacting surface in the suspension and increase the rate of biodegradation
2. Removal from wastewater or concentration of hazardous substances by sedimentation, centrifugation, filtration, flotation, adsorption, extraction, ion exchange, evaporation, distillation, freezing, and separation
3. Preliminary oxidation by H_2O_2, ozone, or Fenton's reagent to produce active oxygen radicals; preliminary photo-oxidation by UV and electrochemical oxidation of hazardous substances

3.3. Aerobic Treatment of Gaseous Wastes

The main applications of biotechnology for the treatment of gaseous wastes include the bioremoval of biodegradable organic solvents, odors, and toxic gases, such as hydrogen sulfide and other sulfur-containing gases from the exhaust ventilation air in industry and farming. Industrial ventilated air containing formaldehyde, ammonia, and other low molecular weight substances can also be effectively treated in a bioscrubber or biofilter. Gaseous xenobiotics, which can be treated biotechnologically, include the following: chloroform, trichloroethylene, 1,2-dibromoethane, 1,2-dibromo-3-chloropropane, carbon tetrachloride, xylenes, dibromochloropropane, toluene, methane, methylene chloride, 1,1-dichloroethene, bis(2-chloroethyl) ether, 1,2-dichloroethane, chlorine, 1,1-trichloroethane, ethylbenzene, 1,1,2,2-tetrachloroethane, bromine, methylmercury, trichlorofluoroethane,

1,1-dichloroethane, 1,1,2-trichloroethane, ammonia, trichloroethane, 1,2-dichloroethene, carbon disulfide, chloroethane, p-xylene, hydrogen sulfide, chloromethane, 2-butanone, bromoform, acrolein, bromodichloroethane, nitrogen dioxide, ozone, formaldehyde, chlorodibromomethane, ethyl ether, and 1,2-dichloropropane.

Gaseous pollutants of gas or air streams must pass through bioscrubbers containing suspensions of biodegrading microorganisms or through a biofilter packed with porous carriers covered by biofilms of degrading microorganisms. Depending on the nature and volume of polluted gas, the biofilm carriers may be cheap porous substrates, such as peat, wood chips, compost, or regular artificial carriers, such as plastic or metal rings, porous cylinders and spheres, fibers, and fiber nets. The bioscrubber's contents must be stirred to ensure a high mass transfer between gas and microbial suspension. The liquid that has interacted with the polluted gas is collected at the bottom of the biofilter and recycled to the top part of the biofilter to ensure adequate contact of polluted gas and liquid and optimal humidity of biofilter. The addition of nutrients and fresh water to a bioscrubber or biofilter must be made regularly or continuously. Fresh water can be used to replace water that has evaporated in the bioreactor. If the mass transfer rate is higher than the biodegradation rate, the absorbed pollutants must be biodegraded in an additional suspended bioreactor or biofilter connected in series to the bioscrubber or absorbing biofilter.

4. ANAEROBIC TREATMENT OF WASTES

There are anaerobic (living without oxygen), facultative anaerobic (living under anaerobic or aerobic conditions), microaerophilic (preferring to live under low concentrations of dissolved oxygen) and obligate aerobic (living only in the presence of oxygen), microorganisms. Some anaerobic microorganisms, called tolerant anaerobes, have mechanisms protecting them from exposure to oxygen. Others, called obligate anaerobes, have no such mechanisms and may die after several seconds of exposure to aerobic conditions. Obligate anaerobes produce energy from: a) fermentation (destruction of organic substances without external acceptor of electrons); b) anaerobic respiration using electron acceptors such as CO_2, $NO_3^-NO_2^-$, Fe^{3+}, SO_4^{2-}; 3) anoxygenic ($H_2S \rightarrow S$) or oxygenic ($H_2O \rightarrow O_2$) photosynthesis. The advantage of anaerobic treatment is that there is no need to supply oxygen in the treatment system. This is useful in cases such as bioremediation of clay soil or high-strength organic waste. However, anaerobic treatment may be slower than aerobic treatment, and there may be significant outputs of dissolved organic products of fermentation or anaerobic respiration.

The following sequence arranges respiratory processes according to increasing energetic efficiency of biodegradation (per mole of transferred electrons): fermentation $\rightarrow$ CO_2 respiration ("methanogenic fermentation") $\rightarrow$ dissimilative sulfate-reduction $\rightarrow$ dissimilative iron reduction ("iron respiration") $\rightarrow$ nitrate respiration ("denitrification") $\rightarrow$ aerobic respiration.

Facultative anaerobes can produce energy from these reactions or from the aerobic oxidation of organic matter and may be useful when integrated together with aerobic and anaerobic microorganisms in microbial aggregates. However, this function is still not well studied. One interesting and useful feature in this physiological group is the ability in some representatives (e.g., *Escherichia coli*) to produce an active oxidant, hydrogen peroxide, during normal aerobic metabolism (8).

Anaerobic respiration is more effective in terms of output of energy per mole of transferred electrons than fermentation. Anaerobic respiration can be performed by different groups of prokaryotes with such electron acceptors as NO_3^-, NO_2^-, Fe^{3+}, SO_4^{2-}, and CO_2. Therefore, if the concentration of one such acceptor in the hazardous waste is sufficient for the anaerobic respiration and oxidation of the pollutants, the activity of the related bacterial group can be used for the treatment. CO_2-respiring prokaryotes (methanogens) are used for methanogenic biodegradation of organic hazardous wastes. Sulfate-reducing bacteria can be used for anaerobic biodegradation of organic matter or for the precipitation/immobilization of heavy metals of sulfate-containing hazardous wastes. Iron-reducing bacteria can produce dissolved Fe^{2+} ions from insoluble Fe(III) minerals. Anaerobic biodegradation of organic matter and detoxication of hazardous wastes can be significantly enhanced as a result of precipitation of toxic organics, acids, phenols, or cyanide by Fe(II). Nitrate-respiring bacteria can be used in denitrification, i.e., reduction of nitrate to gaseous N_2. Nitrate can be added to the hazardous waste to initiate the biodegradation of different types of organic substances, for example polycyclic aromatic hydrocarbons (9). Nitrogroups of hazardous substances can be reduced by similar pathway to related amines.

Anaerobic fermenting bacteria (e.g., from genus *Clostridium*) perform two important functions in the biodegradation of hazardous organics: (a) they hydrolyze different natural polymers and (b) ferment monomers with production of alcohols, organic acids, and CO_2. Many hazardous substances, for example chlorinated solvents, phthalates, phenols, ethyleneglycol, and polyethylene glycols can be degraded by anaerobic microorganisms (4, 10–12). Fermenting bacteria perform reductive anaerobic dechlorination, thus enhancing further biodegradation of xenobiotics. Different biotechnological systems perform anaerobic biotreatment of wastewater: biotreatment by suspended microorganisms, anaerobic biofiltration, and biotreatment in upflow anaerobic sludge blanket (UASB) reactors (4, 5).

Organic and inorganic wastes can be slowly transformed by anaerobic microorganisms in landfills (13). Organic matter is hydrolyzed by bacteria and fungi. Amino acids are degraded using ammonification with formation of toxic organic amines and ammonia. Amino acids, nucleotides, and carbohydrates are fermented or anaerobically oxidized with formation of organic acids, CO_2, and CH_4. Xenobiotics and heavy metals may be reduced, and subsequently dissolved or immobilized. These bioprocesses may result in the formation of toxic landfill leachate, which can be detoxicated by aerobic biotechnological treatment to oxidize organic hazards and to immobilize dissolved heavy metals.

A combined anaerobic/aerobic biotreatment can be more effective than aerobic or anaerobic treatment alone. The simplest approach for this type of treatment is the use of aerated stabilization ponds, aerated and non-aerated lagoons, and natural and artificial wetland systems, whereby aerobic treatment occurs in the upper part of these systems and anaerobic treatment occurs at the bottom end. A typical organic loading is 0.01 kg BOD/m^3 day and the retention time varies from a few days to 100 days (7). A more intensive form of biodegradation can be achieved by combining aerobic and anaerobic reactors with controlled conditions, or by

integrating anaerobic and aerobic zones within a single bioreactor. Combination or alteration of anaerobic and aerobic treatments is useful in the following situations:

1. Biodegradation of chlorinated aromatic hydrocarbons including anaerobic dechlorination and aerobic ring cleavage
2. Sequential nitrogen removal including aerobic nitrification and anaerobic denitrification
3. Reduction of sulfate or Fe(III) with production of H_2S or Fe(II) which are active reagents for the precipitation of heavy metals, organic acids, and nutrients

5. TREATMENT OF HEAVY METALS-CONTAINING WASTES

Liquid and solid wastes containing heavy metals may be successfully treated by biotechnological methods. Some metals can be reduced or oxidized by specific enzymes of microorganisms. Microbial metabolism generates products such as hydrogen, oxygen, H_2O_2, which can be used for oxidation/reduction of metals. Reduction or oxidation of metals is usually accompanied by metal solubilization or precipitation. Solubilization or precipitation of metals may also be mediated by microbial metabolites. Microbial production of organic acids in fermentation or inorganic acids (nitric and sulfuric acids) in aerobic oxidation will promote formation of dissolved chelates of metals. Microbial production of phosphate, H_2S, and CO_2 will stimulate precipitation of non-dissolved phosphates, carbonates, and sulfides of heavy metals such as arsenic, cadmium, chromium, copper, lead, mercury, nickel; production of H_2S by sulfate-reducing bacteria is especially useful to remove heavy metals and radionuclides from sulfate-containing mining drainage waters, liquid waste of nuclear facilities, drainage from tailing pond of hydrometallurgical plants; wood straw or saw dust. Organic acids, produced during the anaerobic fermentation of cellulose, may be used as a source of reduced carbon for sulfate reduction and further precipitation of metals.

The surface of microbial cells is covered by negatively charged carboxylic and phosphate groups, and positively charged amino groups. Therefore, depending on pH, there may be significant adsorption of heavy metals onto the microbial surface (5). Biosorption, for example by fungal fermentation residues, is used to accumulate uranium and other radionuclides from waste streams.

Metal-containing minerals such as sulfides can be oxidized and metals can be solubilized. This approach is used for the bioleaching of heavy metals from sewage sludge (14, 15) before landfilling or biotransformation. Some metals, arsenic and mercury for example, may be volatilized by methylation due to the activity of anaerobic microorganisms. Arsenic can be methylated by methanogenic Archaea and fungi to volatile toxic dimethylarsine and trimethylarsine or can be converted to less toxic non-volatile methanearsonic and dimethylarsinic acids by algae (16). Hydrophobic organotins are toxic to organisms because of their solubility in cell membranes. However, many microorganisms are resistant to organotins and can detoxicate them by degrading the organic part of organotins (17).

In some cases, the different biotechnological methods may be combined. Examples would include the biotechnological precipitation of chromium from Cr (VI)-containing wastes from electroplating factories by sulfate reduction to precipitate chromium sulfide. Sulfate reduction can use fatty acids as organic substrates with no accumulation of sulfide. In the absence of

fatty acids but with straw as an organic substrate, the direct reduction of chromium has been observed without sulfate reduction (18).

6. ENHANCEMENT OF BIOTECHNOLOGICAL TREATMENT OF WASTES

Several key factors are critical for the successful application of biotechnology for the treatment of hazardous wastes:

1. Environmental factors, such as pH, temperature, and dissolved oxygen concentration, must be optimized
2. Contaminants and nutrients must be available for action or assimilation by microorganisms
3. Content and activity of essential microorganisms in the treated waste must be sufficient for the treatment

Optimal growth temperatures ranging from 10 to 90°C must be maintained for effective biotreatment by certain physiological groups of microorganisms. The heating of the treated waste can come from microbial oxidation or fermentation activities, providing sufficient heat generation and good thermal isolation of treated waste from the cooler surroundings. The bulking agent added to solid wastes may also be used as a thermal isolator.

The pH of natural microbial biotopes vary from 1 to 11: volcanic soil and mine drainage have pH values between 1 and 3; plant juices and acid soils have pH values between 3 and 5; fresh water and sea water have pH values between 7 and 8; alkaline soils and lakes, ammonia solutions, and rotten organics have pH values between 9 and 11. Most microbes grow most efficiently within the pH range from 5 to 9 and are called neutrophiles. Species that have adapted to grow at pH values lower than 4 are called acidophiles. Species that have adapted to grow at pH values higher than 9 are called alkaliphiles. Therefore, the pH of a treatment medium must be maintained at optimal values for effective biotreatment by certain physiological groups of microorganisms. The optimum pH may be maintained physiologically by the addition of a pH buffer or pH regulator in the following ways: (a) control of organic acid formation in fermentation; (b) prevention of formation of inorganic acids in aerobic oxidation of ammonium, elemental sulfur, hydrogen sulfide or metal sulfides; (c) assimilation of ammonium, nitrate, or ammonium nitrate, leading to decreased pH, increased pH, or neutral pH, respectively; (d) pH buffers such as $CaCO_3$ or $Fe(OH)_3$ can be used in large-scale waste treatment; and (e) solutions of KOH, NaOH, NH_4OH, $Ca(OH)_2$, HCl, or H_2SO_4 can be added automatically to maintain the pH of liquid in a stirred reactor. Maintenance of optimum pH in treated solid waste or bioremediated soil may be especially important if there is a high content of sulfides in waste or acidification/alkalization of soil in the bioremediation process.

The major elements found in microbial cells are C, H, O, N, S, and P. An approximate elemental composition corresponds to the formula $CH_{1.8}O_{0.5}N_{0.2}$. Therefore, nutrient amendment may be required if the waste does not contain sufficient amounts of these macroelements. The waste can be enriched with carbon (depending on the nature of the pollutant that is treated), nitrogen (ammonium is the best source), phosphorus (phosphate is the best source) and/or sulfur (sulfate is the best source). Other macronutrients (K, Mg, Na, Ca, and Fe) and micronutrients (Cr, Co, Cu, Mn, Mo, Ni, Se, V, and Zn) are also essential for microbial growth and enzymatic activities and must be added into the treatment systems if present

in low concentrations in the waste. The best sources of essential metals are their dissolved salts or chelates with organic acids. The source of metals for the bioremediation of oil spills may be lipophilic compounds of iron and other essential nutrients that can accumulate at the water–air interface where hydrocarbons and hydrocarbon-degrading microorganisms can also occur (19). In some biotreatment cases, growth factors must also be added to the treated waste. Growth factors are organic compounds, such as vitamins, aminoacids, and nucleosides, that are required in very small amounts and only by some strains of microorganisms called auxotrophic strains. Usually, those microorganisms that are commensals or parasites of plants and animals require growth factors. However, sometimes these microorganisms may have the unique ability to degrade some xenobiotics.

Substances may be protected from microbial attack by physical or chemical envelopes. These protective barriers must be destroyed mechanically or chemically to produce fine particles or waste suspensions to increase the surface area for microbial attachment and subsequent biodegradation. Another way to increase the bioavailability of hydrophobic substances is through the washing of waste or soil by water or a solution of surface-active substances. The disadvantage of this technology is the production of secondary hazardous waste due to the resistance of chemically produced surfactants to biodegradation. Therefore, only easily biodegradable or biotechnologically produced surfactants can be used for the pretreatment of hydrophobic hazardous substances.

Extracellular enzymes produced by microorganisms are usually expensive for large-scale biotreatment of organic wastes. However, enzyme applications may be cost-effective in certain situations. Toxic organophosphate waste can be treated using the enzyme parathion hydrolase produced and excreted by a recombinant strain of *Streptomyces lividans*. The cell-free culture fluid contains enzymes that can hydrolyze organophosphate compounds (20). Future applications may involve cytochrome-P450-dependent oxygenase enzymes that are capable of oxidizing different xenobiotics (21).

Low concentrations of dissolved oxygen (0.01–10 mg/L) can be rapidly depleted during waste biotreatment with oxygen consumption rates ranging from 10 to 2,000 g O_2/Lxh. Therefore, oxygen must be supplied continuously in the system. The air supply in liquid waste treatment systems is achieved by aeration and mechanical agitation. Different techniques are employed to supply sufficient quantities of oxygen in fixed biofilm reactors, in viscous solid wastes, in underground layers of soil or in aquifers polluted by hazardous substances. Very often the supply of oxygen is the critical factor in the successful scaling-up of bioremediation technologies from laboratory experiments to full-scale applications (22). Air sparging in situ is a commonly used bioremediation technology, which volatilizes and enhances aerobic biodegradation of contamination in groundwater and saturated soils. Successful case studies include a 6–12 month bioremediation project that targeted both sandy and silty soils polluted by petroleum products and chlorinated hydrocarbons (23). The application of pure oxygen can increase the oxygen transfer rate by up to five times, and this can be used in situations with a strong acute toxicity of hazardous wastes and low oxygen transfer rates, to ensure sufficient oxygen transfer in polluted waste.

In some cases, hydrogen peroxide has been used as an oxygen source because of the limited concentrations of oxygen that can be transferred into the groundwater using above-ground

aeration followed by reinjection of the oxygenated groundwater into the aquifer or subsurface air sparging of the aquifer. However, because of several potential interactions of H_2O_2 with various aquifer material constituents, its decomposition may be too rapid, making effective introduction of H_2O_2 into targeted treatment zones extremely difficult and costly (24). Pre-treatment of wastewater by ozone, H_2O_2, by TiO_2-catalized UV-photooxidation, and electrochemical oxidation can significantly enhance the biodegradation of halogenated organics, textile dyes, pulp mill effluents, tannery wastewater, olive-oil mills, surfactant-polluted wastewater and pharmaceutical wastes, and diminish the toxicity of municipal landill leachates. In some cases, oxygen radicals generated by Fenton's reagent ($Fe^{2+} + H_2O_2$ at low pH), and iron peroxides (Fe (VI) and Fe(V)) can be used as oxidants in the treatment of hazardous wastes.

Many microorganisms can produce and release to the environment such toxic metabolites of oxygen as hydrogen peroxide (H_2O_2), superoxide radical (O_2^-), and hydroxyl radical ($OH\cdot$). Lignin-oxidizing "white rot" fungi can degrade lignin and all other chemical substances due to intensive generation of oxygen radicals which oxidize the organic matter by random incorporation of oxygen into molecule. Not much is known about the biodegradation ability of H_2O_2-generating microaerophilic bacteria.

Dissolved acceptors of electrons such as NO_3^-, NO_2^-, Fe^{3+}, SO_4^{2-}, and HCO_3^- can be used in the treatment system when oxygen transfer rates are low. Selection of the acceptor is determined by economical and environmental reasons. Nitrate is often proposed for bioremediation (9) because it can be used by many microorganisms as an electron acceptor. However, it is relatively expensive and its supply to the treatment system requires strict control because it can pollute the environment. Fe^{3+} is an environmentally friendly electron acceptor. It is naturally abundant in clay minerals, magnetite, limonite, goethite, and iron ores, but its compounds are usually insoluble and it diminishes the rate of oxidation in comparison with dissolved electron acceptors. Sulfate and carbonate can be applied as electron acceptors only in anaerobic environments. Another disadvantage of these acceptors is that these anoxic oxidations generate toxic and foul-smelling H_2S or "greenhouse" gas CH_4.

The addition of microorganisms (inoculum) to start up or to accelerate a biotreatment process is a reasonable strategy under the following conditions:

1. If microorganisms, that are necessary for hazardous waste treatment, are absent or their concentration is low in the waste
2. If the rate of bioremediation performed by indigenous microorganisms is not sufficient to achieve the treatment goal within the prescribed duration
3. If the acclimation period is too long
4. To direct the biotreatment to the best pathway from many possible pathways
5. To prevent growth and dispersion in waste treatment system of unwanted or non-determined microbial strains such as pathogenic or opportunistic organisms. The application of defined and safe microbial strain(s) as a starter culture is especially important for biotechnological systems using aggregated bacterial cells in biofilms, flocs, or granules for two reasons: a) aggregation can be facilitated and enhanced; and b) self-aggregated or co-aggregated bacterial cells often are pathogens or opportunistic pathogens

Currently, a common environmental engineering practice is to use part of the treated waste or enrichment culture as an inoculum. However, applications of defined pure starter cultures have the following advantages:

1. Greater control over desirable processes
2. Lower risk of release of pathogenic or opportunistic microorganisms during biotechnological treatment
3. Lower risk of accumulation of harmful microorganisms in the final biotreatment product. Pure cultures that are most active in biodegrading specific hazardous substances can be isolated by conventional microbiological methods, quickly identified by molecular–biological methods, and tested for pathogenicity and biodegradation properties
4. Inoculum can be produced industrially
5. Regular additions of active microbial culture may be useful to maintain a constant rate of biodegradation of toxic substances in case of high death rates of microorganisms during treatment

Microorganisms suitable for the biotreatment of hazardous substances can be isolated from the natural environment. However, their ability for biodegradation can be modified and amplified by artificial alterations of their genetic (inherited) properties. The description of the methods is given in many books on environmental microbiology and biotechnology (4, 5). Natural genetic recombination of the genes (units of genetic information) occurs during DNA replication and cell reproduction, and includes the breakage and rejoining of chromosomal DNA molecules (separately replicated sets of genes) and plasmids (self-replicating mini-chromosomes containing several genes).

Recombinant DNA techniques or genetic engineering can create new, artificial combinations of genes, and increase the number of desired genes in the cell. Genetic engineering of recombinant microbial strains suitable for the biotreatment usually involves the following steps:

1. DNA is extracted from cells and cut into small sequences by specific enzymes
2. Small sequences of DNA can be introduced into DNA vectors
3. A vector (virus or plasmid) is transferred into the cell and self-replicated to produce multiple copies of the introduced genes
4. Cells with newly acquired genes are selected based on activity (e.g., production of defined enzymes, biodegradation capability) and stability of acquired genes

Genetic engineering of microbial strains can create (transfer) the ability to biodegrade xenobiotics or amplify this ability through the amplification of related genes. Another approach is the construction of hybrid metabolic pathways to increase the range of biodegraded xenobiotics and the rate of biodegradation (25). The desired genes for biodegradation of different xenobiotics can be isolated and then cloned into plasmids. Some plasmids have been constructed containing multiple genes for the biodegradation of several xenobiotics simultaneously. Strains containing such plasmids can be used for the bioremediation of sites heavily polluted by a variety of xenobiotics. The main problem in these applications is maintaining the stability of the plasmids in these strains. Other technological and public concerns include the risk of application and release of genetically modified microorganisms in the environment.

Self-aggregated microbial cells of biofilms, flocs, and granules, and artificially aggregated cells immobilized on solid particles are often used in the biotreatment of hazardous wastes. The advantages of microbial aggregates in hazardous waste treatment are as follows:

1. Upper layers and matrix of aggregates protect cells from toxic pollutants due to adsorption or detoxication; therefore microbial aggregates or immobilized cells are more resistant to toxic xenobiotics than suspended microbial cells
2. Different or alternative physiological groups of microorganisms (aerobes/anaerobes, heterotrophs/nitrifiers, sulfate-reducers/sulfur-oxidizers) may co-exist in aggregates and increase the diversity of types of biotreatments, leading to higher treatment efficiencies in one reactor
3. Microbial aggregates may be easily and quickly separated from treated water. Microbial cells immobilized on carrier surfaces such as granulated activated carbon that can adsorb xenobiotics will degrade xenobiotics more effectively than suspended cells (26)

However, dense microbial aggregates may encounter problems associated with diffusion limitation, such as slow diffusion of both nutrients into, and the metabolites out of, the aggregate. For example, dissolved oxygen levels can drop to zero at some depth below the surface of microbial aggregates so that obligate anaerobic bacteria can grow inside the biofilm of an aerated reactor (27). This distance clearly depends on factors such as the specific rate of oxygen consumption and the density of biomass in the microbial aggregate. When environmental conditions within the aggregate become unfavorable, cell death may occur in zones that do not receive sufficient nutrition or that contain inhibitory metabolites. Channels and pores in aggregate can facilitate transport of oxygen, nutrients and metabolites. Channels in microbial spherical granules have been shown to penetrate to depths of 900 μm (28) and a layer of obligate anaerobic bacteria was detected below the channeled layer (27). This demonstrates that there is some optimal size or thickness of microbial aggregates appropriate for application in the treatment of hazardous wastes.

7. BIOSENSORS

An important application of environmental biotechnology is biomonitoring, including monitoring of biodegradability, toxicity, mutagenicity, concentration of hazardous substances, and monitoring of concentration and pathogenicity of microorganisms in wastes and in the environment. Simple or automated off-line or on-line biodegradability tests can be performed by measuring CO_2 or CH_4 gas production or O_2 consumption (29). Biosensors may utilize either whole bacterial cells or enzyme to detect specific molecules of hazardous substances. Toxicity can be monitored specifically by whole cell sensors whose bioluminescence may be inhibited by the presence of hazardous substance.

The most popular approach uses cells with an introduced luminescent reporter gene to determine changes in the metabolic status of the cells following intoxication (30). Nitrifying bacteria have multiple-folded cell membranes, which are sensitive to all membrane-disintegrating substances: organic solvents, surfactants, heavy metals, and oxidants. Therefore, respirometric sensors measuring the respiration rates of these bacteria can be used for toxicity monitoring in wastewater treatment (31). Biosensors measuring concentrations of hazardous substances are often based on the measurement of bioluminescence (32). This

toxicity sensor is a bioluminescent toxicity bioreporter for hazardous wastewater treatment. It is constructed by incorporating bioluminescence genes into a microorganism. These whole-cell toxicity sensors are very sensitive and may be used on-line to monitor and optimize the biodegradation of hazardous soluble substances.

Similar sensors can be used for the measurement of the concentration of specific pollutants. A gene for bioluminescence has been fused to the bacterial genes coding for enzymes that metabolize the pollutant. When this pollutant is degraded, the bacterial cells will produce light. The intensity of biodegradation and bioluminescence depend on the concentration of pollutant and can be quantified using fiber-optics on-line. Combinations of biosensors in array can be used to measure concentration or toxicity of a set of hazardous substances.

The mutagenic activity of chemicals is usually correlated with their carcinogenic properties. Mutant bacterial strains have been used to determine the potential mutagenicity of manufactured or natural chemicals. The most common test, proposed by Ames in 1971 (33), utilizes back mutation in auxotrophic bacterial strains that are incapable of synthesizing certain nutrients. When auxotrophic cells are spread on a medium that lacks the essential nutrients (minimal medium), no growth will occur. However, cells that are treated with a tested chemical that causes a reversion mutation can grow in a minimal medium. The frequency of mutation detected in the test is proportional to the potential mutagenicity and carcinogenicity of the tested chemical. Microbial mutagenicity tests are used widely in modern research (34–36).

Cell components or metabolites capable of recognizing individual and specific molecules can be used as the sensory elements in molecular sensors (37). Sensors may be enzymes, sequences of nucleic acids (RNA or DNA), antibodies, polysaccharides or other "reporter" molecules. Antibodies, specific for a microorganism used in the biotreatment, can be coupled with fluorochromes to increase sensitivity of detection. Such antibodies are useful in monitoring the fate of bacteria released into the environment for the treatment of a polluted site. Fluorescent or enzyme-linked immunoassays have been derived and can be used for a variety of contaminants, including pesticides and chlorinated polycyclic hydrocarbons. Enzymes specific for pollutants and attached to matrices detecting interactions between enzymes and pollutants are used in on-line biosensors of water and gas biotreatment (38, 39).

A useful approach to monitor microbial populations in the biotreatment of hazardous wastes involves the detection of specific sequences of nucleic acids by hybridization with complementary oligonucleotide probes. Radioactive labels, fluorescent labels, and other kinds of the labels are attached to the probes to increase sensitivity and simplicity of the hybridization detection. Nucleic acids which are detectable by the probes include chromosomal DNA, extrachromosomal DNA such as plasmids, synthetic recombinant DNA such as cloning vectors, phage or virus DNA, rRNA, tRNA and mRNA transcribed from chromosomal or extrachromosomal DNA. These molecular approaches may involve the hybridization of whole intact cells, or extraction and treatment of targeted nucleic acids prior to probe hybridization (40–42). Microarrays for simultaneous semi-quantitative detection of different microorganisms or specific genes in the environmental sample have also been developed (43–45).

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Microbiology of Environmental Engineering Systems

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Abstract Type of energy generation is the major feature in physiological classification of prokaryotes. Chemotrophs can be separated within four groups by the type of electron acceptor: (a) anaerobic fermenting prokaryotes, producing biologically available energy by intramolecular oxidation-reduction; (b) anaerobically respiring prokaryotes, using other than oxygen electron acceptors; (c) microaerophilic bacteria, producing energy by aerobic respiration at low concentration of oxygen; (d) obligate aerobes, producing biologically available energy with oxygen as electron acceptor. There are also intermediary subgroups, which are using different types of energy production, depending on conditions. Phototrophs also can be classified into related physiological groups by the type of electron donor: (a) electron donors are products of anaerobic fermentation (organic acids, alcohols, and H2); (b) electron donors are products of anaerobic respiration (H2S, Fe2+); (c) electron donors are products of microaerophilic respiration (S); (d) electron donors are products of aerobic respiration (H2O). To overcome contradiction between the physiological groups and rRNA gene sequencing-based phylogenetic groups, the periodic table of prokaryotes comprising and explaining the existence of all physiological groups of prokaryotes was proposed. The main feature of the periodic table of prokaryotes is three parallel phylogenetic lines: (a) prokaryotes with Gram-negative type cell wall, habiting mainly in aquatic systems with stable osmotic pressure; (b) prokaryotes with Gram-positive type cell wall, habiting mainly in terrestrial systems with varied osmotic pressure; (c) Archaea that lack conventional peptidoglycan and habiting mainly

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in extreme environments. There are four periods in the periodic table of prokaryotes: anaerobic fermentation, anaerobic respiration, microaerophilic respiration, and aerobic respiration. Three phylogenetic lines and four periods create 12 groups comprising all chemotrophic and phototrophic prokaryotes. Existence of Gram-positive phototrophic bacteria using products of anaerobic, microaerophilic, and aerobic respiration as electron donors was predicted using this periodic table of prokaryotes. Evolutionary parallelism in phylogenetic lines of prokaryotes could be hypothetically explained by synchronous evolution of aquatic, terrestrial, and extreme ecosystems and horizontal exchange of genes between these ecosystems. The periodic table of prokaryotes helps to understand microbial physiological diversity of environmental engineering systems and can be used in the design of environmental engineering processes.

Key Words Prokaryotes • physiological classification • evolutionary parallelism • environmental engineering systems.

1. MICROBIAL GROUPS AND THEIR QUANTIFICATION

Microbiology is a branch of biology devoted to the study of microorganisms (microbes), which include both unicellular and multicellular organisms. These microorganisms are not visible without the aid of a microscope because they are smaller than 70–100 μm. Microbiological sciences, such as industrial microbiology, medical microbiology, veterinary microbiology, agricultural microbiology and environmental microbiology, are specified by their objects of study. Environmental microbiology studies microbes in parts of biosphere such as lithosphere, hydrosphere and atmosphere. The microbiology of environmental engineering systems is a subset of environmental microbiology. The objects of this science are the engineering systems of water, wastewater, solid wastes, soil and gas biotreatment.

The microbiology of environmental engineering systems pursues practical goals such as:

1. Development of biotechnologies for the microbial treatment of water, wastewater, solid wastes, soil and gas.
2. Development of methods to prevent the outbreaks of water-borne, soil-borne, vector-borne, and airborne infectious diseases.
3. Development of methods to monitor and control environmental engineering systems.

However, achievement of such practical goals is not possible without studying the following general problems of environmental microbiology:

1. Classification and identification of microorganisms.
2. Physical, chemical and biological interactions between microorganisms and macroorganisms.
3. Physical and chemical interactions of microorganisms and environment.
4. Biochemical, physiological and cellular adaptations and regulations in microbial systems.

This chapter is intended for environmental engineers as well as environmental engineering students who do not possess an in-depth microbiological background. We will address the basic principles of microbiology of environmental engineering systems, with special attention paid to the interconnections and diversity of microbial groups as well as their functions in

environmental engineering systems. A more in-depth description of these topics is given in specialized chapters of this book.

1.1. Groups of Microorganisms

The objects of the microbiology of environmental engineering systems include bacteria (prokaryotes), microscopic fungi, microscopic algae, protozoa and other microscopic objects such as viruses, metazoa and cysts of the helminthes. All living organisms are composed of cells. Prokaryotic cells are relatively simple in structure; they lack a true nucleus covered by the membrane. The most common cell shapes are spherical and rod-shaped. A eukaryotic cell's structure is more complex because it contains organelles that serve as compartments for special metabolic functions.

Viruses are particles assembled from the biopolymers, which are capable of multiplying and assembling as new virus particles inside living prokaryotic or eukaryotic cells. Viruses are not traditionally included in biological classifications because they are obligate intracellular parasites of cells, and thus, cannot self-reproduce. Extracellular virus particles are metabolically inert. The typical virus size ranges from 0.02 to 0.2 μm. Viruses contain a single type of nucleic acid, either DNA or RNA. There are known virus-like agents called prions, which are infectious proteins. Viruses are important for environmental engineering because of the following reasons:

1. Pathogenic viruses must be removed, retained or destroyed during water and wastewater treatment.
2. Viruses of bacteria (bacteriophages) can infect and degrade the bacterial cultures.
3. Bacteriophages can be used for the detection of specific microbial pollution of environment.
4. Viruses may be a vector (carrier) of the genes in artificial or natural genetic recombinations.

Prokaryotes are microorganisms with prokaryotic type cells. They consist of two phylogenetic groups: Bacteria and Archaea. The typical size of these cells is between 1 and 2 μm, but there have been cells known to be smaller or bigger than this range. Prokaryotes are most active in the degradation of organic matter and are used in wastewater treatment and soil bioremediation. However, there are many bacteria that are harmful to human, animal and plant health, and the removal or killing of these pathogenic bacteria in water, wastewater or solid waste is an important task of environmental engineering.

Energy sources for the growth of prokaryotes include:

1. Chemical substances (chemotrophy) or light (phototrophy).
2. Utilization of organic substances (heterotrophy) or inorganic substances (lithotrophy).

Other physiological properties also vary:

1. Source of carbon may be carbon dioxide (autotrophy) or organic substances (organotrophy).
2. Optimal temperature for growth varies from 0°C to higher than 100°C.
3. Optimal pH for growth varies from two to nine.

Relation to oxygen is one of the main features of prokaryotes. Generation of biologically available energy in a conducted cell is due to oxidation–reduction reactions. Oxygen is the most effective acceptor of electrons in energy generation from oxidation of substances, but not all

microorganisms can use it. The following groups of microorganisms differ in their relation to oxygen:

1. Obligate anaerobic prokaryotes, producing energy by fermentation (it is intramolecular oxidation–reduction without an external acceptor of electrons); they die after contact with oxygen because they lack protection against oxygen radicals produced during the contact of cells with oxygen.
2. Tolerant anaerobes produce energy only by fermentation but survive after contact with oxygen due to protective mechanisms against oxygen radicals.
3. Facultative anaerobic bacteria, which are capable to produce energy by fermentation if oxygen is absent or by aerobic respiration if oxygen is present.
4. Microaerophilic bacteria, which prefer low concentration of dissolved oxygen in a medium.
5. Obligate aerobes produce energy by aerobic respiration only.

Anoxic (anaerobic) respiration is typical for prokaryotes only and is the oxidation of organic or inorganic substances by electron acceptors other than oxygen. Different electron acceptors are used for energy generation by specific physiological groups of prokaryotes, including:

1. Nitrate (NO_3^-) and nitrite (NO_2^-) are used by denitrifying bacteria (denitrifiers).
2. Sulphate (SO_4^{2-}) is used by sulphate-reducing bacteria.
3. Sulphur (S) is used by sulphur-prokaryotes.
4. Ferric ions (Fe^{3+}) is used by iron-reducing bacteria.
5. Ions of different oxidized metals are used as acceptor of electrons.
6. Carbon dioxide (CO_2) is used by methanogens.

Fungi are eukaryotic microorganisms, mostly multicellular, which assimilate organic substances and absorb nutrients through the cell surface. The typical cell size is between 5 and 20 μm. Cells are often combined in the branched filaments called hyphaes, which are combined in a web known as mycelium. Fungi are important degraders of polymers and are used in the composting and biodegradation of toxic organic substances. Fungi are used in environmental engineering in composting, soil bioremediation and biodegradation of xenobiotics. Mycelium effectively penetrates solid wastes and soil. There are five major groups of fungi:

1. *Oomycetes* (water molds).
2. *Zygomycetes* (molds).
3. *Ascomycetes* (sac fungi and yeasts) reproduced by spores stored in the sac called ascus or spores called conidia.
4. *Basidiomycetes* (club fungi and mushrooms).
5. *Deuteromycetes* (or *Fungi imperfecti*) have no known sexual stage.

Molds are filamentous fungi (from *Zygomycetes* and *Ascomycetes*) that have widespread occurrence in nature. They have a surface mycelium and aerial hyphae that contain asexual spores (conidia). These spores are airborne allergens in damp or poorly constructed buildings. Yeasts (from *Ascomycetes*) are fungi that grow as single cells, producing daughter cells either by budding (the budding yeasts) or by binary fission (the fission yeasts). Mushrooms are filamentous fungi that form large above-ground fruiting bodies, although the major portion of the biomass consists of hyphae below ground.

Algae are floating eukaryotic microorganisms that assimilate energy from light. The typical size of a cell is 10–20 μm. Algae carry out oxygenic photosynthesis:

$$CO_2 + H_2O + \text{light} \rightarrow CH_2O < \text{organic matter} > + O_2 \quad (1)$$

Algae live primarily in aquatic habitats and on the soil surface. Algae should not be confused with cyanobacteria, which are prokaryotes. The classification of algae is based on the type of chlorophyll and other pigments, cell wall structure and nature of carbon reserve material:

1. *Chlorophyta* (green algae).
2. *Chrysophita* (golden-brown algae).
3. *Euglenophyta*, have no cell.
4. *Pyrrophyta* (dinoflagellates).
5. *Rhodophyta* (red algae).
6. *Phaeophyta* (brown algae).

Algae are important for environmental engineering for the following reasons:

1. They remove nutrients from water and are active microorganisms in waste stabilization ponds.
2. Some algae are fast-growing in polluted water and produce toxic compounds; these cause the "red tides" in polluted coastal areas.
3. Selected species of microscopic algae in natural waters are used for the indication of water quality.
4. There may be value-added products, for example, pigments and unsaturated fatty acids from algae grown in wastewater.

Protozoa are unicellular organisms that absorb and digest organic food inside a cell. The typical cell size is from 10 to 50 μm. Some protozoa are pathogenic and must be removed from water and wastewater. Four major groups of protozoa are distinguished by their mechanism of motility: amoebas move by means of false feet; flagellates move by means of flagella; ciliates use cilia for locomotion; and some protozoa have no means of locomotion. Examples are given in Table 2.1.

Protozoa are unicellular organisms that obtain nutrients by ingesting other microbes, or by ingesting macromolecules. The cells form cysts under adverse environmental conditions

Table 2.1
Examples of parasitic protozoa

Group	Example of parasitic species from this group	Disease caused by this species
Sarcodina (amoeboids)	*Entamoeba histolytica*	Amebiasis
Mastigophora (flagellates)	*Giardia intestinalis*	Giardiasis
Ciliophora (ciliates)	*Balantidium coli*	Balantidiasis
Sporozoa (no means of locomotion)	*Plasmodium vivax*	Malaria
	Cryptosporidium spp. (more than ten species)	Cryptosporidiosis

and are resistant to desiccation, starvation, high temperature and disinfection. Changes in the protozoan community reflect the operating conditions of aerobic wastewater treatment:

1. Amoebas can be found in high concentrations of organic matter (at high values of biochemical oxygen demand – BOD).
2. Flagellated protozoa and free-swimming ciliates are associated with high bacterial concentrations in activated sludge and medium concentration of BOD values.
3. Protozoa contribute significantly to the reduction of bacteria, including pathogens in activated sludge.
4. Stalked ciliates occur at low bacterial and BOD concentrations in water.

Helminthes are parasitic worms that survive in humans and animals. Many of these parasitic worms have microscopic cysts (seeds). The removal or inactivation of these cysts in water, wastewater and solid wastes is a goal of environmental engineering. Due to the high hydrophobicity of the cyst surface, cysts can be accumulated in the landfill leachate, foam of aeration tanks, or float up during the storage or primary treatment of sewage.

1.2. Microbiological Methods Used in Environmental Engineering

Specific microbiological methods are used to study microorganisms in environmental engineering systems:

1. Isolation, cultivation, identification and quantification of pure cultures.
2. Selection of strains and construction of recombinant microbial strains.
3. Selection and quantification of enrichment cultures.
4. Identification and quantification of microorganisms in environmental samples without cultivation.
5. Extraction, cloning, enrichment and identification of microbial genes and their products in environmental engineering systems.

Isolation of pure culture (microbial strain) is usually performed by spreading a diluted microbial suspension on a Petri dish with a semi-solid medium to produce 10–50 colonies on the dish after several days of cultivation. Cells of one colony are picked up for the next round of cultivation on a semi-solid or liquid medium. However, the following methods can also be effectively used for the isolation of pure microbial culture:

1. Mechanical separation of cells by micromanipulator.
2. Sorting of cells or microbeads with immobilized cell, using flow cytometer.
3. Magnetic or immunomagnetic separation.
4. Cell chromatography.

A microbial population that originates from one colony is called a microbial strain. A microbial population that originates from one cell is called a microbial clone.

Selection is the screening of microbial variants with specific desirable characteristics within the population of one strain. These variants may include:

1. Faster or more efficient growth (positive selection).
2. Faster or more efficient biochemical function (positive selection).
3. Slower or less efficient biochemical function (negative selection).
4. Better survival under harmful conditions (positive selection).
5. Weaker resistance to some factors of the environment (negative selection).

The differences between variants are caused by natural spontaneous mutations, i.e., changes in the DNA sequences of genes. Mutagenic chemicals, ultraviolet rays and ionizing radiation are used to increase the rate of mutagenesis and to increase probability of desirable variant formation. The screening of the desirable variant can be replaced by the creation of selection pressure, i.e., conditions favorable for growth, survival or development of desirable variant. Therefore, this variant will be accumulated in a microbial population and can be detected during the screening.

The cultivation of microorganisms is performed under suitable conditions, usually at optimal temperature, pH, osmotic pressure and concentration of gases (oxygen, carbon dioxide, hydrogen), on a semi-solid or liquid medium, containing all necessary substances for the growth of the strain. The elemental composition of biomass can be shown approximately by the formula $CH_{1.8}O_{0.5}N_{0.2,}$ but half of the known elements are used in the synthesis of microbial biomass and must be present in the medium. Suitable conditions and essential substances for the growth of some strains are not known yet and the cultivation of these microorganisms has not been successful to date. Additionally, some microorganisms are living in strong symbiotic or parasitic relationships with other microorganisms or macroorganisms that cannot be cultivated separately from these organisms. Therefore, not all microorganisms can be isolated and cultivated.

The *identification* of microorganisms involves the determination of relationship of a studied strain (taxon) with some known group, which is then accepted and approved by an international scientific committee. Measurement or qualitative evaluation of the relationship between compared microbial groups is performed by the methods of phenotypic classification (conventional taxonomy) and genotypic classification (phylogenetic taxonomy).

Phenotypic classification (conventional taxonomy) is based on the phenotypic characteristics, i.e., visible or measured characteristics determined by an organism's interaction with the environment:

1. Cytological characteristics such as size, shape, cell structure, typical cell aggregates, membrane structures, intracellular structures and cell organelles. One of most important cytological characteristics of prokaryotes is the Gram-positive or Gram-negative type of cell wall. A Gram-positive cell well is a thick and rigid 3-D layer of polymer. A Gram-negative cell wall is a thin and more elastic layer of polymer, which is covered by an outer membrane and a lipopolysaccharide layer.
2. Physiological characteristics such as type of energy production, relation to oxygen, pH, temperature, chemical content of cell wall and membranes, production of specific metabolites and enzyme profile.
3. Ecological characteristics such as habitats, econiches, colonial structures and interrelationships with other organisms.

Genotypic classification (phylogenetic taxonomy) is based on the analyses of genetic characteristics of the organisms, which are stored in the sequences of DNA. Genotypic characteristics include:

1. G + C content in DNA.
2. Sequences of genes (the sequences of DNA, which store information on the biopolymers of homologous, similar function in different species are compared).
3. Sequences of homologous (similar) proteins.
4. Level of hybridization between the sequences of DNA and RNA of compared strains.

Collections of strains and clones. The properties of strains and clones are the primary data used for classification. Strains are stored in microbial collections in the form of suspension, colonies on solid medium, or in dry or frozen state. There are many specialized and national collections of microorganisms. The purpose of such collections is to acquire, authenticate, preserve, develop and distribute biological materials, information, technology, intellectual property and standards for the advancement, validation and application of scientific knowledge to private industry, government and academic organizations. For example, large culture collections include the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) and American Type Culture Collection (ATCC). A strain is identified by its assigned number in a microbial collection and the name of the species. For example, *Bacillus subtilis* ATCC6633 refers to a strain of species *Bacillus subtilis* stored under number 6333 in the ATCC.

A *species* is a primary unit of phenotypic classification. It is defined by the phenotypic and genotypic characteristics of a collection of similar strains. The name of any species is given and read in *Latin* and includes the name of the genus (first) and the name of the species (second): *Saccharomyces cerevisiae*, *Bacillus subtilis*.

Higher levels of phenotypic classification are genus (collection of similar species), family (collection of similar genera), order (collection of similar families) and kingdom (collection of similar orders). Prokaryotic groups of conventional taxonomy are described in Bergey's Manual of Systematic Bacteriology (1). The manual contains phenotypic characteristics, which are used to classify prokaryotes by conventional taxonomy. Groups of viruses, fungi, protozoa and algae are described in specific manuals approved by related international scientific associations. Prokaryotic groups of phylogenetic taxonomy can be defined through the comparison of the sequences of ribosomal RNA (rRNA), especially 16S rRNA. It is thought that the number of differences in the sequence reflects the evolutionary distance of the origin of compared sequences from a common ancestor sequence.

Selection of enrichment culture refers to the selection of the population with one dominated strain or one dominated microbial community, which is accumulated in the system of cultivation because of the preferred conditions (selection pressure) for this strain or community. Enrichment cultivation is often used in environmental engineering to select microorganism(s) capable of particular metabolic transformations. Selective conditions (selection pressure) for the production of enrichment culture are as follows:

1. Source of energy.
2. Source of carbon.
3. Source of nitrogen and phosphorus.
4. Temperature.
5. pH.
6. Concentration of heavy metals.
7. Presence of specific antibiotic in a medium.
8. Concentration of dissolved oxygen.
9. Osmotic pressure of a medium.
10. Spectrum and intensity of light, etc.

Quantification of microbial biomass, i.e., determination of cell number (enumeration) or quantity of cell biomass, can be performed by the following methods:

1. Microscopic or flow cytometric enumeration of cells.
2. Physical measurement of microbial cells and biomass concentration.
3. Chemical measurement of microbial cells and biomass concentration.
4. Biological methods of cell and viruses enumeration.
5. Physiological measurement of biomass.
6. Molecular-biological methods of cells and viruses enumeration.

Factors that affect the choice of the method include:

1. Cost and length of time required for analysis.
2. Sensitivity and specificity of the method.
3. Availability of the equipment.
4. Characteristics of the interest.

Microscopic enumeration of cells and virus particles is performed using light microscopes (bright field, phase contrast and fluorescence microscopy), confocal laser scanning microscopes (CLSM), transmitted electron microscopes (TEM), scanning electron microscopes (SEM) and other kinds of microscopes that are able to visualize a number of microbial cells or virus particles on a defined area. The particular cell structure may be specifically stained, cell or virus surface may be labeled by immunochemical methods and DNA or RNA of cells can be hybridized with oligonucleotide probes labelled by fluorescence, radioactive or other labels. This specific staining can ensure cell enumeration altogether, with cell identification and measurement of cell physiological state.

Flow cytometry enumeration is used to quantify cells by staining specific cells with fluorescent-labelled antibodies, oligonucleotide probes and specific fluorochromes, which are excited by lasers in the flow of a small diameter (2). The fluorescence of individual cells is then measured by photomultipliers and the signals are collected and treated by a computer. In addition to cell number, three to six other parameters of thousands of individual microbial cells or virus particles can be analyzed in seconds.

Physical methods for microbial biomass measurement are based on the determination of weight, optical density, turbidity, fluorescence or radioactivity of microbial suspensions and solid matter. A convenient method of suspended biomass estimation is turbidity measurement. The share of scattered light is proportional to the cell concentration in the sample. Autofluorescence of microbial cell components (chlorophyll of algae, bacteriochlorophyll and carotenoids of cyanobacteria, F_{420} of methanogens) or fluorescence of stained cells also can be used for the measurement of biomass. Fluorescence spectrometry can be used to quantify microorganisms in environmental engineering systems, using determination of the binding of specific oligonucleotide probes (3).

Chemical methods for microbial biomass measurement include the analysis of protein, DNA or the components of cell wall, ATP, photopigments, cytochromes, coenzymes $NADH_2$ or F_{420}. ATP measurement is a sensitive indicator of a small quantity of viable microorganisms. The chemical changes in the medium caused by the microbial growth can be monitored using electrochemical sensors and fiber optic sensors.

Physiological methods of microbial biomass measurement are based on the measurements of physiological activity of the cells, for example, respiration rate, biochemical transformation rate and ATP concentration.

Biological methods of cell enumeration are as follows:

1. Plate count, i.e., cultivation on a semi-solid medium and enumeration of colony-forming units (CFU). It is assumed that one cell produces one colony, but this assumption is often not right. There may be 10^3–10^{12} cells in 1 mL of the sample. Therefore, it should be diluted in a sterile medium before being spread onto a Petri dish to produce not more than 100–300 colonies per plate.
2. Most probable number count, i.e., identification of the maximum dilution at which the growth or microbial activity can be easily detected by the colour change, precipitation or formation of gas bubbles. For example, if the maximum dilution to detect microbial activity in 1 mL of specific medium is 5×10^{-4}, the most probable number of cells in the sample is 2×10^3 cells/mL. It is assumed that multiplication of one cell in the tube with maximum dilution can produce the detectable result (color change, gas bubbles), but this assumption is often incorrect.

If the studied cells and viral particles have a low cell concentration, they must be concentrated using the following methods:

1. Filtration of the sample through a sterile membrane filter having a pore size < 0.45 μm to retain bacterial cells and $<$ 2–5 μm to retain eukaryotic cells.
2. Precipitation or centrifugation of cells or viral particles of the sample.
3. Chromatography of the sample.
4. Adsorption of cells and viral particles in the column with a specific adsorbent.

Viruses are enumerated biologically by spreading a diluted suspension on the surface of a lawn of actively growing cells susceptible to the virus. As a virus particle infects and reproduces, the produced viruses kill surrounding cells, forming a zone of clearing in the cell layer.

Molecular-biological methods for microbial biomass quantification are as follows:

1. Immunochemical quantification of microbial biomass due to colour change in the reaction between specific antibodie(s) and the cell surface.
2. Molecular-biological quantification of microbial biomass due to color change in the in situ reaction between specific oligonucleotide probe(s) and cell RNAs or DNA (3).
3. Quantitative polymerase chain reaction (PCR) called real-time PCR. It involves the extraction of DNA from the sample and amplification of specific genes with its quantification after every cycle of amplification. This method is especially important for bacterial groups that cannot be cultivated in the laboratory because the medium or growth conditions for them were not yet defined, or which are symbiotic or parasitic species.

Viruses can be enumerated by immunochemical methods or by PCR of specific DNA/RNA.

1.3. Comparison of Physical, Chemical, Physico-chemical and Microbiological Processes

Environmental engineering problems, i.e., waste or wastewater treatment, soil bioremediation and biopurification of exhaust gases, can be usually solved by physical, chemical, physico-chemical and biological/microbiological technologies. An optimal technology can

Table 2.2
Advantages and disadvantages of different environmental engineering technologies

Type of technology	Advantages	Disadvantages
Physical technologies (sedimentation, filtration, volatilization, fixation, evaporation, heat treatment, radiation, etc.)	Required time is from some seconds to some minutes; high predictability of the system	Low specificity and high energy demand
Chemical technologies (oxidation, incineration, reduction, chemical immobilization, chelating, chemical transformation)	Required time is from some seconds to some minutes; high predictability of the system	High expenses for reagents, energy, and equipment; air pollution due to incineration, formation of secondary wastes
Physico-chemical treatment (adsorption, absorption, chromatography)	Required time is from some minutes to some hours	High expenses for adsorbents; formation of secondary waste
Microbiological technologies (biooxidation, biotransformation, biodegradation)	Low volume or absence of secondary hazardous wastes; process can be initiated by natural microorganisms or small quantity of added microbial biomass; high process specificity; wide spectrum of degradable substances and diverse methods of biodegradation	High expenses for aeration, nutrients, and maintenance of optimal conditions; required time is from some hours to days; unexpected or negative effects of microorganisms-destructors; low predictability of the system because of complexity and high sensitivity of biological systems

be selected, based on economical or environmental criteria. Some general advantages and disadvantages of different environmental engineering technologies are shown in Table 2.2.

2. MICROBIAL ECOSYSTEMS

2.1. Structure of Ecosystems

An *ecosystem* comprises biotic (biological) and abiotic (physical, chemical) components, interacting with each other and isolated from the environment by a boundary.

The *hierarchy of life units* in microbial ecosystems can be represented in order of increasing spatial and biological complexity of ecosystems and the sequence of their combination:

1. Suspended cells (unicellular organisms) of one species.
2. Suspended cells (unicellular organisms) of microbial community.
3. Aggregated cells and multicellular microorganisms.
4. Ecosystems of located biotop.
5. Ecosystems of whole biosphere.

The *boundary* between an ecosystem and its surrounding environment is a steep gradient of physical and/or chemical properties. The physical boundary is formed by an interphase between solid and liquid phases, solid and gas phases, liquid and gas phases. For example, the microbial ecosystem of an aerobic tank for wastewater treatment is separated from the environment by the reactor walls and air–water interphase. The steep gradient of chemical substances, for example, oxygen, ferrous, hydrogen sulphide, etc., forms a chemical barrier. Such barriers separate, for example, aerobic and anaerobic ecosystems in a lake. The steep gradient of conditions can also be created by cell aggregation in flocs, granules or biofilms. The main function of the boundary is to maintain integrity of an ecosystem by controlled isolation from the environment, and to protect an ecosystem from the destructive effects of the environment.

The boundaries of unicellular organism are as follows:

1. The cell membrane (cytoplasmic membrane) performs selective and controlled exchange of molecules between cell and environment. It is the most sensitive boundary because even a small break in the cell membrane will destroy the isolating and energy-generating properties of a cell membrane. Surface-active substances, organic solvents, oxidants and high temperature destroy the integrity of a cell membrane.
2. The cell wall protects a cell from changes in osmotic pressure and mechanical impulses. Bacteria with a thick cell wall are stained as Gram-positive cells.
3. Bacteria that are stained as Gram-negative cells have a thin cell wall covered by an outer membrane. Lipopolysaccharides of outer membrane of Gram-negative bacteria are very specific. These molecules interact with the human body's immune system and are often toxic or allergenic for humans. Gram staining is just one, and not always reliable method to differentiate bacteria with Gram-positive and Gram-negative types of cell walls.
4. Some prokaryotes, for example, mycoplasmas, have no cell wall.
5. Fungi and algae often have cell walls containing polysaccharides such as cellulose or chitin. Some algae have inorganic compounds such as calcium carbonate or silica in their rigid walls. Animal cells often have no cell walls.
6. The glycocalyx (capsule) is an extracellular polysaccharide, covering microbial cells of some species. Its functions include attachment of the cells to the surface; aggregation of cells; protection of cells against drying, oxidants, heavy metals and antibiotics.

A *multicellular aggregate* is formed and separated from its surrounding environment due to:

1. Aggregation by hydrophobic force, electrostatic interactions or salt bridges.
2. Loose polysaccharide or inorganic matrix (iron hydroxide, for example), combining the cells altogether by mechanical embedding, chemical bonds, hydrogen bonds, electrostatic forces or hydrophobic interactions.
3. Formation of mycelia, which is a net of branched cell filaments.
4. Polysaccharide matrix with a filamentous frame.
5. Structured matrix with layers parallel to the boundary or subaggregates, which are perpendicular to the boundary (4).
6. Coverage by a common sheath of organic (polysaccharides, proteins) or inorganic origin (iron hydroxide, silica, calcium carbonate).
7. Coverage by a common sheath ("skin" of microbial aggregate) consisting of dead cells.

A microbial aggregate can be considered as a multicellular organism if its parts have different coordinated or synchronized physiological functions, i.e., growth, motility, sexual interactions, assimilation of atmospheric nitrogen, production of extracellular polysaccharides, transport and distribution of nutrients and reduction of oxygen.

Microbial communities of environmental engineering systems are usually suspended or adhered to surface cells and microbial aggregates such as fixed biofilms and suspended flocs or granules. The boundaries of these ecosystems are as follows:

1. Side walls of the equipment with a fixed microbial biofilm.
2. Bottom of the equipment with the sediment of microbial aggregates.
3. Gas–liquid interphase with accumulated hydrophobic substances (lipids, hydrocarbons, aromatic aminoacids) and cells or aggregates with high hydrophobicity of their surface or cells and aggregates containing gas vesicles (5).

Diversity of a microbial ecosystem refers to the heterogeneity of genotypes (diversity of strains, species, physiological groups), in space (different zones, layers, aggregates and chemical or physical gradients), and in time (temporal changes in diversity of genotypes and spatial structure of ecosystem). Succession refers to the typical sequence of temporal changes in an ecosystem. Stagnation or climax is a state of ecosystem characterized by weak changes caused by poor environment, degeneration or ageing of the system.

There are known numerous mathematical expressions to quantify diversity. For example, Shannon-Weaver index (H) is:

$$H = \sum_{i=0}^{i=s} \left[\mathrm{p}_i - \ln(\mathrm{p}_i) \right] \tag{2}$$

where p_i is the proportion of the i-th group in the community, and S is a number of the groups in the community. Evenness index (E) is a measure of how similar the abundances of different groups are:

$$E = H / \ln S \tag{3}$$

When there are similar proportions of all groups, then the evenness index is one. The evenness index is larger than one when the abundances are very dissimilar. An example of quantitative characterization of microbial diversity in an anaerobic digester of activated sludge is given below.

Example: diversity in an anaerobic digester. There are at least five microbial groups involved in anaerobic digestion:

1. Hydrolytic bacteria degrading polymers (polysaccharides, proteins, nucleic acids) to monomers (glucose, aminoacids, nucleosides).
2. Acidogenic bacteria fermenting monomers to organic acids and alcohols.
3. Acetogenic bacteria producing acetate from other organic acids and alcohols.
4. Acetotrophic methanogens, producing methane from acetate.
5. Hydrogenotrophic methanogens, producing methane from hydrogen and carbon dioxide.

If the cell concentration of these organisms per 1 mL is 4×10^7, 7×10^8, 2×10^7, 5×10^8 and 1×10^8, respectively, the Shannon-Weaver index (H) of microbial diversity by physiological functions will be 13, and the evenness index (E) will be 8.1. The diversity indices are related to the process efficiency and stability and can be used in environmental engineering to compare the processes with different operational parameters.

2.2. *Interactions in Microbial Ecosystems*

The types of interactions between the biotic elements of a microbial ecosystem (cells of microbial population, microbial populations, microorganisms and macroorganisms) are positive and negative. Positive interactions are as follows:

1. Commensalism (only one biotic element has benefits).
2. Cooperation, mutualism (both elements have benefits).
3. Essential mutualism, symbiosis (both elements cannot live separately).

Negative interactions are as follows:

1. Neutral competition (organisms compete in the rate and efficiency of nutrients consumption, growth rate or in the resistance to unfavourable for growth environmental factors).
2. Antagonism (both abiotic elements suffer from interaction because they produce specific factors that negatively affect growth rate or other physiological or biochemical properties of competitors).
3. Amensalism (only one element suffers from the interaction).
4. Predation and parasitism; it is interaction when one element (prey) suffers and the other element (predator) benefits.

There may be neutralism, i.e., absence of positive or negative interactions between biotic elements.

The population density or average distance between biotic elements determines the type of interaction (Fig. 2.1).

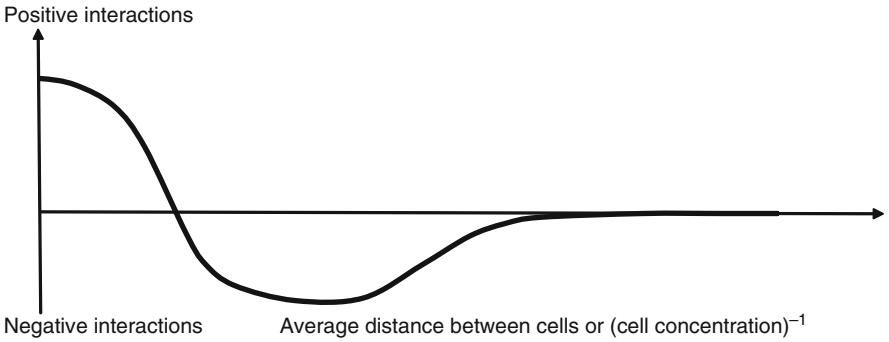

Fig. 2.1. Microbial interactions depending on cell concentration in ecosystem or the distance between cells in community.

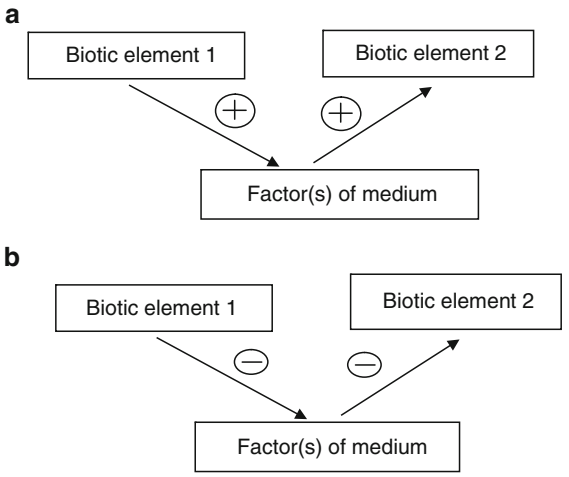

Fig. 2.2. Examples of microbial commensalism: (**a**), one group of microorganisms produces growth factor(s) essential for another group; (**b**), facultative anaerobes use oxygen and create anaerobic habitat suitable for the growth of obligate anaerobes.

When the population density is low, organisms have neither positive nor negative interactions. When the population density is medium, organisms compete among themselves for the availability of resources, by rate or efficiency of growth, and by production of metabolites, which negatively affect the growth of competitors. When the population density is high, cells usually aggregate and cooperate between themselves. Both competition and cooperation are carried out mainly because of the changes of chemical factors of environment such as concentration of nutrients, pH and redox potential of the medium, excretion of antibiotics, extracellular digestive enzymes, or heavy metals binding exopolysaccharides and simultaneous biodegradation of substances.

Commensalism, a microbial system relationship in which only one biotic element benefits, is realized by different ways (Fig. 2.2). There are thousands of examples of this interaction in environmental biotechnological systems. Some of them are as follows:

1. Facultative anaerobes use oxygen and create the conditions for the growth of obligate anaerobes; this interaction is important in the formation of anaerobic layer in microbial aggregates existing under aerobic conditions (6).
2. One group of microorganisms produces a growth factor essential for another group; this interaction is an obvious condition for the outbreak of pathogenic *Legionella pneumophila*, originated from such engineering systems as air conditioners, cooling towers and fountains.
3. Sequential biodegradation of xenobiotics by different groups of microorganisms; the microbial group performing biodegradation does not depend on the activity of the groups degrading its product of metabolism.

Mutualism is a type of interaction in which both biotic elements (microbial groups) have advantages from their interaction (Fig. 2.3).

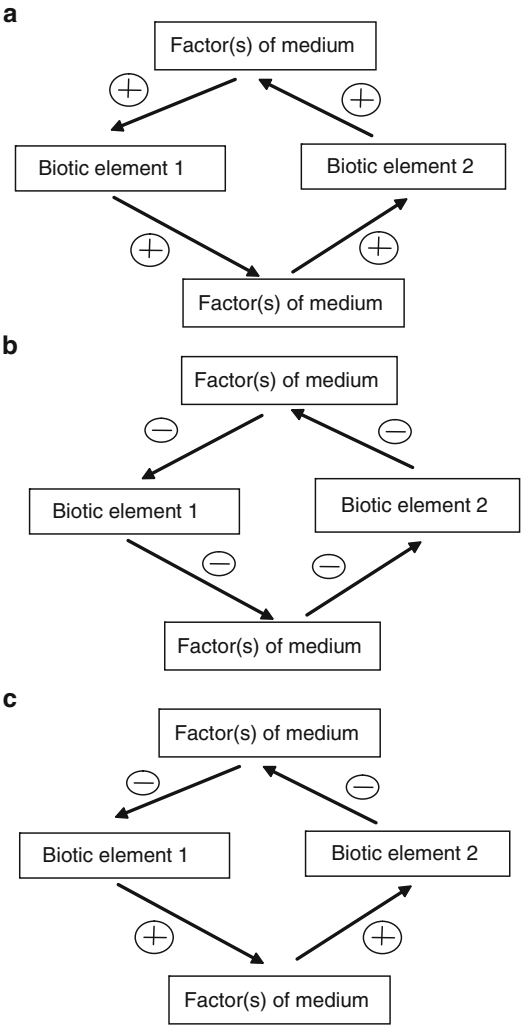

Fig. 2.3. Examples of mutual microbial interactions: (**a**), both microbial groups are exchanging with growth factors or nutrients; (**b**), both microbial groups diminish negative factors of medium; (**c**), removal of oxygen by group 2 creates anaerobic conditions for fixation of nitrogen by group 1 which supplies nitrogen compounds for group 2.

Mutual interactions are facilitated by close physical proximity in microcolonies, biofilms and flocs. Physiological cooperation in biofilm or aggregates is supplemented and supported by its spatial structure, i.e., formation of microhabitats for individual populations. Some examples of mutualism are as follows:

1. Syntrophy ("co-eating"), both microbial groups supply growth factors or nutrients.
2. Sequential biodegradation of xenobiotics when the product of biodegradation inhibits biodegradation.

3. Cycling of element by two microbial groups:

$$\text{Phototrophic bacteria} + \text{light} + H_2S + CO_2 \rightarrow S + \text{organics} \quad (4)$$

$$\text{Facultative anaerobic bacteria} + S + \text{organics} \rightarrow H_2S + CO_2 \quad (5)$$

4. Removal of oxygen by heterotrophic bacteria creates anaerobic conditions for fixation of nitrogen by phototrophic cyanobacteria, which supply nitrogen compounds for heterotrophic bacteria.

Positive interactions between animals and microorganisms are common and often essential for animals. Microorganisms can improve the digestion and assimilation of food by animals; produce growth factors, like vitamins and essential aminoacids for animals and keep out the pathogenic microorganisms from the surface and cavities of macroorganisms. For example, ants and termites cultivate cellulose-degrading fungi in their chambers to enhance the feeding value of plant material. Insects provide cellulose-degrading fungi with favorable conditions, and a supply of cellulose and mineral components. Another example is interactions between microorganisms and human organisms. The human body contains complex and stable microbial communities on the skin, hairs, body cavities, and within the gastrointestinal tract. Macroorganisms provide favorable conditions and supply nutrients to microorganisms, which produce some vitamins and keep out the pathogenic microorganisms from the skin and surface of cavities.

Symbiotic mutualism, or simply symbiosis, means that two groups cannot live separately. This is commonly the case in the interaction between macroorganisms and microorganisms. Many protozoa have symbiotic relations with bacteria and algae, often including them into the cell as endosymbionts. Bacterial endosymbionts supply the growth factors to the protozoan partner. A well-known example is the symbiosis of ruminant animals (cow, deer, sheep) and anaerobic, cellulose-degrading microorganisms in their rumen. Ruminant animals ensure crushed organics and mineral components, optimal pH and temperature, and microorganisms hydrolyze and transform cellulose to assimilated fatty acids.

Positive interactions between plants and microorganisms are common and often essential for plants. Epiphytic microorganisms live on aerial plant structures such as stems, leaves and fruits. The habitat and microorganisms on the plant leaves is called phyllosphere. The yeasts and lactic acid bacteria, for example, dominate in the phyllosphere. They receive carbohydrates and vitamins from the plant. High microbial activity occurs also in the soil surrounding the roots, called the rhizosphere. Organic compounds that stimulate heterotrophic microbes are excreted through the roots. Some fungi are integrated into the roots and contribute to plant mineral nutrition. This type of symbiotic interaction is called mycorrhizae. An example of mycorrhizae is the interaction between pine and fungi. Fungi integrated into the roots of pine contribute to plant mineral nutrition in exchange for a supply of organic nutrition from the plant.

Symbiotic mutualism of plants and microorganisms is a common interaction. A well-known example is the symbiotic fixation of atmospheric nitrogen, which is a major reservoir of nitrogen for life. The roots of some plants are invaded by nitrogen-fixing bacteria, mainly from the genus *Rhizobium*, which form tumor-like aggregates (nodule), where the bacteria are transformed into large cells (bacteroids) capable of fixing N_2 from air. A plant supplies

the bacteroids with organic and mineral feed and the bacteroids supply organic nitrogen to the plant. Symbiotic relations ensure the existence of lichens where photosynthetic algae or cyanobacterial component of lichens produce organic matter and microscopic fungi provide mineral nutrient transport and the mechanical frame for the photosynthetic organisms. Most lichens are resistant to extreme temperatures and drying and are capable of fixing nitrogen and occupying hostile environments.

Neutral competition between the biotic elements (organisms/populations/groups) means competition by the rate of nutrients consumption or growth rate. There may also be neutral competition by affinity with the nutrients or by resistance to environmental factors unfavorable for growth. It is the most typical interaction between aquatic natural ecosystems and wastewater treatment engineering systems.

Amensalism is an active competition in which one biotic element produces a substance that inhibits the growth of another biotic element. There may be, for example, changes in pH caused by the production of inorganic and organic acids by one population. Neutral competition and amensalism are the main mechanisms for forming an enrichment culture where one or some species dominate after cultivation of an environmental sample. The production of antibiotics is a specific application of amensalism because antibiotic is a substance able to, at low concentrations, negatively affect the growth of sensitive cells. Antibiotic-producing microorganisms dominate in rich environments with optimal conditions for growth, i.e., in the biotops where neutral competition is not sufficient to ensure domination of one biotic element. These biotops are soil, phyllosphere, skin or cavities of animals, but not aquatic biotops with a low concentration of nutrients.

Antagonism is the active competition between two biotic elements, i.e., competition enhanced by specific tools such as excretion of chemical substances, including antibiotics, by two competing biotic elements.

Predation occurs when one organism engulfs and digests another organism. A typical predator–prey relationship exists between predator protozoa and bacteria. Therefore, the predator protozoa improve the bacteriological quality of the effluent after aerobic wastewater treatment because it helps to reduce the number of free-living bacteria.

Parasitism is a very common interaction between microorganisms and macroorganisms, and between different microorganisms. The benefiting parasite derives its nutritional requirements from the host, which is the harmed organism. All viruses are parasites of bacteria, fungi, algae, plants and animals. Some prokaryotes are parasites of prokaryotes. For example, *Bdellovibrio spp.*, small curved cells, are parasites of Gram-negative bacteria, and *Vampirococcus spp.* sucks the cytoplasm out of another bacterium. Enumeration of microbial parasites in the environmental sample by the zones of lysis on a Petri dish with a layer of specific bacteria is the simplest nondirect way to evaluate the pollution of environment with these bacteria. Growth of bacterial viruses (bacteriophages) can deteriorate the industrial cultivation process because of spontaneous lyses of bacterial cells. Bacteriophages are widely used in genetic engineering of bacterial strains as the vector for transfer of defined genes into bacterial cells.

Plant parasites are represented by phytopathogenic viruses, prokaryotes and fungi. These parasites cause plant diseases. Typical stages of disease are as follows:

1. Contact of the microorganism with the plant.
2. Entry of the pathogen into the plant.
3. Growth of the infecting microorganism.
4. Development of plant disease symptoms.

Microbial pathogens disrupt normal plant functions by producing enzymes, toxins and growth regulators. Some plant pathogens such as white-rot fungi or bacteria from genus *Pseudomonas* can degrade xenobiotics and are widely used in environmental engineering. Therefore, the risk of plant infection must be accounted for in environmental biotechnology operations, especially during soil bioremediation.

Parasites of human and animals are represented by pathogenic viruses, prokaryotes, fungi or protozoa. The pathogenic (infectious) microorganisms grow in animal tissue and can cause diseases in macroorganisms. Saprophytic microorganisms feed on dead organic matter. Opportunistic pathogens are normally harmless but have the potential to be pathogens for debilitated or immunocompromised organisms.

Infection refers to the disease transmission caused by the transfer of pathogenic microorganisms from the environment or from one macroorganism to another. Infectious microorganisms can enter a human through direct contact between individuals or reservoir-to-person contact. The diseases may be conventionally distinguished as air-borne, water-borne, soil-borne and food-borne infectious diseases. When infectious agents are spread by an insect such as a mosquito, flea, lice, biting fly or tick, they are referred to as vectors.

Infectious diseases still account for 30–50% of deaths in developing countries because of poor sanitation. By comparison, mortality from infectious diseases is 10 times smaller in developed countries. Transmission of water-borne diseases is directly related to the bacteriological quality of water and effluent of wastewater treatment plants. Sources of pathogens other than sewage outlets are wildlife watersheds, farms and landfills. The prevention of outbreaks of water-borne and air-borne diseases is one of the main goals of environmental biotechnology. Environmental engineers and epidemiologists must work closely to identify the reason of outbreak, find its source (reservoir), define the major means of transmission of infectious microorganisms, and to develop a way to stop or diminish the scale of outbreak.

Factors of pathogenicity include the following abilities of microorganisms:

1. Production of exotoxins, which are extracellular proteins. In this case, host damage can occur at sites far removed from a localized focus of infection. For example, anaerobic bacteria *Clostridia tetani* can be introduced from the soil into the body with deep puncture wounds. If the wound becomes anaerobic, the microorganism can grow and release its tetanus toxin, which causes spastic paralysis.
2. Production of enterotoxins, which are the exotoxins that act in the small intestine. These cause diarrhea, the secretion of fluid into the intestinal passage.
3. Production of endotoxins, which are lipopolysaccharides of the outer membrane of Gram-negative bacteria. Endotoxins are less toxic than exotoxins.
4. Formation of microstructures (fimbriae, flagellum) and macromolecules for specific adherence of microbial cells or viruses to a host cell.
5. Formation of cell structures (capsule) and macromolecules (O-antigen) protecting microbial cells from the reaction of a host macroorganism.

Water-borne pathogens enter the host body by ingestion of cells, cysts or viral particles. The most common water-borne pathogenic bacteria are pathogenic strains of *Escherichia coli*, *Leptospira spp*., *Vibrio cholera*, *Shigella spp*., *Salmonella spp*., and *Campylobacter spp*. Two protozoans of major concern as water-borne pathogens are *Giardia intestinalis*, *Cryptosporidium spp.* and *Entamoebia histolytica*. One to ten ingested cysts of *Giardia* can cause diarrhea. There are over 100 known water-borne human enteric viruses.

An *indicator microorganism* is a conventionally selected microorganism or group of microorganisms used to determine the risk of water-borne infection associated with fecal contamination of water from humans or animals. There is a great variety of pathogenic organisms in water, and detection of each one in order to monitor water quality is an expensive operation. An indicator microorganism must be of the same origin and have similar physiological properties as some group of pathogens, and can be easily detected or enumerated in a water sample. Common indicators of water pollution with enteropathogens (main agents of water-borne diseases) from feces of warm-blooded animals are the numbers of the cells of *E. coli* (fecal coliforms), some *Streptococcus spp*. (fecal streptococci) or anaerobic *Clostridium spp*. The concentration of coliforms is usually less than 1 cell/mL in treated drinking water and more than some million (10^6) cells/mL in sewage. The concentration of heterotrophic bacteria in water determined by heterotrophic plate count (HPC) is also important bacteriological parameter of water quality. The concentration of anaerobic bacteria from genera *Clostridium*, *Bifidobacterium* or *Bacteroides* may be considered a good indicator of fecal pollution of water because their content in feces in some orders larger than the content of coliforms. There are no indicator organisms for protozoan cysts and viruses because of the specific release and survival of every strain.

3. MICROBIAL GROWTH AND DEATH

3.1. Nutrients and Media

The *elemental composition* of biomass can be shown approximately by the formula $CH_{1.8}O_{0.5}N_{0.2}$. The average content of carbon in a microbial biomass is approximately 50%. The exact elemental composition can be determined by an automatic COHN analyzer and used in the design of the biotechnological process. For example, the aerobic growth of biomass ($CH_{1.8}O_{0.5}N_{0.2}$) and biodegradation of carbohydrates shown by formula CH_2O can be described by the following equations:

$$X_1 < CH_2O > + X_1O_2 \rightarrow X_1H_2O + X_1CO_2 \tag{6}$$

which shows oxidation of carbohydrates to generate energy used for growth;

$$X_2 < CH_2O > + X_20.5O_2 \rightarrow X_2\,[2H] + X_2CO_2 \tag{7}$$

which shows oxidation of carbohydrates to generate reducing equivalents [2H] used for biomass synthesis; and

$$X_3CH_2O + X_30.2NH_3 + X_30.1\,[2H] \rightarrow X_3CH_{1.8}O_{0.5}N_{0.2} + X_30.5H_2O \tag{8}$$

which shows assimilation of carbon from carbohydrates to biomass.

To ensure supply of all essentials for biological activity elements, a medium must contain so-called macronutrients (C, H, O, N, P, S, K, Na, Mg, Ca and Fe) and micronutrients (Cr, Co, Cu, Mn, Mo, Ni, Se, W, V and Zn). The recommended media for specific groups of microorganisms are given in numerous microbiological manuals.

The *chemical content* of microbial biomass varies moderately. The water content in microbial cells is 70–80%. The average content of dry matter in bacterial cells is as follows: protein, 55%; RNA, 15%; polysaccharides, 10%; lipids, 5%; DNA, 5%; monomers and inorganic ions, 10%. The quantity of DNA per cell is stable; quantities of RNA and protein per cell are larger at higher growth rate. Cells of algae or fungi grow at a lower rate than the majority of prokaryotes and include a larger quantity of cell wall material such as cellulose, silica and calcium carbonate. Therefore, the content of RNA and protein in biomass of fungi and algae may be lower than that of bacterial biomass.

"*Storage*" *compounds*. Quantities of "storage" compounds, which accumulate in cells in a rich medium under unfavorable conditions for growth, vary in a wide range. For example, the content of polyhydroxibutirate (PHB), which accumulates in bacterial cells under an excess of carbon source and oxygen limitation, can reach 80% of dry mass. Other "storage" compounds are such intracellular polysaccharides as glycogen (for prokaryotes and eukaryotes), starches (for eukaryotes), extracellular polysaccharides (for prokaryotes), storage lipids (for eukaryotes), polyphosphate and sulphur granules in prokaryotes. Therefore, the content of any component (c_i) of microbial biomass may be described by the following function:

$$(c_i) = (Ci)/\,[C_1 + C_2 f_1(\mu) + C_3 f_2(M)] \tag{9}$$

where Ci is the average quantity of i-component in cell, C_1 is average quantity of components in cell (DNA, material of cell wall), which weakly depend on growth rate and medium conditions; C_2 is the average quantity of components (RNA, protein) in cell, which significantly depend on specific growth rate (μ); C_3 is the average quantity of "storage" components in cell, which significantly depend on medium conditions (M).

Medium content must reflect the needs in elements and substances for desired microbial activity and growth. It can be defined medium, which is a mixture of pure mineral salts and organic substances or so-called complex medium containing organic and inorganic substances due to the digestion or extraction of natural ingredients such as meat, plant biomass, manure and food waste. Medium may be in liquid, solid, gaseous states or in their combinations. Microbial cultivation in laboratory is often performed on the surface of a solid gel medium.

Carbon sources for growth. Prototrophic microorganisms can use one source of carbon and energy to synthesize all organic components of a cell. Auxotrophic microorganisms require supply of growth factors, i.e., some components of biomass, such as vitamins, aminoacids, nucleosides or some fatty acids from the medium. They lose their ability to synthesize these substances due to presence of these substances in their natural habitats. Autotrophic microorganisms can use only CO_2 to synthesize all organic components of the cell. Heterotrophic microorganisms use organic sources of carbon to synthesize cell biomass.

3.2. Growth of Individual Cells

Growth, proliferation and differentiation. Growth is defined as an increase in individual cell mass or the mass of cell population. Proliferation (cell division) is defined as an increase in cell number. Cell proliferation by binary fission is the most common type. However, there may be "unbalanced" proliferation, which consists of multiple fissions of cells without their growth. There may be also "unbalanced" growth without proliferation. This appears as elongation or enlargement of cells without their divisions. Cell differentiation is the transformation of microbial cells into specialized cells. Examples of such cells are as follows:

1. An endospore is an anabiotic (i.e., temporarily not active) cell with low content of water and covered by thick envelope, serving for survival under unfavorable conditions for growth, e.g., starvation, dry environment and high temperature.
2. An exospore is similar to an endospore by its properties, but not forming in the mother cell; the main functions of these cells are to increase survival and dispersion of cells in the environment.
3. An anabiotic cyst is an enlarged cell with the main function of increased survival.
4. Nitrogen-fixing cysts and bacteroides are enlarged cells whose main function is the transformation of atmospheric nitrogen into aminogroups of organic substances.

Eukaryotic cell cycle. There is strict coordination of a cycle of individual cell growth and division with DNA replication cycle in a eukaryotic cell. A eukaryotic cell cycle (mitotic cycle) has the following phases:

1. G_1-phase is a period between cell division and initiation of DNA replication; the duration of mitotic cycle is usually proportional to the duration of G_1-phase; differentiation of cells starts from G_1-phase.
2. S-phase is a period of chromosomal DNA replication.
3. G_2-phase is a period between termination of DNA replication and mytosis (splitting of nucleus).
4. M-phase is a period of mytosis, splitting of nucleus.

Prokaryotic cell cycle. There is certain coordination between the cell division cycle (period between consecutive cell divisions) and DNA replication cycle (period between initiation and termination of chromosomal DNA replication) in a prokaryotic cell. However, this coordination is not as strong as in eukaryotes. Depending on growth or proliferation rates, there may be some cycles of DNA replication within a cell division cycle, or even a cell division cycle without a DNA replication cycle accompanying the formation of DNA-free daughter cells (7).

Coordination of cell cycle events. There are many levels of coordination between biochemical and physiological cell activities during a cell cycle:

1. Individual RNAs and enzymes synthesis and degradation.
2. Regulation of enzyme activity by metabolites and co-factors.
3. Regulation of catabolism and energy storage.
4. Regulation of whole-cell activity by different cell regulators.

Periods of exotrophy and endotrophy in cell cycle. A simple theory explaining coordination of cell cycle events is alternation of the periods of exotrophy and endotrophy in cell cycle (8). It was demonstrated experimentally that G_1- and G_2- phases of mitotic cycle comprise the phases of exotrophy when the external source of carbon and energy is extensively transformed

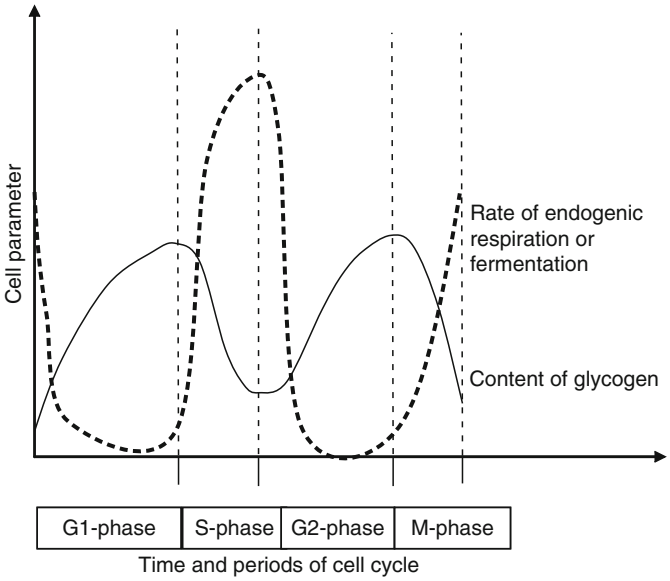

Fig. 2.4. Example of alterations of exo- and endotrophy in cell cycle of eukaryotes.

into energy and carbon store (glycogen, starch, lipids). S- and M-phases, which are the most sensitive periods of a cell cycle, are the phases of endotrophy. During endotrophy, the accumulated store of energy and carbon is utilized for DNA replication and mytosis, respectively (Fig. 2.4).

External sources of energy and carbon are not assimilated during these periods. The alternations between the periods of exotrophy and endotrophy are performed due to the increase or decrease of intracellular concentration of cyclic AMP and are accompanied by alternation of the charge of membrane potential (proton-motive force).

Different sensitivity of cells in the states of exo-and endotrophy. Environmental factors, which are unfavorable for DNA replication, retain cells in phases of endotrophy, G_1- or G_2 -phases. An extended period of exotrophy leads to enormous intracellular accumulation of carbon and energy sources (8). Exotrophic and endotrophic cells are distinguished by their biochemical and physiological properties so greatly that it would be useful to study these two different groups of cells.

Cell age and cell trophic state distributions in microbial population. Due to asynchronous cell cycles of individual microbial cells, there is a distribution of cells with different ages and cell trophic states in population. Cell size, DNA content, and percentage of exotrophic or endotrophic cells can be used to monitor cell population by flow cytometry. For example, duration of exotrophy (Δt_{ex}) and duration of G_1-phase (Δt_{G1}) of yeasts are linearly related to the duration of cell cycle (T) (7, 8):

$$\Delta t_{ex} = 0.5\ T - 1.0 \tag{10}$$

and

$$\Delta t_{G1} = 0.7\ T - 0.9 \tag{11}$$

Using these equations, the specific growth rate in population (μ) can be determined from the microscopic view or flow cytometry distribution, taking into account that $T = \ln 2/\mu$. G_1-cells of the budding yeasts can be determined as cells without buds. Exotrophic and endotrophic cells of bacteria can be also distinguished after adding a small quantity of cooxidizing substrate, producing toxic products of oxidation. For example, allyl or amyl alcohol can be added to cells that utilize ethanol. As a result, cells will produce allyl or amyl aldehyde, which cannot be further oxidized and therefore, kill cells. Exotrophic cells die after this incubation but endotrophic cells remain alive because they do not consume and oxidize external sources of carbon and energy. The share of exotrophic cells increases during starvation and other unfavorable conditions because the S-phase cannot be started until intracellular accumulation of sufficient quantity of carbon and energy sources is finished (8).

3.3. Growth of Population

Exponential growth and proliferation. Growth is an increase of biomass concentration or content (X). Proliferation is an increase in cell concentration or content (N). The balanced growth of microorganisms is followed by a proportional increase of cell number and biomass in the studied system. Under unfavorable conditions, the proliferation can be stopped, but not growth, resulting in long or large cells in the population. Under some conditions, these large cells will stop growing and split into smaller cells. The time required to form two new cells from one cell during the balanced growth is generation time, t_g. Exponential and balanced proliferation and growth are described by the equations:

$$N = N_0 2^n \tag{12}$$

or

$$\mathrm{X} = \mathrm{X}_0 2^{\mathrm{n}} \tag{13}$$

where N_0 is the initial cell concentration, n is the number of generations, X is concentration of the biomass after n generations, X_0 is initial concentration of biomass. Exponential growth is conventionally described by the equations:

$$\mathrm{d}X/\mathrm{d}t = \mu X \tag{14}$$

or

$$\mu = (\ln X - \ln X_0)/t, \tag{15}$$

where μ is specific growth rate and t is the duration of exponential growth. Typical specific growth rates are from 0.2 to 1.0/h^{-1} for unlimited growth of heterotrophic bacteria and from 0.01 to 0.2/h^{-1} for the growth of microscopic fungi. In such specific cases as apical (on the tip) elongation of thread-like, nonbranching hypha of mycelial fungi or actinomycetes, growth can be described by a linear equation.

Growth efficiency is determined by a growth yield that is a ratio between quantity of produced biomass and consumed nutrient ($Y_{X/S}$) or energy ($Y_{X/E}$).

For the batch system, it is determined by the equation:

$$Y_{X/S} = (X_t - X_0)/(S_0 - S_t) \tag{16}$$

where S_t is substrate concentration in the system at the end of period t, S_0 is the initial substrate concentration. Growth yield ($Y_{X/S}$) for the continuous system without recycling of the biomass is determined by the following equation:

$$Y_{X/S} = X/(S_i - S_e) \tag{17}$$

where X is a biomass concentration, S_i and S_e are the concentrations of substrate in the influent and effluent.

Microbial batch culture. A semiclosed system of cultivation is called a batch culture. There is usually a supply of air, release of gaseous products and additions of titrant and antifoam substance during this type of cultivation. Due to exhaustion of nutrients and accumulation of biomass and metabolites, the sequence of following phases is typical in batch culture:

1. Lag-phase or phase of cells adaptation and self-control of environment; its duration depends on the concentration of inoculated biomass and magnitude of the difference between previous and current conditions of cultivation.
2. Short log-phase or phase of exponential growth.
3. Transitional period between log-phase and stationary phase.
4. Stationary phase is characterized by slow growth or its absence due to exhaustion of nutrients or accumulation of metabolites.
5. Death phase is characterized by an increasing number of dead cells and their lysis.

Microbial continuous culture is open for exchange by gases and liquids. There is a large diversity of aerobic and anaerobic bioreactors for continuous cultivation, for example:

1. Bioreactors of complete mixing; the most common type is a chemostat where the dilution rate (D), which is a ratio between flow rate (F) and working volume of the reactor (V), is maintained constant.
2. Plug-flow systems.
3. Consecutively connected bioreactors of complete mixing, which form a plug-flow system.
4. Fixed biofilm reactor or retained biomass reactor with the flow of medium through it; biomass is retained in the reactor due to adhesion, sedimentation or membrane filtration.
5. Complete mixing or plug-flow continuous cultivation with recycling of microbial biomass.
6. Semicontinuous and sequencing batch cultivation, which is a continuous cultivation with the periodical addition of nutrients and removal of suspension.

3.4. Effect of Environment on Growth and Microbial Activities

Macro- and microenvironments. There is no gradient of parameters that depends on activity of microorganisms in a macroenvironment; however, there is a gradient of parameters depending on the microbial activity in a microenvironment. The typical scale of such microbial microenvironments as aggregate, biofilm, or microbial mat is between 0.1 and 100 mm. An artificial microenvironment is created due to adhesion of cells on carrier or cell incorporation

into carrier. Cells can be concentrated not only on liquid–solid interphase but also on liquid–gas interphase because the nutrient concentrations are higher there than in the bulk of liquid. A medium (pl. *media*) is an artificial environment for the cultivation of microorganisms in the form of solution, suspension or solid matter.

Effects of nutrients on growth rate. Kinetic limitation means that specific growth rate depends on the concentration of limiting nutrient. Usually, it is one limiting nutrient, but there may be simultaneous limitation by some nutrients. If one nutrient limits the specific growth rate, this dependency is often expressed by Monod's equation:

$$\mu = \mu_{\max}\left[S/(S + K_s)\right] \tag{18}$$

where $\mu_{\max}$ is the maximum of specific growth rate; S is concentration of the nutrient (substrate), limiting growth rate; K_s is a constant. However, there are hundreds of other known models describing μ as $f(S_i)$. A double limitation of μ by donor of electrons and oxygen is typical for the cases when the initial step of catabolism is catalyzed by oxidase or oxygenase incorporating atom(s) of oxygen into a carbon molecule or energy source.

Effects of nutrients on yield. The stoichiometric limitation of growth means that the dosage of the nutrient in the medium linearly determines the yield of biomass. For some groups of prokaryotes, the sources of carbon and energy are separated. Growth efficiency depends on energy extracted during catabolism of the energy source. Growth yield reflects the balance of energy produced in catabolism and energy assimilated in biosynthesis. Typically, in microbial growth there is no feedback regulation between the rates of biosynthesis and catabolism. Therefore, limitation of biosynthesis due to nutrient limitation or unfavorable physical factors of environment diminishes growth yield. However, there may be paradoxical increase of growth yield under unbalanced biosynthesis and catabolism and excess of carbon source. This can be caused by the redirection of carbon flow under excess of carbon source to the synthesis of storage carbohydrates or PHB, which require less energy for their synthesis than cell biomass. Another portion of energy, in the case of unbalanced catabolism and biosynthesis, can be used for the synthesis of extracellular polysaccharides or intracellular accumulation of polyphosphates, polypeptides or low molecular weight osmoprotectors.

Effect of starvation on microorganisms. There are three typical responses of microorganisms to starvation, i.e., shortage of some nutrients in a medium. The bacteria known as R-tactics are fast growing in a rich medium but can quickly die under a shortage of nutrients. Typical representatives of this group are *Pseudomonas spp*. The L-tactics bacteria are fast growing in a rich medium, but under starvation, they form dormant spores and cysts. Typical representatives of this group are *Bacillus spp*. K-tactics bacteria are adapted to grow slowly in a medium with a low concentration of nutrients. Typical representatives are the oligotrophes, *Hyphomicrobium spp.*

Effect of oxygen on growth. Aerobes are microorganisms that grow at the atmospheric pressure of oxygen (0.21 atm). Microaerophiles prefer a low concentration of oxygen because they have oxygen-sensitive molecules. Aerotolerant anaerobes have no need for oxygen to grow but can tolerate its presence in the medium. Obligate (strict) anaerobes are sensitive to oxygen because they have no protection against such toxic products of oxygen reduction as hydrogen peroxide (H_2O_2), superoxide radical (O_2^-) and hydroxyl radical (OH·). The

relationship to oxygen can be easily determined in the laboratory and is one of the most important identification properties of microorganisms because it was created in microbial evolution in parallel with the planet's evolution from an anaerobic to an aerobic atmosphere. The concentration of dissolved oxygen for the specific growth rate of aerobic microorganisms is usually limited to below 0.1 mg/L, but in the cases where the initial step of catabolism is catalyzed by oxidase or oxygenase, it can be significantly higher, up to 1 mg/L.

Effect of temperature on growth. The maximum temperature for growth depends on the thermal sensitivity of secondary and tertiary structures of proteins and nucleic acids. The minimum temperature depends mainly on the freezing temperature of the lipid membrane. The optimal temperature is close to the maximum temperature. Different physiological groups of microorganisms adapt to different temperatures. Psychrophiles have optimal temperatures for growth below 15°C. Mesophiles have optimal growth temperatures in the range between 20 and 40°C. Thermophiles grow best between 50 and 70°C. There are known thermoextremophyles growing at temperatures higher than 70°C.

Effect of pH on growth. Natural biotops have differing pH values: pH 1–3 (gastric juice, volcanic soil, mine drainage); pH 3–5 (plant juices, acid soils); pH 7–8 (fresh and sea water); pH 9–11 (alkaline soils and lakes). Acidophiles grow at pH lower than 5, neutrophiles grow within the pH range from 5.5 to 8.5, and alkalophiles grow at pH higher than 9. Intracellular pH is an approximately neutral pH. Extracellular pH affects the dissociation of carboxylic-, phosphate-, and amino-groups of a cell's surface, thus changing its charge and adhesive properties. This feature is important for the sedimentation of activated sludge, cell aggregation, and formation of microbial biofilm.

Effect of osmotic pressure on growth. The majority of microorganisms can live with a concentration of salts in the medium up to 30 g/L. A higher concentration of salts or organic substances can cause water to diffuse out of the cell by osmosis. However, some groups of microorganisms adapt to high osmotic pressure or low activity of water. These halophiles require the addition of NaCl in the medium during their isolation and cultivation. Extreme halophiles require a high concentration of NaCl (15–30%) in the medium. Xerophiles are able to live in a dry environment. The main adaptation characteristic of halophiles and xerophiles is their ability for intracellular accumulation of such low molecular weight hydrophilic osmoprotectors as polyoles, oligosaccharides and aminoacids.

3.5. Death of Microorganisms

Natural death of microorganisms. The equally splitting cells of bacteria are considered almost immortal creatures. However, some bacterial cells die even in pure culture at optimal growth conditions. Hypothetically, the death of microorganisms at optimal conditions for growth can be caused by a small asymmetry always determined in cell division and accumulation or depletion of asymmetrically separated cell components. The asymmetry of the division of eukaryotic cells is visible so that mother and daughter cells can be often distinguished. There may be accumulation or depletion of inert or essential cell components in this asymmetrical division, budding or splitting of eukaryotic cells. Therefore, the percentage of dead cells in the population of pure eukaryotic culture under optimal conditions for growth can be from 1 to 5%. Other reasons for natural cell death may include the production of

toxic oxygen radicals, shortage of essential cell components due to starvation, changes in structure of cell biopolymers and lipid components due to unfavorable physical parameters of the microenvironment.

Fate of released microorganisms in environment. The natural death of pathogenic microorganisms released to the environment is the most important factor in the termination of infectious diseases outbreaks. Active biodegraders, which are used in environmental engineering, are often opportunistic pathogens or genetically modified strains. Therefore, the study of environmental fate, death rate and survivability of microorganisms, which are used in bioremediation of polluted soil or spills in marine environment and released to environment, is essential for determining process feasibility. A rule of the thumb is that applied microorganisms must have some reasonable limits of lifetime in the treated and surrounding areas. This short lifetime can prevent accumulation or spread of unwanted microorganisms in the environment.

Control of microbial death. The control of unwanted microbial growth can be performed by physical or chemical inhibition of growth, killing of the microorganisms or their removal from the environment. Antimicrobial agents kill cells of bacteria, fungi or inactivate viral particles; thus, the terms bactericidal, fungicidal and viricidal agents are used. The most sensitive targets of the microbial cell are as follows:

1. Integrity of cytoplasmic membrane.
2. Active centers of enzymes.
3. Secondary, tertiary and quaternary structures of enzymes.
4. Primary and secondary structures of nucleic acids.

Heat treatment. The rate of cell death under heat treatment is a function of the first order:

$$\mathrm{d}X_\mathrm{d}/\mathrm{d}t = k(X_0 - X_\mathrm{d}) \tag{19}$$

or

$$\ln(1 - X_\mathrm{d}/X_0) = -kt \tag{20}$$

where X_d and X_0 are the numbers of dead cells and initial number of alive cells, respectively; t is the time of exposure, and k is a constant of decay. Another parameter, the decimal reduction time (D), which is the time required for tenfold reduction of the population, is used in practice:

$$D = (\ln\ 0.1)/(-k) = 2.3/k \tag{21}$$

Pasteurization kills the vegetative cells of bacteria, fungi and protozoa. Vegetative cells of bacteria have a decimal reduction time in the range of 0.1–0.5 min under a temperature of 65°C. During bulk pasteurization, the liquid is exposed at 65°C for 30 min. During flash pasteurization, the liquid is heated to 71°C for 15 s and then rapidly cooled. Pasteurization reduces the level of microorganisms in the treated staff but does not kill all of them. Heat sterilization kills all microorganisms in the treated staff. It is often performed in autoclave. Bacterial endospores cannot be killed at the temperature of boiling water. Therefore, the autoclave uses steam under the pressure of 1.1 atm, which corresponds to a temperature of 121°C. The time of exposure of the sterilized material in autoclave under 121°C must be from 10 to 20 min.

Sterilization by radiation. Electromagnetic irradiation such as microwaves, ultraviolet (UV) radiation, X-rays, gamma rays and electrons are also used to sterilize materials. UV

irradiation, which does not penetrate solid, opaque or light absorbing materials, is useful for disinfection of the surfaces, air, and water. Gamma and X-rays, which are more penetrating, are used in the sterilization of heat-sensitive materials, especially biomedical plastics.

Sterilization by filtration of cells and viruses is performed in the bulk of filtration material by using particles adsorption or polymer membranes with a defined diameter of pores. Depth filters consist of a random array of overlapping fibers. Depth filters adsorb particles on the fiber surface. Membrane filters contain a large number of pores with a diameter smaller than cell size. Thus, microorganisms are trapped on the surface of the membrane. Sterilization of liquids by filtration through the membrane preserves sensitive substances, usually biological polymers, antibiotics and vitamins that are easily inactivated by heat. Bulk filtration (adsorption) is used mainly for the removal of fungi spores, bacterial cells and virus particles from air. Filtration, coagulation and settling processes, which are used in the treatment of drinking water, help reduce and remove pathogens with the sediments and consumption of particles by protozoa living on the surface of filtration material.

Conservation (preservation) refers to the prevention of microbial spoilage of organic materials by the following means:

1. Lowering the storage temperature.
2. Lowering pH.
3. Drying.
4. Addition of salt or organic substances to decrease water activity.
5. Addition of organic solvent (ethanol).
6. Formation of anaerobic conditions.

Disinfectants and antiseptics. Disinfectants such as chlorine gas, chloramine, ozone and quaternary ammonium compounds are chemical antimicrobial agents that are used on inanimate objects. Antiseptics such as iodine, 3% solution of hydrogen peroxide and 70% solution of ethanol, are chemical antimicrobial agents that are used on living tissue. Antimicrobial activity can be characterized by minimum inhibitory concentration (MIC), which is the amount of antimicrobial agent required to inhibit the growth of the test organism. MIC may be determined by the tube dilution technique or by the agar diffusion method.

Disinfectants and antiseptics participate in the following modes of action:

1. Destruction of integrity of cell membrane by organic solvents, for example, ethanol, acetone and hexane.
2. Destruction of integrity of cell membrane by anionic, cationic and nonpolar surfactants (detergents).
3. Destruction of active centers of enzymes by oxidants (iodine, chlorine, ozone) and heavy metals (Hg, Cu, Ag).
4. Destruction of structure of proteins and nucleic acids by oxidants (iodine, chlorine, ozone), heavy metals (Hg, Cu, Ag) and organic solvents (ethanol, phenols).

Antibiotics are microbial or chemically synthesized substances that are used to treat infectious diseases because of their ability to inhibit specific microbial species. The mode of action is specific for each thermophiles grow best antibiotic and is based usually on the inhibition of specific enzyme activity or inactivation of an active center of specific enzyme. There are

thousands of known antibiotics but only some hundreds are applicable in medicine because of the toxic effects of antibiotics on humans. Antibiotics are used in environmental biotechnology to select specific strains of microorganisms and in the construction of recombinant strains.

Disinfection is the chemical or physical treatment of water or wastewater treatment plant effluent by strong oxidants such as chlorine, chloramines, chlorine dioxide, ozone, ferrate or by UV, with the aim to diminish the concentration of defined microorganisms and viruses to some level.

The rate of cell death ideally should follow first-order kinetics:

$$dX/dt = -kt \tag{22}$$

or

$$\ln(X/X_0) = -kt \tag{23}$$

where X and X_0 are the final and initial numbers of living cells, t is time of exposure, and k is a constant of decay that depends on the conditions of the disinfection. However, in the actual kinetics of disinfection, the order of the equation and a constant of decay change during the disinfection process are due to the presence of microorganisms with different resistance levels to the disinfectant.

Resistance of different groups of microbes to disinfection. Generally, the resistance of microbes to disinfections follows this order: vegetative bacteria (most sensitive group) → viruses → spore-forming bacteria → protozoan cysts (most resistant). Test microorganisms can be used, in some cases, to study water disinfection kinetics and to compare different disinfectants and regimes of disinfection instead of the pathogens. Indicator microorganisms are used often as test microorganisms. For example, cells of *Escherichia coli* can be used as test organisms in the disinfection of media containing pathogenic enterobacteria.

Comparison of chemical disinfectants. Disinfectants can be compared under the same conditions and microorganisms, by k, a constant of decay, or other technical or economical parameters. A benefit of chlorination is that chlorine residue remains in the water during distribution, which protects against recontamination. An undesirable side effect is that chlorination forms trihalomethanes, some of which are suspected carcinogens and bad-smelling chlorophenols. The efficiency of disinfection by chlorine gas decreases with pH because nondissociated hypochlorous acid (OHCl) is more active than hypochlorite ion (OCl^-). Chloramines (NH_2Cl and $NHCl_2$) are weaker disinfectants than chlorine but are effective in the control of microbial biofilm with an exopolysaccharide matrix. Ozone is more effective against viruses and protozoa than chlorine, but it is more expensive because it is generated by electrical discharge in a dry air stream at the site of application. Another disadvantage is that there is no residual antimicrobial activity after ozonation. Therefore, ozonation and chlorination are commonly used in sequence.

UV disinfection of water. UV radiation damages microbial DNA at a wavelength of 260 nm. Microbial inactivation is proportional to the UV dose. Humic substances, phenolic compounds and suspended solids interfere with UV transmission. Microbial cells can be reactivated by repairing DNA damages with exposure to visible light. Therefore, UV-treated water should not be exposed to light during storage.

Chemical and physical interferences with chemical disinfection. Ferrous and manganese ions, nitrites, sulphides and organic substances reduce the concentration of oxidizing disinfectants, thus reducing the inactivation of microorganisms during disinfection. Therefore, these substances must be removed or preoxidized before disinfection. The particles of clay, silt, iron hydroxides, aggregation of cells, their incapsulation in slime, in macroorganisms, or coverage of microorgansisms by the sheath, significantly reduce the inactivation of microorganisms and viruses during disinfection because of a steep gradient of oxidant in aggregates and its low concentration in cells. Therefore, particles and aggregates must be removed from water by coagulation and filtration or aggregation prior to disinfection.

4. DIVERSITY OF MICROORGANISMS

4.1. Physiological Groups of Microorganisms

Evolution of prokaryotes and atmosphere. According to geological data, the age of Earth is about 4.6×10^9 years old. The first organisms, prokaryotes, appeared about $3.5–3.8 \times 10^9$ years ago. There was no oxygen in that atmosphere, so the first organisms were anaerobes. The next step was an accumulation of atmospheric oxygen by oxygenic (oxygen-producing) phototrophic prokaryotes on the boundary $2.2–2.0 \times 10^9$ years ago. Eukaryotes appeared about $1.8–1.5 \times 10^9$ years ago, probably because of the intracellular symbiosis of smaller and bigger cells. The aerobic atmosphere led to the formation of the ozone barrier for intensive UV radiation on the Earth's surface. It was the primary condition for the creation of terrestrial life and multicellular organisms $0.6–0.5 \times 10^9$ years ago.

Diversity of energy generation types. Due to their long-term evolution, microorganisms have two major mechanisms for biological energy generation and related physiological groups:

1. Chemotrophy is the generation of biologically available energy due to the oxidation and reduction of chemical substances.
2. Phototrophy is the generation of biologically available energy due to the capture and transformation of light energy.

There are two types of chemotrophy and related physiological groups:

1. Organotrophy (chemoorganotrophy to be exact) is the generation of biologically available energy due to the oxidation of organic substances.
2. Lithotrophy (lithochemotrophy to be exact) is the generation of biologically available energy due to the oxidation of inorganic substances, for example, Fe^{2+}, H_2S, S, NH_4^+, NO_2^-.

There are two types of phototrophy and related physiological groups of prokaryotes:

1. Anoxygenic photosynthesis is the generation of biologically available energy from light energy under anaerobic conditions, using sulphide as an electron donor:

$$2H_2S + CO_2 + \text{light energy} \rightarrow < CH_2O > + 2S + H_2O \tag{24}$$

where $<CH_2O>$ is the conventional formula of organic matter produced from CO_2.

2. Oxygenic photosynthesis is the generation of biologically available energy and oxygen from light energy, using oxygen of water as an electron donor:

$$H_2O + CO_2 + \text{light energy} \rightarrow < CH_2O > + O_2 \quad (25)$$

Diversity of energy generation by organotrophes. There are different ways to generate biologically available energy by organotrophes and related physiological groups:

1. Fermenting organisms produce biologically available energy under anaerobic conditions (i.e., absence of oxygen and other acceptors of electrons) by fermentation, which is an intramolecular oxidation/reduction; one part of the molecule is oxidized and another part is simultaneously reduced.
2. Anaerobic respiring organisms produce biologically available energy under anoxic conditions (i.e., absence of oxygen but presence of other acceptors of electrons) by anaerobic respiration, i.e., oxidation of organic matter by acceptor of electrons other than oxygen, for example, Fe^{3+}, SO_4^{2-}, CO_2.
3. Aerobically respiring organisms produce biologically available energy under aerobic conditions (i.e., presence of oxygen) by aerobic respiration.

4.2. Phylogenetic Groups of Prokaryotes

Phylogenetic taxonomy compares the gene sequences of macromolecules of homologous (similar) function from different species. The phylogenetic relationships between microbial groups have been determined by the comparison of rRNAs, part of ribosome, which is a conservative, slow-evolving cell component used for protein synthesis. Ribosomal RNA is often considered the best tool to infer prokaryotic phylogeny because it is one of the most constrained and ubiquitous molecules available, and thus, the most informative (9). The established branching order shows the three domains of life: Bacteria (Eubacteria), Archaea and Eucarya (Eukaryotes). The last domain includes the kingdoms of plants, animals, fungi and protists.

Application of rRNA sequences in Microbiology of Environmental Engineering Systems. The time scale of rRNA sequence changes can be expressed in terms of 10^9 years. The discrepancy between rRNA sequences, A and B, is conventionally called the evolutionary distance (E_{AB}). It may be determined after computer or manual alignment as a number of the differences between the sequences (number of the mutations) per 100 sites (positions) of the compared sequences, i.e., as percentage of the mutations in compared sequences. The dissimilarity or similarity between the sequences of some organisms can be shown in a table (matrix), or in the form of a tree showing the hypothetical branching order of the organisms during their evolution. Different kinds of phylogenetic trees can be generated by computer analysis. The main applications of rRNA sequencing in environmental engineering are as follows:

1. The sequences of rRNA genes of strain or clone can be determined by the DNA sequencing machine, and the strain can be identified by comparison with known sequences downloaded from the databases.
2. The collections of rRNA gene sequences are used to study phylogenetic (evolutionary) relationships between microorganisms.

3. Specific parts of 16S rRNA sequences can be used as short (10–25-mer) labeled oligonucleotide probes for the detection of strains, genera, families or higher taxonomic units by the specific binding (hybridization) between target sequence rRNA and the probe.
4. Whole cell fluorescence in situ hybridization (FISH) with rRNA-targeted, fluorescent oligonucleotide probes is a popular approach to study the microbiology and spatial structure of complex microbial communities.

Major rRNA Phylogenetic Divisions of Archaea can be found in the classification of the Ribosomal Database Project (RDP-II) (10). The number of known sequences (data taken from http://rdp.cme.msu.edu/) reflects the abundance of the group and research interest to this group is shown in parentheses:

[1] ARCHAEA (1173)
[1.1] EURYARCHAEOTA (822)
[1.1.1] METHANOCOCCALES (45)
[1.1.2] METHANOBACTERIALES (206)
[1.1.3] METHANOMICROBACTERIA AND RELATIVES (504)
[1.1.4] THERMOCOCCALES (66)
[1.1.5] METHANOPYRALES (1)
[1.2] CRENARCHAEOTA (351)
[1.2.1] THERMOPHILIC CRENARCHAEOTA (160)
[1.2.2] NONTHERMOPHILIC CRENARCHAEOTA (189)
[1.2.3] ENVIRONMENTAL CLONE PJP27 SUBGROUP (2)

Major rRNA phylogenetic divisions of Bacteria can be found in the classification of the Ribosomal Database Project (RDP-II) (10). The number of known sequences (data were taken from http://rdp.cme.msu.edu/) reflects the abundance of the group and research interest to this group is shown in parentheses:

[2] BACTERIA (15104)
[2.1] THERMOPHILIC OXYGEN_REDUCERS (42)
[2.2] THERMOTOGALES (33)
[2.3] CTM PROTEOLYTICUS_GROUP (2)
[2.4] STRAIN EM 19 (1)
[2.5] ENVIRONMENTAL CLONE OPB45 GROUP (11)
[2.6] STR.SBR2095 (1)
[2.7] GREENNON-SULFUR BACTERIA AND RELATIVES (165)
[2.8] ENVIRONMENTAL CLONE OPB80 GROUP (14)
[2.9] LEPTOSPIRILLUM-NITROSPIRA (92)
[2.10] PROSTHECOBACTER_GROUP (99)
[2.11] ANR.THERMOTERRENUM GROUP (29)
[2.12] ENVIRONMENTAL CLONE OPB2 GROUP (3)
[2.13] NITROSPINA_SUBDIVISION (197)
[2.14] FLS SINUSARABICI ASSEMBLAGE (13)
[2.15] FLEXIBACTER-CYTOPHAGA-BACTEROIDES (781)

[2.16] GREEN SULFUR BACTERIA (48)
[2.17] ENVIRONMENTAL CLONE G37 GROUP (2)
[2.18] ENVIRONMENTAL CLONE WCHB1–31 GROUP (52)
[2.19] ENVIRONMENTAL CLONE UN104 GROUP (8)
[2.20] PLANCTOMYCES AND RELATIVES (281)
[2.21] CYANOBACTERIA AND CHLOROPLASTS (523)
[2.22] ENVIRONMENTAL CLONE 1611 (1)
[2.23] ENVIRONMENTAL CLONE PAD1 GROUP (3)
[2.24] ENVIRONMENTAL CLONE PAD39 (1)
[2.25] FIBROBACTER AND ACIDOBACTERIUM (173)
[2.26] ENVIRONMENTAL CLONE NH25–19 (1)
[2.27] SPIROCHETES AND RELATIVES (648)
[2.28] PROTEOBACTERIA (6893)
[2.29] FUSOBACTERIA AND RELATIVES (40)
[2.30] GRAM POSITIVE BACTERIA (4947)

Division of Proteobacteria comprises the majority of prokaryotes with Gram-negative type of cell wall (*Gracilicutes* by conventional taxonomy) and includes the following subdivisions by the classification of the Ribosomal Database Project (RDP-II):

[2.28] PROTEOBACTERIA (6893)
[2.28.1] ALPHA_SUBDIVISION (1968)
[2.28.2] BETA SUBDIVISION (1085)
[2.28.3] GAMMA SUBDIVISION (2949)
[2.28.4] DELTA SUBDIVISION (545)
[2.28.5] EPSILON SUBDIVISION (317)
[2.28.6] UNCULTURED MAGNETOTACTIC CLONES (27)
[2.28.7] ENVIRONMENTAL CLONE_A8 (1)
[2.28.8] UNNAMED DELTA PROTEOBACTERIUM (1)

Division of Gram-positive bacteria comprises prokaryotes with Gram-positive negative type of cell wall (*Firmicutes* by conventional taxonomy) and includes the following subdivisions by the classification of the Ribosomal Database Project (RDP-II):

[2.30] GRAM POSITIVE_BACTERIA (4947)
[2.30.1] HIGH_G + C_BACTERIA (2320)
[2.30.2] THERMOANAEROBACTER AND RELATIVES (146)
[2.30.3] SPOROMUSA AND RELATIVES (133)
[2.30.4] EUBACTERIUM AND RELATIVES (299)
[2.30.5] C.PURINOLYTICUM_GROUP (62)
[2.30.6] ANAEROBIC_HALOPHILES (39)
[2.30.7] BACILLUS-LACTOBACILLUS-STREPTOCOCCUS_SUBDIVISION (1248)
[2.30.8] MYCOPLASMA AND RELATIVES (377)
[2.30.9] CLOSTRIDIUM AND RELATIVES (323)

4.3. Connection Between Phylogenetic Grouping and G + C Content of Chromosomal DNA

G + C content in DNA and phylogenetic grouping. The G + C content of chromosomal DNA of prokaryotes ranges from 25 to 80 mol%. The current thinking in prokaryotic taxonomy is that if organisms in the same taxon are too dissimilar in G + C content, a taxon should be divided. It is a common opinion in prokaryotic classification that the distributions of G + C content of DNA is important for taxonomy (grouping) but not for phylogenetic classification. The reason for this is an absence of theory connecting the G + C content in DNA with the evolutionary distance between the rRNA sequences.

A model connecting G + C content in DNA with the rRNA-based phylogenetic distances includes the following assumptions:

1. A speciation (formation of new species) results in the formation of additional phylogenetic branches on the main branch. Figure 2.5a shows the two modern representatives of main and additional branches.

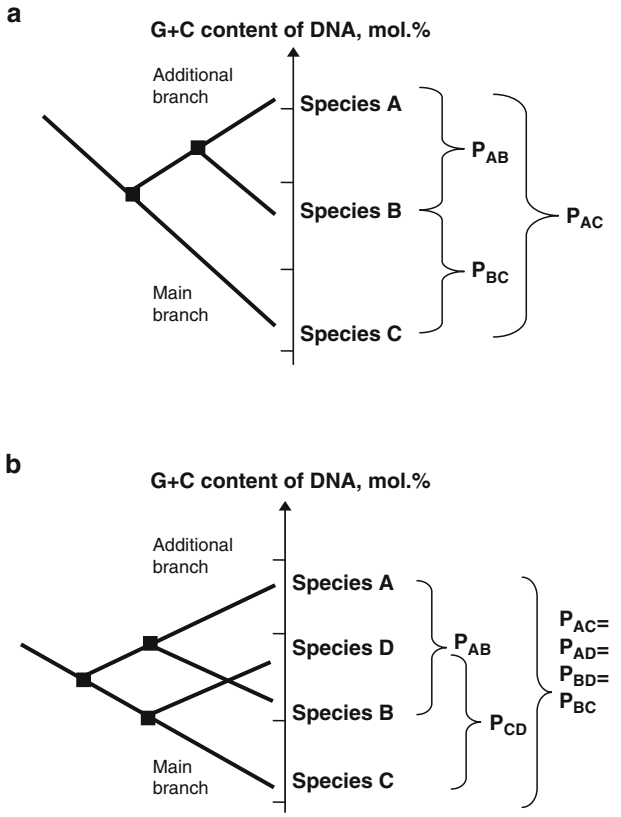

Fig. 2.5. Determination of evolutionary distance (P) between species in terms of G + C content in chromosomal DNA of modern representatives A,B, C, and D on main and additional braches.

2. The G + C contents in the DNA of the representatives in the main and additional evolutionary branches are changed in opposite directions. One branch and its modern representatives can be called GC^+, while another branch and modern representative can be called GC^- (Fig. 2.5a). Hypothetically, the bias to GC^+ or GC^- mode of DNA sequence evolution can be mediated by such conditions of environment as temperature and salinity.
3. The evolutionary distance (in terms of G + C content in chromosomal DNA) between modern representatives of main and additional branches ("GC distance") is the absolute value of the difference between G + C content of modern representatives of these branches (Fig. 2.5a, b).
4. The evolutionary distance (in terms of G + C content in chromosomal DNA) between modern representatives of any two branches (G + C content distance) is the absolute value of the difference in G + C contents in DNA of modern representatives of their main branches (Fig. 2.5a, b).

One proof of this model is that the pattern of the branching of rRNA-based phylogenetic tree for some species corresponds to the G + C content in the DNA of these species. Some demonstrations of this correlation for small numbers of species of methanogens, nitrifiers and sulphate-reducers are shown in Figs. 2.6 and 2.7. Small numbers of species was selected to demonstrate the correlation clearly.

16S rRNA phylogenetic distance trees and the trees based on evolutionary distances in terms of G + C content in DNA show the same order of branching. Therefore, this correlation can be used for the production phylogenetic tree showing not only the evolutionary distances between the sequences of 16S rRNA but also the branching order, which is related to the G + C content of DNA of studied microorganisms. The hypothesis explaining the evolutionary formation of GC^+ and GC^- branches can be used for the prediction of prokaryotes not yet discovered.

4.4. Comparison of rRNA-Based Phylogenetic Classification and Conventional Phenotypic Taxonomy

The *main contradiction* between phenotypic taxonomy and modern phylogenetic classification, based on comparison of rRNA sequences is that physiological groups often do not correspond to rRNA-based phylogenetic groups. Some examples are the grouping of microaerophilic and aerobic prokaryotes in one β-subdivision of proteobacteria and grouping of facultative-anaerobic and aerobic prokaryotes in the γ-subdivision of proteobacteria. Almost all divisions and subdivisions consist of species with a mixture of physiological and cytological features.

The small evolutionary distance between two species of different physiological groups reflects a short evolutionary time after speciation. An example is the small evolutionary distance between *Nitrobacter winogradski* and *Rhodopseudomonas palustris*, which are an aerobic chemolithotroph and an anaerobic phototroph, respectively. The small evolutionary distance between their 16S rRNAs can be explained as thus: the branch of *Nitrobacter winogradski*, originated from the line of *Rhodopseudomonas palustris* a short evolutionary time ago.

Physiological twins and phylogenetic twins. The contradiction between phenotypic taxonomy and rRNA-based phylogenetic classification is due to the stability of newly formed

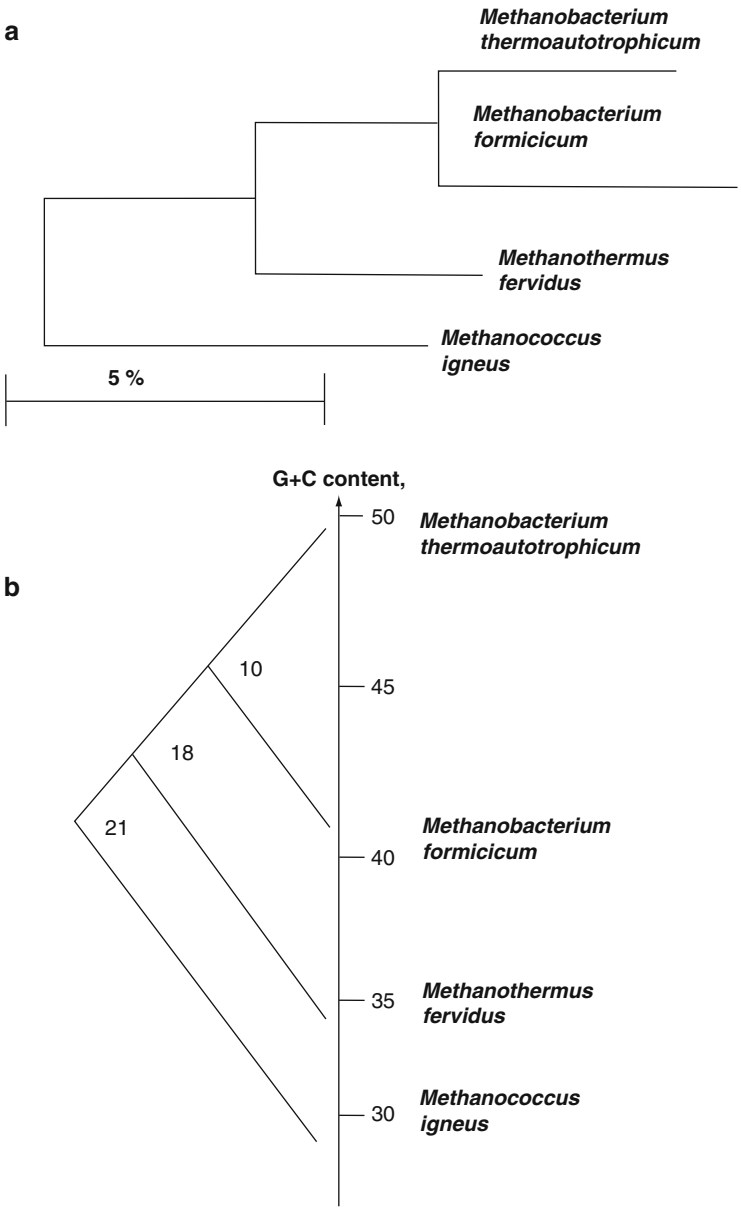

Fig. 2.6. Demonstration of similarity in the branching of 16S rRNA distance tree (**a**) and the distance tree accounting G + C content of DNA (**b**) of some methanogens. Evolutionary distances (P) between the species in terms of G + C content in chromosomal DNA of modern representatives are shown in bear the points of branching.

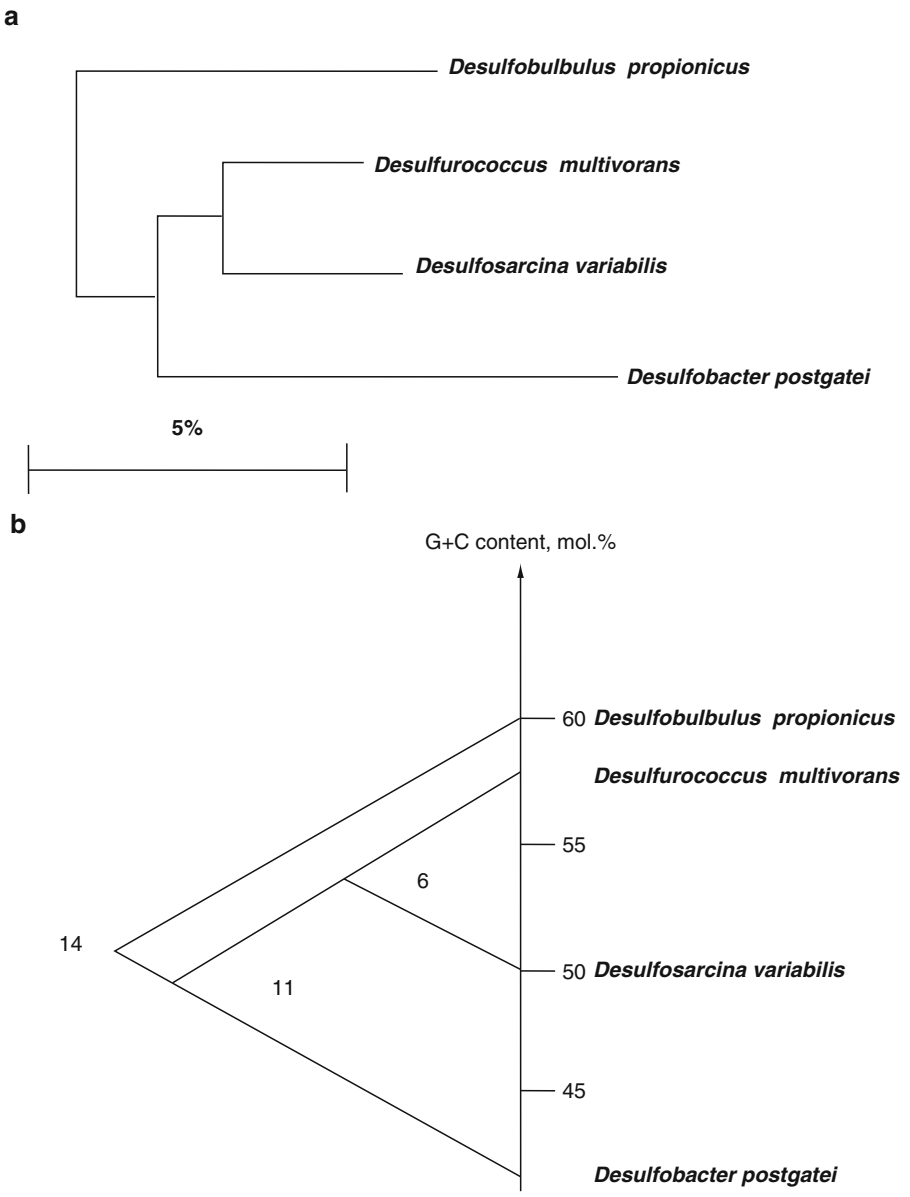

Fig. 2.7. Demonstration of similarity in the branching of 16S rRNA distance tree (**a**) and the distance tree accounting G + C content of DNA (**b**) of some sulphate-reducing bacteria. Evolutionary distances (P) between the species in terms of G + C content in chromosomal DNA of modern representatives are shown in bear the points of branching.

basic physiological properties during evolution. This stability is especially clear for such basic physiological properties as types of energy generation and the relationship of microorganisms to oxygen. At the same time, rRNA and other polynucleotide and polyaminoacid sequences changed significantly during evolution. Therefore, the distance between rRNA sequences, which is used in phylogenetic classification, reflects the time after divergency (branching) of phylogenetic lines but not the physiological discrepancies between their representatives. For example, such basic physiological characteristics as the ability for anaerobic respiration was created at an early stage of evolution and preserved in modern representatives. The physiological properties of some of these representatives may be very similar, but the dissimilarity between their rRNAs would be very large because of the accumulation of a large number of mutations in 16S rRNA over a long period of evolution (Fig. 2.8a).

If the main and additional evolutionary branches separated a long time ago but their representatives developed under the same conditions, they can have similar basic physiological features. These representatives, with a large phylogenetic distance, in terms of 16S rRNA sequences and large evolutionary distance, in terms of G + C content in DNA but similar physiological properties, can be considered physiological twins because they belong to the same physiological group (Fig. 2.8a).

If the main and additional evolutionary branches did not separate a long time ago, their modern representatives can have different basic physiological features but a small phylogenetic distance in terms of 16S rRNA sequences and small evolutionary distance in terms of G + C content in DNA (Fig. 2.8b). These organisms, with a small phylogenetic distance in terms of 16S rRNA sequences and a small evolutionary distance in terms of G + C content in DNA but with large physiological differences, can be considered phylogenetic twins because they belong to the same phylogenetic group (Fig. 2.8b).

The existence of the groups of GC^+ and GC^- physiological twins can be proven by symmetry of the distribution of G + C content in DNA of species within the groups of fermenting, anaerobic respiring and aerobic prokaryotes (Fig. 2.9a, b).

The existence of physiological twins and phylogenetic twins comprises the difference between phenotypic taxonomy and rRNA-based phylogenetic classification. Another cause of this difference is that the most popular present phylogenetic grouping is based on the evolution of one gene of 16S rRNA. Evolutionary classification based on the group of genes may be different, especially if these genes will be related to energy generation and oxidation–reduction reactions. However, even for such complicated phylogenetic analysis, the reason for the basic contradiction between phylogenetic and physiological groupings will remain: physiological and cytological features acquired in evolution remain basically the same, but the gene sequences will be significantly changed during evolution.

Identification of prokaryotes by the sequencing of 16S rRNA gene is faster and simpler than identification based on physiological features. Additionally, the sequences from poorly cultivated prokaryotic strains, or those not cultivated in the laboratory, can also be used for the identification of prokaryotes. That is why phylogenetic classification, based on comparison of 16S rRNA gene, is so popular at present. However, due to the contradictions between phylogenetic classification and physiological grouping, the mechanistic classification of prokaryotes, using only similarities of 16S rRNA gene produces the groups, which are

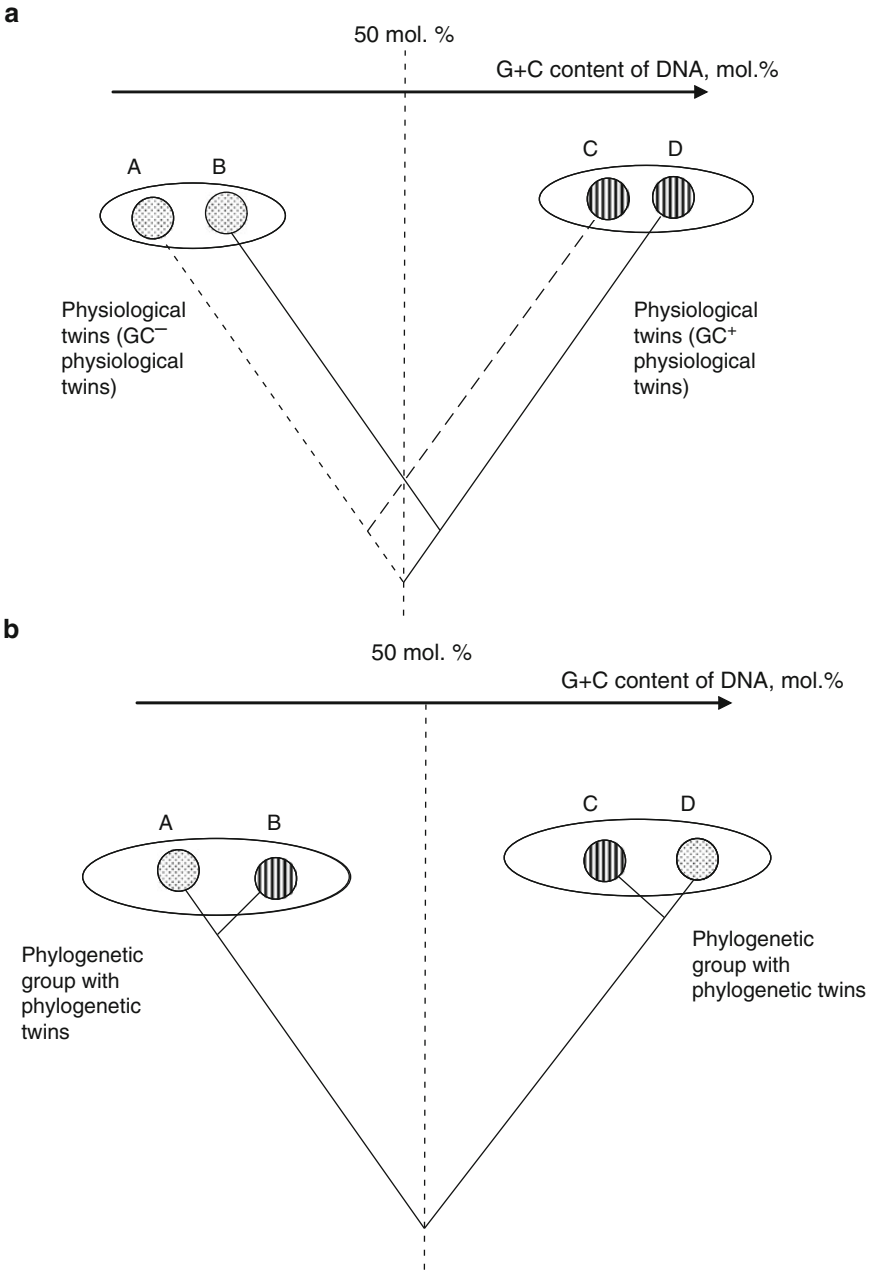

Fig. 2.8. Formation of physiological twins (**a**) and phylogenetic twins (**b**).

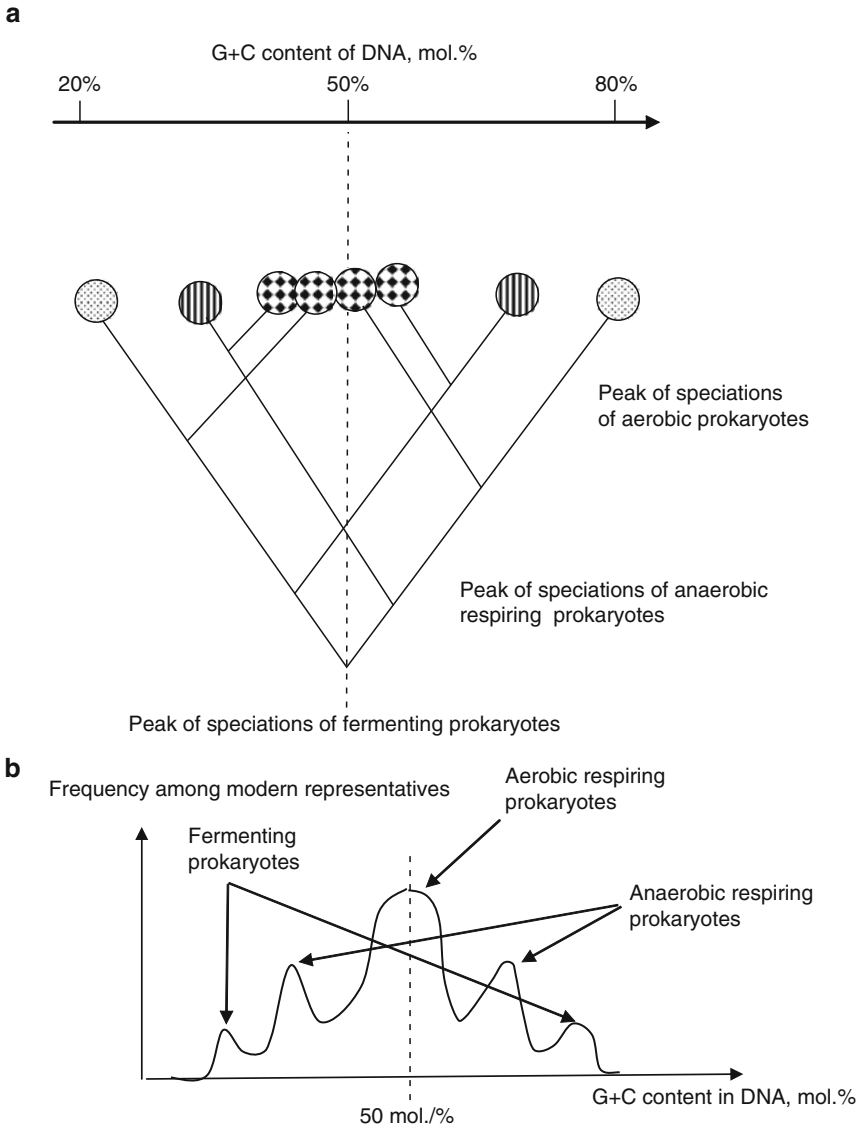

Fig. 2.9. Waves of speciations (**a**) and symmetry of the distribution of G + C content in DNA in the groups of fermenting, anaerobic respiring, and aerobic prokaryotes (**b**).

often the mixture of species with different basic physiological properties. Thus, to classify prokaryotes without contradictions between physiological and phylogenetical grouping, data on the evolution of basic physiological properties and DNA sequences must be combined and classified altogether.

Parallelism in evolution of genes. Another source of difference between phenotypic taxonomy and rRNA-based phylogenetic classification is the parallelism in the evolution of genes. In rRNA-based phylogenetic classification, it is thought that the number of differences in sequence of rRNA reflects the evolutionary distance of the origin of compared sequences from a common ancestor sequence. However, it was proved in experiments that there may be lateral transfers of genes in the environment, i.e., transfer of genes not only from ancestor to descendant but also between neighboring organisms. By our hypothesis of parallelism of prokaryotic evolution described below, the frequency of lateral transfer of the genes between major phylogenetic lines of *Gracilicutes*, *Firmicutes* and *Archaea* can by synchronized by the evolutionary changes of atmosphere and the waves of organic matter accumulation due to the waves of glaciations on the planet.

4.5. Periodic Table of Prokaryotes

Absence of predictive power in rRNA-based phylogenetic classification. The existing classification of 16S rRNA gene shown in the Ribosomal Database Project (RDP-II) and other databases, for example, BLAST of National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov/), is very useful for the experimental identification of microbial species. Identification can be currently performed by PCR of 16S rRNA gene and gene sequencing for several hours. Using conventional taxonomy methods to identify species requires a significantly longer and more laborious procedure performed by experienced researchers. Therefore, rRNA-based classification is more popular in experimental research. However, there are no physiological connections between phylogenetic groups and no predictive power in the current rRNA-based phylogenetic classification. As a result of the widespread use of 16S rRNA- based phylogenetic classification in experimental research, microbial diversity is often perceived by new users and students as a random mixture of microbial species and groups.

Main features of periodic table of prokaryotes. The hypothetical periodic table of prokaryotic phylogeny (Table. 2.3) was proposed to give predictive power to prokaryotic classification, clarify the physiological and evolutionary connections between microbial groups and give a logical basis for students to understand microbial diversity. (8, 11).

The main features of the periodic table of prokaryotic phylogeny are described below.

A. Three basic phylogenetic lines (related to the divisions of Bergey's Manual of Systematic Bacteriology) underwent parallel synchronized evolution:
   1. L1, *Gracilicutes* (prokaryotes with thin cell walls, implying a Gram-negative type cell wall).
   2. L2, *Firmicutes* (procaryotes with a thick, strong skin, indicating a Gram-positive type cell wall).
   3. L3, *Mendosicutes* (Archaea, prokaryotes that lack a conventional peptidoglycan).

The divisions of *Tenericutes* from Bergey's Manual (procaryotes without rigid cell wall) and oligotrophs (prokaryotes adapted for growth at a low concentration of carbon source in a medium) are not considered basic phylogenetic lines because they both do not have chemo- and phototrophic modern representatives with all types of energy generation.

Table 2.3
Phylogenetic lines, periods, and groups of periodic table of prokaryotes (selected examples of conventional genera are shown in the groups)

Evolutionary line of prokaryotes	Sub-line	Periods of evolution			
		P1	P2	P3	P4
Prokaryotes with Gram-negative type of cell wall (*Gracilicutes*)	L1c	$P1 - L1c$ *Bacteroides* *Prevotella* *Ruminobacter*	P2-L1c *Desulfobacter* *Geobacter* *Wolinella*	P3-L1c *Escherichia* *Shewanella* *Beggiatoa*	P4-L1c *Pseudomonas* *Acinetobacter* *Nitrosomonas*
	L1p	P1-L2p Not known	P2-L1p *Chlorobium* *Rhodocyclus* *Chromatium*	P3-L1p *Chloroflexus*	P4-L1p cyanobacteria *Prochloron*
Prokaryotes with Gram-positive type of cell wall (*Firmicutes*)	L2c	P1-L2c *Clostridium*, *Peptococcus* *Eubacterium*	P2-L2c *Desulfotomaculum* *Desulfitobacterium* *Bacillus infernus*	P3-L2c *Microthrix*, *Nocardia*, *Streptococcus*	P4-L2c *Bacillus* *Arthrobacter* *Streptomyces*
	L2p	P1-L2p *Heliobacterium* *Heliobacillus*	P2-L2p Not known	P3-L2p Not known	P4-L2p Not known
Archaea	L3c	P1-L3c *Desulfurococcus* *Thermosphaera* *Pyrodictium*	P2-L3c *Methanobacterium* *Thermococcus* *Haloarcula*	P3-L3c *Metallosphaera* *Sulfolobus* *Acidianus*	P4-L3c *Picrophilus* *Ferroplasma*

B. Phototrophic and chemotrophic sublines underwent parallel synchronized evolution in every phylogenetic line, with the exemption of the phototrophic line of *Archaea*. Therefore, there are five phylogenetic sublines in the periodic table:
   1. L1c, chemotrophic gracilicutes.
   2. L1p, phototrophic gracilicutes.
   3. L2c, chemotrophic firmicutes.
   4. L2p, phototrophic firmicutes.
   5. L3c, chemotrophic archaea.

Some representatives of *Archaea* possess light energy assimilating pigments but there are no phototrophic representatives in this line, probably because of the extreme environmental conditions for which *Archaea* were adapted.

C. New chemotrophic ways of biologically available energy production were created in every chemotrophic subline and related period of evolution in the following sequence:
   1. P1c, anaerobic fermentation.
   2. P2c, anaerobic respiration.
   3. P3c, microaerophilic respiration or alteration of fermentation and respiration.
   4. P4c, aerobic respiration.

D. New phototrophic ways of biologically available energy production were created in every phototrophic subline and related period of evolution in the following sequence:
   1. In the period P1p, products of anaerobic fermentation (organic acids and alcohols) are used as electron donor and carbon sources.
   2. In period P2p, products of anaerobic respiration are used as electron donors (H_2S, Fe^{2+}) and carbon sources (CO_2).
   3. In period P3p, products of microaerophilic respiration are used as electron donors (S) and carbon sources (CO_2).
   4. In period P4p, products of aerobic respiration are used as electron donors (H_2O) and carbon sources (CO_2).

Reasons of parallelism and periods in periodic table of prokaryotes. The existence of three evolutionary lines can be explained by parallel synchronized evolution in three different habitats:

1. *Gracilicutes* (Gram-negative bacteria) were adapted to life in biotops with constant osmotic pressure such as seawater or animal fluids.
2. *Firmicutes* (Gram-positive bacteria) were adapted to life in biotops with changeable osmotic pressure such as soil and shallow basins.
3. *Archaea* were adapted to life in hyper-extreme environments with high temperature and salinity.

The evolutionary parallelism in phylogenetic lines can be explained by the geological synchronization of the frequency of speciations in three phylogenetic lines. Hypothetically, the synchronization could have been caused by the waves of glaciations in the Pre-Cambrian era (8, 11). Every period of glaciation decreased the microbial population in the biosphere, decreased the concentration of CO_2 in the atmosphere and increased the accumulation of dead organic matter, thus creating conditions for a new wave of speciations in a warm period of geological evolution on Earth.

Another factor in the synchronization of the evolutionary periods in phylogenetic lines was the evolution of the anaerobic atmosphere to an aerobic one. Therefore, the periods of prokaryotic evolution and related groups of anaerobic fermenting, anaerobic respiring, microaerophilic and aerobic prokaryotes could be synchronized by the changes of oxygen concentration in the atmosphere. The creation of new groups of chemotrophs likely produced the conditions for the creation of a new group of phototrophs because the final products of this new group were used as electron acceptors and carbon sources for a related new group of phototrophs. Also likely, the horizontal gene transfers between three habitats enhanced the synchronization of parallel evolution in three phylogenetic lines.

Importance of the periodic table of prokaryotic phylogeny. The periodic table of prokaryotic phylogeny, shown in Table. 2.3, provides a theoretical understanding of microbial diversity. Due to the logical connection between the Earth's evolution and the parallel evolution in three lines of prokaryotes, the table possesses predictive power. Some groups of prokaryotes have not been discovered yet. Using the periodic table of prokaryotic phylogeny, it would be possible to forecast the discovery of aerobic and microaerophilic phototrophic *Firmicutes* (Gram-positive bacteria).

The periodic table of prokaryotic evolution gives a general overview and is not suitable for practical taxonomy and identification. However, it would be possible to produce more detailed identification tables, taking into account the parallelism of not only physiological

but additional cytological, biochemical, ecological and molecular-biological features of prokaryotes. To avoid the contradictions between the classification by the periodic table and phylogenetic classification, based on 16S rRNA gene sequences, the existence of physiological twins and phylogenetic twins described above must be taken into account.

5. FUNCTIONS OF MICROBIAL GROUPS IN ENVIRONMENTAL ENGINEERING SYSTEMS

5.1. Functions of Anaerobic Prokaryotes

These microorganisms are related to the period P1 of the periodic table. Their natural biotops are the sediments of aquatic ecosystems, tissues of macroorganisms, anaerobic microzones of soil and hot springs.

Chemotrophic subline of Gracilicutes (group P1-L1c). The main functions of anaerobic chemotrophic gram-negative fermenting bacteria (group P1-L1c) in environmental biotechnology are as follows:

1. Fermentation of saccharides to organic acids, alcohols and hydrogen.
2. Syntrophic formation of acetate from other organic acids during anaerobic digestion of organic matter.
3. Indication of fecal pollution of water.

Examples of the functions of selected genera are shown below.

Bacteroides spp. are the predominant organisms in the human colon and are generally isolated from the gastrointestinal tract of humans and animals; some species are pathogenic. Their functions in environmental biotechnology include: (a) anaerobic degradation of polysaccharides in an anaerobic digester and the anaerobic zones of microbial biofilms; and (b) indication of fecal pollution in water. *Prevotella spp.* are mainly pathogenic organisms but some species can be used for the biodegradation of collagen-containing wastes. *Ruminobacter spp.* facilitate anaerobic fermentation in the rumen and can be used for anaerobic fermentation of organic wastes. *Acetogenium spp.* and *Syntrophococcus spp.* are so-called acetogens, capable of producing acetate from other organic acids. This function ensures a supply of acetate to acetotrophic methanogens in the anaerobic digestion of organic wastes. S*yntrophococcus spp*. can also metabolize some C_1-compounds (methanol, formaldehyde, formic acid, carbon monoxide) and remove metoxilic groups from lignin. *Syntrophobacter wolinii* also produces acetate by degradation of propionate only in coculture (syntrophically) with hydrogen-utilizing prokaryotes. The acetate produced is sequentially used by acetotrophic methanogens during anaerobic digestion of organic wastes. *Veillonella spp*. are parasitic microorganisms. Some species can be used for the reduction of nitroaromatic compounds, including 2,4,6-trinitrotoluene (TNT), as first step in their biodegradation.

Chemotrophic subline of Firmicutes (group P1-L2c). The main functions of anaerobic chemotrophic gram-positive fermenting bacteria (group P1-L2c) in environmental biotechnology are as follows:

1. Hydrolysis of biopolymers.
2. Fermentation of saccharides and aminoacids to organic acids, alcohols and hydrogen.

3. Formation of acetate from hydrogen and carbon dioxide during the anaerobic digestion of organic matter.
4. Indication of fecal pollution in water.

Examples of the functions of selected genera are shown below. Anaerobic chemotrophic Gram-positive fermenting bacteria (group P1-L2c) perform the stages of anaerobic digestion of organic wastes such as the hydrolysis of biopolymers and fermentation of monomers to organic acids, alcohols, hydrogen and carbon dioxide. The existence of GC antipodes is especially clear in this group. This group is subdivided in both phylogenetic classification and conventional taxonomy for the divisions with low and high G + C content of DNA. Examples of the functions of selected genera from the subdivision with high GC content of DNA are shown below.

Species from the genus *Clostridium* are able to form endospores; usually they have no tolerance towards oxygen but the spores can survive in an aerobic environment. Both pathogenic and nonpathogenic species exist. The pathogenic species, for example, *Clostridium botulinum* and *Clostridium tetani*, are agents of severe diseases and produce strong toxins that can be considered as bioweapons. Clostridia can hydrolyze biopolymers, ferment monomers and aminoacids, produce alcohols, organic acids and hydrogen. It is the first rate-determining step in the anaerobic digestion of organic waste. Some thermophilic clostridia can be used for the hydrolysis of cellulose and production of fuel ethanol. Some clostridial species may be used for the reductive dechlorination of xenobiotics, for example, pesticides and herbicides. *Clostridium perfringens* is an indicator species in water quality evaluation. Spores of *Clostridium spp*. are used as test cultures in disinfection studies.

Species from the genera *Clostridium*, *Peptococcus*, *Peptostreptococcus* and *Eubacterium* ferment saccharides to form fatty acids (butyric, propionic, lactic, succinic and acetic acids), ethanol, hydrogen and carbon dioxide. It is the second step in the anaerobic digestion of organic waste.

Species from genera *Acetobacterium* and *Acetogenium*, together with some species from the genus *Clostridium*, are homacetogenic bacteria; they are able to reduce carbon dioxide and produce acetate that serves as a substrate for acetotrophic methanogens. It is the third important step in the anaerobic digestion of organic wastes. The production of organic acids by anaerobic fermenting bacteria can contribute to microbially induced corrosion of metal engineering systems.

Chemotrophic subline of Archaea (group P1-L3c). There are known species of anaerobic fermenting *Archaea*. Obligate anaerobic species from genera *Desulfurococcus*, *Thermosphaera*, *Pyrodictium* and some others from the phylum *Creanarchaeota* are able to ferment organic substances, but some of them can generate energy using sulphur as an electron acceptor. Those species capable of sulphur respiration can be classified also in the group of anaerobic *Archaea* (group P2-L3c of periodic table). These species are not currently used in environmental engineering systems but they may be potentially effective in the anaerobic biodegradation of organic wastes in engineered hot ecosystems.

Phototrophic subline of Gracilicutes (group P1-L1p). In the future, such organisms may be found among filamentous anoxygenic phototrophs (FAPs), belonging to the family *Chloroflex-*

aceae. Anaerobic gram-negative phototrophic bacteria using oxidized products of fermentation (organic acids and alcohols) can perform anaerobic photobiodegradation of organics in stabilization ponds and microbial mats of shallow rivers or springs.

Phototrophic subline of Firmicutes (group P1-L2p). There is one family *Heliobacteriaceae* in this group of obligate anaerobic gram-positive phototrophic bacteria. The genera of heliobacteria are *Heliobacterium*, *Heliobacillus*, *Heliophilum* and *Heliorestis.* Heliobacteria catabolize mainly fermentation products such as pyruvate, lactate, acetate, butyrate, ethanol and carbon dioxide. They can perform fermentation of pyruvate with the formation of acetate and CO_2 as products. Heliobacteria reside in soil, especially in paddy fields, mainly in the ryzosphere of plants, and can form spores for survival under unfavourable growth conditions. Symbiotic relationships may exist between heliobacteria performing strong nitrogen fixation for rice plants and rice plants supplying organic substances for heliobacteria. Heliobacteria would be useful in the biodegradation of organics in soil or microbial mats of springs, and in nitrogen fixation in paddy soils and soils under bioremediation.

5.2. Functions of Anaerobic Respiring Prokaryotes

These microorganisms are related to the period P2 of the periodic table. Their natural biotops are anoxic zones of aquifers, aquatic sediments, hot springs and anoxic microzones of soil.

Chemotrophic subline of Gracilicutes (group P2-L1c). There are many important functions of anaerobic chemotrophic gram-negative anaerobic respiring bacteria (group P2-L2c) in environmental biotechnology:

1. Biotechnologies, coupled with the reduction of sulphate: removal of heavy metals or sulphate.
2. Biotechnologies, coupled with the reduction of nitrate and nitrite: denitrification of wastewater; anoxic biodegradation of organic substances.
3. Biotechnologies coupled with the reduction of Fe^{3+}: removal of phosphate, anoxic biodegradation of organic substances.

Examples of the functions of selected genera are shown below.

Dissimilatory sulphate-reducing bacteria are obligate anaerobes that use organic acids, alcohols and hydrogen as donor of electrons and sulphate or other oxoanions of sulphur as acceptors of electrons, for example:

$$CH_3COOH + SO_4^{2-} \rightarrow 2CO_2 + H_2S + 2OH^- \quad (26)$$

$$4H_2 + SO_4^{2-} \rightarrow H_2S + 2H_2O + 2OH^- \quad (27)$$

Hydrogen sulphide is the toxic product of this anaerobic respiration. There is a large diversity of morphological forms (spherical, ovoid, rod-shaped, spiral, vibrioid-shaped cells, etc.), physiological varieties and related genera in phenotypic taxonomy, for example, *Desulfococcus*, *Desulfobacter*, *Desulfobacterium*, *Desulfobulbus*, *Desulfosarcina*, *Desulfovibrio*, etc. Sulphur-reducing bacteria from genera *Desulfurella* and *Desulfuromonas* are unable to reduce sulphate or other oxoanions of sulphur. The groups of sulphate-reducing and sulphur-reducing bacteria are concentrated in a delta subdivision of Proteobacteria in phylogenetic classifications. Typical habitats are anoxic sediments or bottom waters of freshwater, marine

or hypersaline aquatic environments; thermophilic species occur in hot springs and submarine hydrothermal vents. Sulphate-reducing bacteria are the agents of corrosion of steel and concrete constructions, and engineering equipment in oil and gas industry and in wastewater treatment. These bacteria can be used in environmental engineering to precipitate undissolved sulphides of heavy metals from solutions or for the removal of sulphate from wastewater with a high concentration of sulphate.

Close phylogenetic relatives of sulphate-reducing bacteria in a delta subdivision include a group of genus, *Syntrophus*, benzoate-reducing bacteria, oxidizing fatty acids with benzoate as an electron acceptor in syntrophic association with hydrogen-using microorganisms such as methanogen *Methanospirillum hungatei*.

Iron-reducing bacteria can reduce different Fe(III) compounds, using organic substances. These bacteria are important in the anaerobic biodegradation of organic matter in the aquifers because they can reduce different Fe(III) natural compounds such as iron-containing clay minerals:

$$4Fe^{3+} + CH_2O + H_2O \rightarrow 4Fe^{2+} + CO_2 + 4H^+ \quad (28)$$

This group of bacteria includes many genera such as *Geobacter.* Species of genus *Geobacter* are from a delta subdivision of Proteobacteria in the 16S rRNA-based phylogenetic classification. They are phylogenetically similar to sulphate-reducing bacteria and compete with them for electron donors in anaerobic zones. *Geobacter metallireducens* and *Geobacter sulfurreducens* are able to reduce not only Fe(III) but also Mn (VI), U(VI), Tc(VII), Co(III), Cr(IV), Au(III), Hg(II), As(V) and Se(VII), using aliphatic and some aromatic acids and alcohols as electron donors. It is the dominant group of iron-reducing bacteria detected in aquifers and subsurface environments. Therefore, they can be used for the bioremediation of these biotops. Other biotechnologies involving iron-reducing bacteria, are the removal of phosphate, sulphide, and ammonia from return liquor of municipal wastewater treatment plants (12, 13). Two new cultures of iron-reducing bacteria, *Stenotrophomonas maltophilia* and *Brachymonas denitrificans*, have been recently isolated. These cultures were able to remove phosphate and degrade xenobiotics, using iron hydroxide as an oxidant and branched fatty acids of liquid after anaerobic digestion of biomass (13).

Halobacteria from *Dehalobacter* genus are capable to oxidize such electron donors as formate, acetate, pyruvate, lactate and H_2 due to anaerobic reductive dechlorination and can be used for degradation of chlorinated ethenes in soil or wastewater. The ability to reduce Fe(III), Mn (VI), Se (VI) and As (V) and anaerobic reductive dechlorinatation is often a common property amongst bacteria in the group P2-L1.

The species *Wolinella succinogenes* can use fumarate, nitrate, nitrite, nitrous oxide (N_2O) and polysulphide as terminal electron acceptors with formate, molecular hydrogen or sulphide as the electron donors. The species was proven as a bioagent for the treatment of hazardous industrial wastewater containing ammonium perchlorate (AP) and rocket motor components on the sites of demilitarization and disposal of solid rocket motors.

Denitrifying bacteria, which are capable of oxidizing organic substances, hydrogen, Fe^{3+}, H_2S or S using nitrite or nitrate as electron acceptors, are usually not only anaerobic respiring bacteria but facultative anaerobic prokaryotes from the groups P3-L1 and P3-L2.

Chemotrophic subline of Firmicutes (group P2-L2c). The functions of anaerobic chemotrophic Gram-positive anaerobic respiring bacteria (group P2-L2c) in environmental biotechnology are similar to the functions of the bacteria from P2-L1c group, i.e., biotechnologies, coupled with reduction of sulphate, nitrate, Fe(III) and other metals. Sulphate-reducing bacteria in this group are classified in the genus *Desulfotomaculum.* They form heat-resistant endospores and use organic acids and alcohols as electron donors. The *Desulfitobacterium* genus was recognized as an important group of anaerobic dechlorination of such xenobiotics as chlorinated phenols, chlorinated ethenes and polychlorinated biphenyls (PCBs). The species of *Desulfosporosinus* genus may be important in the bioremediation of groundwater, contaminated with benzene, toluene ethylbenzene and xylene (BTEX compounds). *Bacillus infernus* is an anaerobic species that is able to reduce Fe(III) and Mn (VI), using formate or lactate.

Chemotrophic subline of Archaea (group P2-L3c). The majority of *Archaea*, the methanogens and extreme (hyper) thermophiles are representatives of this group. Methanogens are obligate anaerobes, which convert CO_2, molecular hydrogen, methyl compounds or acetate to methane by anaerobic respiration:

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \quad (29)$$

$$CH_3COOH \rightarrow CO_2 + CH_4 \quad (30)$$

Methane is produced by methanogens in the rumen (fore-stomach) of ruminant animals, paddy fields and wetlands. It is also produced and utilized as a fuel during industrial anaerobic digestion of organic wastes on municipal wastewater treatment plants and on the landfills. Representatives of microbial genera *Methanobacterium*, *Methanobrevibacter*, *Methanothermus*, *Methanococcus*, *Methanolobus*, *Methanothrix*, *Methanomicrobium*, *Methanogenium*, *Methanospirillum*, *Methanoplanus*, *Methanocorpusculum*, *Methanoculleus*, *Methanohalobium*, *Methanohalophilus*, *Methanosarcina* and *Methanosphaera* can be easily distinguished under a TEM by the shape of cell or cell arrangement or under CLSM, using specific oligonucleotide probes and FISH with cells of methanogens.

Sulphate-reducers (*Archaeoglobus*) and extremely thermophilic and hyperthermophilic S^0 – metabolizers of *Archaea* (*Desulfurolobus*, *Metallosphaera*, *Pyrobaculum*, *Thermofilum*, *Thermoproteus*, *Hyperthermus*, *Staphylothermus*, *Thermodiscus*, *Desulfurococcus*, *Pyrodictium*, *Thermococcus* and *Pyrococcus*) require temperatures from 70 to 105°C for growth. Some organisms use sulphur as an electron acceptor. Hyperthermophiles are inhabitants of hot and sulphur-rich volcanic springs on the surface or on the ocean floor. They are not used in environmental biotechnology currently, but they may be useful in thermophilic biodegradation of organic wastes, production of environmentally useful enzymes, recovery of metals at a temperature close to boiling water (14), and probably, for the removal of sulphur from coal and oil.

Phototrophic subline of Gracilicutes (groupP2-L1p). The majority of anoxygenic phototrophic bacteria are in this group. Their main functions in environmental biotechnology are as follows:

1. Anaerobic photoremoval of bad-smelling and toxic H_2S during anaerobic treatment of waste.

2. Removal of sulphate from water by a bacterial system consisting of sulphate-reducing bacteria and anoxygenic phototrophic bacteria with the formation of elemental sulphur.
3. Anaerobic removal of nutrients.
4. Removal of Fe^{2+} from water by photooxidation.

The stoichiometry of anoxygenic photosynthesis can be shown by the following equation:

$$2H_2S + CO_2 + \text{energy of light} \rightarrow < CH_2O > + 2S + H_2O \quad (31)$$

where $<CH_2O>$ is a conventional formula, showing synthesized carbohydrates.

The following are groups of anoxygenic phototrophic Gram-negative bacteria by conventional taxonomy:

1. Purple sulphur bacteria with internal or external sulphur granules, e.g., the genus *Chromatium*; by the phylogenetic classification of 16S rRNAs, phototrophic purple bacteria belong to the α-, β- and γ-Proteobacteria.
2. Purple nonsulphur bacteria, e.g., the *Rhodocyclus* genus; according to the phylogenetic classification of 16S rRNAs, phototrophic purple nonsulphur bacteria can be found in the α- and β -Proteobacteria.
3. Green sulphur bacteria, e.g., the genus *Chlorobium*; by the phylogenetic classification of 16S rRNAs phototrophic green sulphur bacteria can be found in the group 2.16 of bacteria; they are typical aquatic microorganisms and grow where light reaches the anaerobic water layer of a lake or sediment.
4. Multicellular filamentous green nonsulphur bacteria, e.g., the genus *Oscillochloris*, live in hot and cold springs, freshwater lakes, river water and sediments, in both marine and hypersaline environments. It is the group 2.7 of bacteria by the phylogenetic classification of 16S rRNAs and is phylogenetically distant from green sulphur bacteria.

Some green no-sulphur bacteria, such as the species of genus *Chloroflexus*, are able to perform aerobic respiration and may be included in the groups of microaerophilic or aerobic phototrophes. Some species of purple bacteria, green sulphur bacteria and *Chloroflexus* oxidize ferrous iron as an electron donor for photosynthesis instead of H_2S .

Phototrophic subline of Firmicutes (group P2-L2p). Representatives of this group of anoxygenic Gram-positive phototrophic bacteria, using products of anaerobic respiration (H_2S, Fe^{2+}) have not been discovered yet. Such species will likely have properties close to heliobacteria and will be similar to 16S rRNA phylogeny to the subgroups of heliobacteria, *Desulfotomaculum* and *Desulfitobacterium*.

5.3. Functions of Facultative Anaerobic and Microaerophilic Prokaryotes

It is probable that the microorganisms created in period P3, at the boundary of the periods of anaerobic and aerobic atmosphere on Earth, selected two strategies of adaptation:

1. The ability to switch methods of energy production between fermentation, anaerobic and aerobic respirations, depending on redox conditions (concentration of oxygen) in their habitat; this ability is characteristic of diverse groups of facultative anaerobic (another term is facultative aerobic) microorganisms; modern representatives of this group dominate in biotops where aerobic and anaerobic conditions frequently change.

2. The ability for aerobic respiration only at an oxygen concentration lower than 1 mg/L; this physiological group consists of microaerophilic prokaryotes; the natural habit of modern representatives of this group is the interphase between aerobic and anaerobic zones of the ecosystem or hot aquatic biotops, where the concentration of dissolved oxygen is low.

Chemotrophic subline of Gracilicutes (group P3-L1c). Facultatively, aerobic Gram-negative bacteria can produce energy by aerobic respiration, anaerobic respiration (usually by denitrification) or fermentation. Most of these bacteria are active destructors of organic substances and are used in environmental biotechnology where aerobic and anaerobic conditions are frequently changed. Many facultative anaerobic species are enterobacteria, i.e., their typical habitat is the human or animal intestine. There are many agents of water-borne diseases in the genera of *Salmonella*, *Shigella* and *Vibrio.* The cell number of the indicator species of *Escherichia coli*, group of physiologically similar coliforms, and enterococci, are common indicators of water pollution by feces or sewage.

The species of the genus *Shewanella* from the gamma-subdivision of Proteobacteria are facultative anaerobic bacteria, which are able to perform anaerobic respiration using thiosulfate, elemental sulfur, nitrate, iron (III), and manganese (VI) as electron acceptors. The growth yield of these processes is low. However, the biomass of these species can be grown aerobically with high yield on fermentation end products, i.e., lactate, formate and some amino acids. *Shewanella spp.* live naturally in association with fermentative prokaryotes that supply them the needed nutrients. Some species have been isolated from the deep sites and are tolerant of high pressures and low temperatures. The main application of *Shewanella spp*. in environmental biotechnology may be the aerobic growth of biomass with a further application for anoxic remediation of polluted sites, using Fe(III) of ferric oxides, hydroxides, or iron-containing clay minerals as electron acceptors. Reduction of iron and manganese makes these metals dissolved but reduction of dissolved U(VI) and Cr(VI) makes U(IV) and Cr(III) insoluble, respectively.

Denitrifying bacteria are capable of oxidizing organic substances, hydrogen, Fe^{3+}, H_2S or S, using nitrite or nitrate as electron acceptors:

$$5 < CH_2O > +4NO_3^- + 4H^+ \rightarrow 2N_2 + 5CO_2 + 7H_2O \tag{32}$$

The ability to reduce nitrate or nitrite is widespread among prokaryotes of the P2 and P3 periods. Active denitrifiers, which are used for the removal of nitrate from groundwater and wastewater, are *Pseudomonas denitrificans* and *Paracoccus denitrificans.* Electron donors for industrial scale denitrification can be methanol or ethanol. Hydrogen or sulphur can be used for industrial scale autotrophic denitrification of drinking water and seawater, respectively:

$$10H_2 + 4NO_3^- \rightarrow 2N_2 + 8H_2O + 4OH^- \tag{33}$$

$$5S + 6NO_3^- + 2H_2O \rightarrow 5SO_4^{2-} + 3N_2 + 4H^+ \tag{34}$$

Denitrifiers can also be used for the anoxic biodegradation of toxic organic substances in the case of fast bioremediation of anoxic clay soil.

Filamentous microaerophilic H_2S-oxidizing bacteria from genera *Beggiatoa* and *Thiothrix*, cause a problem of wastewater treatment called bulking. Bulking or bulking foaming in poorly

aerated or overloaded aerobic tanks may be because of the excessive growth of filamentous bacteria forming loose and poorly settled flocs. Growth of *Beggiatoa spp*. in sulphide rich, microaerophilic environment leads to the formation of sulphur-containing slime. Similar hydrogen and sulphide-oxidizing thermophilic filamentous microaerophilic bacteria include species from *Aquifex* and *Hydrogenobacter* genera.

Neutrophilic iron-oxidizing and manganese-oxidizing bacteria are used in environmental biotechnology for the removal of iron and manganese from water. These were also proposed to be used for the removal or even recovery of ammonia from wastewater instead of nitrification (12). Iron-oxidizing bacteria clog drains, pipes and wells with iron hydroxide deposits. Their natural habitats are springs from iron-rich soil, rocks and swamps. Species from *Siderocapsa* genus are usually suspended or attached to the soil, rock and plant surfaces in springs or lakes with an input of Fe(II) from iron-rich soil, deposits or swamps. Cells are covered by a slime iron hydroxide-containing capsule. Species from *Naumanniella* genus are usually psychrophilic and attached to the walls of the pipes and wells. Cells are slim rods with a thin iron-containing capsule. *Sidero*c*occus spp*. are cocci with capsule-like iron hydroxide deposition. *Siderocystis spp.* form chain-connected spherical ferric hydroxide particles. There are also stalk-forming bacteria and thread-forming sheathed bacteria of *Gallionella*, *Leptothrix* and *Sphaerotilus* genera capable of oxidizing iron (II) and precipitate iron (III) hydroxides in the stalk or sheath. Sheaths of neutrophilic iron-oxidizing bacteria can adsorb heavy metals and radionuclides from hazardous streams. The microaerophic filamentous species of *Sphaerotilus natans* have false branches of sheathed cells with a mycelium-like appearance and is called "sewage fungus." It is also responsible for bulking in poorly aerated or overloaded aerobic tanks. Some microaerophilic bacteria participate in the transformation of metals. Microaerophilic spirilla, for example *Magnetospirillum spp*., can produce magnetite from Fe^{2+}.

Microaerophiles can form H_2O_2 and other reactive oxygen species (ROS), such as superoxide radical and hydroxyl radical as the final products of oxygen detoxication. ROS can oxidize nonspecific xenobiotics. Microaerophilic bacteria are known to cause the biodegradation of benzene, phenol, toluene and naphthalene.

Together with the species useful for environmental biotechnology, there are many pathogenic microaerophilic organisms, for example, some strains of *Escherichia coli* that cause intestinal infections, *Salmonella spp.* and *Campylobacter spp*., that cause life-threatening infections salmonellesis and campylobacteriosis, respectively. Some strains of bacteria from the species *Vibrio cholera* are agents of water-borne infectious disease. Bacteria *Helicobacter pylori* cause stomach ulcer, and *Treponema pallidum* cause syphilis. Some facultative anaerobic bacteria, for example, *Stenotrophomonas maltophila*, are active degraders of xenobiotics but are opportunistic pathogens. These strains are used in environmental engineering but strict biosafety rules must be heeded in their applications.

Chemotrophic subline of Firmicutes (group P3-L2c). Microaerophic filamentous Gram-positive bacteria, for example, *Microthrix parvicella*, *Nocardia spp.*, *Trichococcus*, are common to wastewater-activated sludge; however, the abundance of these organisms is associated with bulking, foaming and scum formation, and finally with wastewater treatment failure.

There are facultative anaerobic Gram-positive bacteria belonging to the genera *Propionibacterium*, *Staphylococcus*, *Streptococcus* and *Enterococcus*, which are closely associated with the surfaces of human body and animals. Some are the agents of infectious diseases. These bacteria, for example, *Enteroccoccus*, are used in environmental engineering as indicators of bacteriological quality of the environment.

Facultative anaerobic species among *Actinomycetes*, a group of Gram-positive bacteria with high G + C content in DNA, are important in the degradation of organic compounds in soil. The microaerophilic representatives of genus *Frankia* are able to fix molecular nitrogen in symbiosis with nonleguminous plants.

Chemotrophic subline of Archaea (group P3-L3c). The species from genera *Sulfolobus*, *Acidianus* and *Metallosphaera* are microaerophilic or facultative anaerobic, grow in sulphur-rich, hot acid biotops and are capable of oxidizing organic substances, S, $S_4O_6^{2-}$, S^{2-} and Fe^{2+}, using oxygen or Fe^{3+} as electron acceptors. Another group of facultatively anaerobic, thermoacidophilic *Archaea*, are the species of the genera *Thermoplasma*, *Picrophilus* and *Ferroplasma.* Applications of acidophilic *Archaea* in environmental engineering may be bioextraction (bioleaching) of heavy metals from sewage sludge at high temperatures. Another potential application is the bioremoval of inorganic and organic sulphur from coal and oil to diminish the emissions of suphur oxides in the atmosphere.

Phototrophic subline of Gracilicutes (group P3-L1p). The oxygenic photosynthetic Gram-negative bacteria comprise the cyanobacteria and the prochlorophytes that are distinguished by their photosynthetic pigments. Some representatives from the *Microcoleus* and *Oscillatoria* genera are facultative anaerobic organisms capable of anoxygenic photosynthesis with hydrogen or sulphide as electron donors for the reduction of CO_2 or for oxygenic photosynthesis:

$$H_2O + CO_2 + \text{energy of light} \rightarrow < CH_2O > + O_2 \tag{35}$$

where $<CH_2O>$ is the conventional formula for synthesized carbohydrates.

The ability of cyanobacteria to grow in both aerobic and anaerobic environments is related to the life of some cyanobacteria in a dense microbial mat, where the conditions are aerobic during the day and become anaerobic at night. During anoxygenic photosynthesis under CO_2 limitation, the electrons from sulphide may be also used for fixation of molecular nitrogen or for the production of molecular hydrogen. This feature can be used in the biogeneration of hydrogen in fuel cells.

Phototrophic subline of Firmicutes (group P3-L2c). There are no currently known microaerophilic or facultatively aerobic, Gram-positive phototrophic bacteria.

5.4. Functions of Aerobic Prokaryotes

Chemotrophic subline of Gracilicutes (group P4-L1c). Strictly speaking, aerobic chemotrophic Gram-negative bacteria are the most active organisms in the biodegradation of xenobiotics, aerobic wastewater treatment, and soil bioremediation. Examples of the functions of selected genera are shown below.

Members of the genus *Pseudomonas* (the pseudomonads), for example *P. putida*, *P. fluorescence* and *P. aeruginosa*, are used in environmental biotechnology as active degraders of xenobiotics in wastewater treatment and soil bioremediation. These organisms oxidize aliphatic

hydrocarbons, monocyclic and polycyclic aromatic hydrocarbons, halogenated aliphatic and aromatic compounds, different pesticides and oxidize or cometabolize halogenated ethanes and methanes. Biodegradation often depends on the presence of specific plasmids. Therefore, both native and genetically engineered strains with amplified and diverse degradation ability are used in environmental engineering. Some selected strains of *Pseudomonas* genus are used instead of chemical biocides to control plant diseases. Other active biodegraders are the species from genera of *Alcaligenes*, *Acinetobacter*, *Burkholderia*, *Comamonas* and *Flavobacterium.* The majority of active biodegraders are opportunistic bacteria, i.e., they can cause diseases in immunosupressive, young or old people. Therefore, all experiments and treatments of water and soil by these bacteria must be performed with precautions against the dispersion of these bacteria in the environment and reasonable biosafety rules must be used in their applications.

There are many pathogens among the above-mentioned genera, for example, *Pseudomonas aeruginosa*, *Burkholderia cepacia*, and *Burkholderia pseudomallei.* Therefore, the test of acute toxicity and other pathogenicity tests of all microbial strains, isolated as the active biodegraders of xenobiotics for environmental engineering applications, must be made after selection and before pilot-scale research. Representatives of genus *Xantomonas* are also active biodegraders, but there are many phytopathogenic species. Therefore, they cannot be used for soil bioremediation, but used as the test cultures to test new biocides for agriculture.

The formation of activated sludge flocs is enhanced by the production of extracellular slime. *Zoogloea ramigera* is considered an important organism in flocs formation because of its strong self-aggregation. Probably the most important role in the formation of activated sludge floc belongs to gliding bacteria from the genera *Flavobacterium*, *Cytophaga*, *Myxobacterium*, *Flexibacterium* and *Comamonas*. They are called gliding bacteria because of cell translocation on a solid surface due to the interaction of cell surface and solid surface. They produce extracellular polysaccharides and have the ability for strong aggregation of their cells.

Species of genus *Acinetobacter* are capable of accumulating polyphosphate granules and are used for biological removal of phosphate from wastewater. This removal diminishes the supply of phosphate ions and dissolved phosphates of heavy metals to the environment. Another important environmental biotechnology feature of these bacteria is the ability to produce extracellular polyanionic heteropolysaccharides that can emulsify hydrocarbons, and thus, enhance their degradation in an aqueous environment. Together with these useful properties, *Acinetobacter spp.* are opportunistic pathogens that cause different infections in immunocompromised patients. These infections are difficult to treat successfully because clinical strains of *Acinetobacter ssp*. acquire resistance to the antibiotics.

Azotobacter and *Azomonas* are the genera of free-living nitrogen fixing soil bacteria that accumulate organic nitrogen and improve soil fertility. Selected strains of genus *Azotobacter* are used for the industrial biosynthesis of PHB and its derivates for biodegradable plastics. Biodegradability of plastic materials is very important for environmental sustainability.

Methanotrophs do not grow on organic compounds and oxidize such single-carbon compounds as methane, methanol, formaldehyde and formate. Methane-oxidizing species, for example, from the *Methylococcus* and *Methylomonas* genera, are important for the removal of methane from the atmosphere, thus, diminish the greenhouse effect due to accumulation of

carbon dioxide and methane:

$$CH_4 + 0.5O_2 \rightarrow CH_3OH \tag{36}$$

A well known application in environmental biotechnology is the bioremoval of halogenated methanes and ethanes from the polluted groundwater by cometabolism. For example, cometabolism of trichloroethylene (TCE) by methylotrophic bacteria is considered an effective approach for the remediation of a polluted aquifer. The main reaction of cometabolism, catalyzed by enzyme methanemonooxygenase of methanotrophs, is described by the following equation:

$$Cl_2C=CHCl + NADH_2 + O_2 \rightarrow Cl_2C-CHCl + H_2O + NAD \tag{37}$$

There are many oligotrophes in the group P4-L1. These organisms are adapted to living in a poor environment with a low concentration of nutrient sources, including carbon and energy. Their adaptation is so stable that many oligotrophes are obligate ones and cannot grow in a medium with a high concentration of carbon and energy sources. Oligotrophic microorganisms are important for the treatment of ground water, sea water and fresh water with a low concentration of carbon source. For example, *Hyphomicrobium spp*. are budding oligotrophic bacteria capable to oxidize single-carbon compounds by oxygen or nitrate. They are used in environmental biotechnology for the removal of nitrate from water, using methanol as an electron donor. Stalked oligotrophic from genus *Caulobacter spp*. are able to survive during long-term starvation. It is thought that they may perform gene transfer between different bacteria participating in water and wastewater treatment because they are often adhered to the cells of other bacteria.

Bdellovibrio spp. are Gram-negative bacteria, which are parasites of other Gram-negative bacteria. Therefore, their presence in water is an indication of water pollution by Gram-negative bacteria. *Bdellovibrio spp*. can be applied in environmental engineering for the biological control of human, animal and plant pathogens in water and soil, and for control of excessive growth of microbial biofilm in fixed biofilm reactors, which are used for water and wastewater treatment.

Rhizobia are bacteria from the genera *Rhizobium*, *Bradyrhizobium*, *Azorhizobium*, which are able to grow and fix atmospheric molecular nitrogen symbiotically with leguminous plants, for example, peas, beans and clover. It is considered that this fixation supplies about a half the nitrogen used in agriculture. The application of specifically selected and industrially cultivated rhizobia for the inoculation of soil, where leguminous plants are planted for the first time, can double the yield of these plants. Therefore, these bacteria are used in environmental engineering for the enhancement of soil fertility after bioremediation of polluted soil.

There are many pathogenic species in the group P4-L1. *Yersinia pestis* is responsible for the devastating outbreaks of plague in Asia and Europe in the sixth, fourteenth, seventeenth, eighteenth and beginning of the twentieth centuries. Another example is *Burkholderia pseudomallei*, agent of melioidosis. This disease is common in Southeast Asia. It affects people exposed to soil and soil aerosols: farmers on rice paddies, construction workers or people

living close to the soil excavation area. The disease may be misidentified as syphilis, typhoid fever or tuberculosis. Pulmonary melioidosis can range from bronchitis to a severe pneumonia. During the period from 1989 to 1996, a total of 372 melioidosis cases, with 147 deaths, were reported in Singapore (15). *Legionella spp.* is an agent of Legionnaires disease, which is a lung infection caused by inhalation of water droplets from poorly maintained cooling towers, air conditioners, fountains and artificial waterfalls.

Pathogens can be removed or killed in environmental engineering systems by the following methods:

1. Bulk or membrane filtration, and UV treatment for air and aerosol disinfection.
2. Coagulation, aggregation, sedimentation, slow filtration of water and wastewater to diminish the concentration of pathogens due to adsorption, sedimentation of microbial aggregates or predation of protozoa.
3. Chemical treatment by chlorine, chlorine dioxide, ozone or UV light for water and wastewater effluent disinfection.
4. Chemical treatment by oxidants, organic solvents, surfactants, salts of heavy metals or UV light for the disinfection of solid surfaces and microbial biofilms.
5. Aeration, biotreatment, thermal treatment, acidification, hydrogen peroxide addition, electromagnetic radiation to disinfect or to diminish the content of pathogens in solid waste or soil.

Chemolithotrophs in chemotrophic subline of Gracilicutes (subgroup of the group P4-L1c). Chemolithotrophs are aerobic prokaryotes, which can use a reduced inorganic compound as an energy source. The species grow as autotrophs but some can also grow as organotrophs.

Nitrifying bacteria comprise two groups of aerobic bacteria: ammonia-oxidizers (*Nitrosomonas spp.*, *Nitrosococcus spp.*, *Nitrosovibrio spp.*, *Nitrosospira spp.*, *Nitrosolobus spp.*) performing the reaction:

$$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + 2H^+ + H_2O \tag{38}$$

and nitrite-oxidizers (*Nitrobacter spp.*, *Nitrococcus spp.* and *Nitrospira spp.* from different subdivisions of 16S rRNA classification) that use the nitrite to form nitrate:

$$NO_2^- + 0.5O_2 \rightarrow NO_3^- \tag{39}$$

Nitrifying bacteria are widely used in environmental biotechnology to transform toxic ammonium to a less toxic nitrate. The nitrate produced can be transformed further to molecular nitrogen by denitrifying bacteria. The problems of large-scale nitrification in wastewater treatment include:

1. Washing out of these microorganisms from a bioreactor of continuous cultivation due to their slow growth rate in comparison with the growth rate of heterotrophs.
2. High sensitivity of nitrifiers to toxic substances, surfactants or organic solvents due to the large folded surface of a cell membrane.

The activity of nitrifying bacteria was the basis of ancient Chinese environmental biotechnology to convert organic wastes and household ashes into fertilizer and gunpowder as a value-added by-product. The transformation of waste to gunpowder can be described by the following sequence of the reactions:

1. Formation of ammonia from aminoacids of proteins by bacteria-ammonifiers in anaerobic microzones of waste

$$RNH_2 \rightarrow NH_4^+ \tag{40}$$

2. Production of nitrate from ammonium by bacteria-nitrifiers in aerobic microzones of waste

$$NH_4^+ + 2O_2 \rightarrow HNO_3 + H_2O + H^+ \tag{41}$$

3. Neutralization of acid solution of nitrate by potassium oxide from ash

$$K_2O + 2HNO_3 \rightarrow 2KNO_3 + H_2O \tag{42}$$

4. Formation of hydrogen sulphide by sulphate-reducing bacteria in anaerobic microzones of waste

$$SO_4^{2-} + CH_2O + 6H^+ \rightarrow H_2S + CO_2 + 3H_2O \tag{43}$$

5. Oxidation of hydrogen sulphide by microaerophilic bacteria

$$H_2S + 0.5O_2 \rightarrow S + H_2O \tag{44}$$

These reactions result in a suspension, containing carbon particles from ash, particles elemental sulphur and potassium nitrate. Drainage of this suspension from the waste pile and drying it under the sun in a drainage collector, produce a mixture of carbon, sulphur and potassium nitrate, which is gunpowder.

Aerobic sulphur-oxidizing chemolithotrophic bacteria oxidize reduced sulphur compounds, producing sulphuric acid:

$$S + 1.5O_2 + H_2O \rightarrow H_2SO_4 \tag{45}$$

$$H_2S + 4O_2 \rightarrow H_2SO_4 \tag{46}$$

These bacteria are used or can be used in environmental biotechnology for the following purposes:

1. Bioremoval of toxic H_2S from gas, water and wastewater.
2. Industrial bioleaching of metals, for example, copper, zinc, or uranium from the ores.
3. Bioleaching of heavy metals from anaerobic sewage sludge before its utilization as an organic fertilizer.
4. Bioleaching of heavy metals and radionuclides from polluted soil.
5. Acidification of alkaline soil.

Sulphur oxidation can cause corrosion of steel and concrete constructions due to the production of sulphuric acid. Some species, for example, *Thiobacillus ferrooooxidans*, (*Acidithiobacillus ferrooxidans*), can grow at an extremely low pH and be isolated from acid mine drainage.

Oxidization of Fe(II) is performed by two groups of aerobic bacteria: acidophilic and neutrophilic iron-oxidizers. Fe(II) is stable in acid solutions, if the pH is lower than 3. Its chemical oxidation by oxygen of air under a low pH is slow. Some bacteria, however, for example, *Thiobacillus ferrooooxidans*, (*Acidithiobacillus ferrooxidans*), can oxidize Fe(II) some thousand times faster than that in a chemical process. Neutrophilic iron- and manganese-oxidizers are usually microaerophilic, and were described above as the representatives of

group P3-L1. The main point for neutrophilic oxidation of iron is that Fe(II) is not stable under aerobic conditions and neutral pH and must be immediately oxidized by oxygen. However, atoms of Fe(II), surrounded by chelated organic acids, are protected from chemical oxidation by oxygen. Therefore, the functions of neutrophilic microaerophilic "iron-oxidizers" are production of H_2O_2 and chemical degradation of organic "envelope" of Fe^{2+} atom by H_2O_2. Precipitation of iron hydroxide by these bacteria can clog pipelines and wells. They are used in environmental engineering for the removal of iron and manganese from drinking water, treated in slow sand filter. Another important application is the removal of ammonia from wastewater by coprecipitation with fine particles of positively charged iron hydroxide produced by neutrophilic "iron-oxidizers" (12).

Chemotrophic subline of Firmicutes (group P4-L2c). Aerobic heterotrophic Gram-positive bacteria have diverse functions in environmental biotechnology. Representatives of genus *Bacillus* dominate in the aerobic treatment of wastewater or solid waste, which is rich in such polymers as starch or protein. The species of genus *Bacillus* produce antibiotics and can degrade different xenobiotics. They produce endospores providing cell survival in variable soil environment and after drying. Therefore, the endospores can be used in commercial compositions to start up soil bioremediation or biodegradation of certain substances during wastewater treatment. The shape and cellular location of endospores are used as identifying characteristics in the differentiation of the species. Some species, for example *Bacillus subtilis*, live in human intestine and are used as a medical application of viable cells as probiotics to normalize microflora of intestine. However, there are also pathogenic species in this genus. For example, *Bacillus anthracis* cause deadly infection anthrax due to the production of strong toxins and are even considered as biological weapons.

Bacteria of the genus *Arthrobacter* are commonly found in soil and are active in the biodegradation of xenobiotics. Their specificity is a rod-coccus growth cycle; the cells are rod-shaped during active growth and cocci-shaped in the stationary phase of batch culture. Saprophytic species of mycobacteria are skilled degraders of xenobiotics but there are also many pathogenic species, for example, *Mycobacterium tuberculosis* (agent of tuberculosis) and *Mycobacterium leprae* (agent of lepra). *Nocardia spp*. and *Rhodococcus spp*. degrade hydrocarbons and waxes; they form mycelium, which breaks into rods and cocci. Excessive growth of *Nocardia asteroides*, *Rhodococcus spp*., and *Gordona amarae* in aerobic tank causes foaming (brown scum) of activated sludge due to high hydrophobicity of cell surface of these species, their production of biosurfactants, or hydrolysis of lipids. This foaming produces nuisance odours and can increase the risk of infection of wastewater workers with opportunistic pathogenic actinomycetes *Nocardia asteroides.*

Actinomycetes are aerobic, heterotrophic Gram-positive prokaryotes with a high G + C content in DNA, growing with aerial mycelia. They are used in the aerobic treatment of wastewater, soil bioremediation, and composting of solid wastes because there are many active degraders of natural biopolymers and xenobiotics. Genus *Streptomyces*, containing half a thousand species, is extremely important for medical biotechnology because many antibiotics are produced by the strains from this genus. A significant part of soil microbial biomass is the biomass of streptomycetes. Therefore, the character odour of moist earth is due to the volatile

substances such as geosmin produced by these microorganisms. Thermophilic actinomycetes play an important role in the composting of organic wastes.

Chemotrophic subline of Archaea (group P4-L3c). Some halophilic Archaea are aerobic microorganisms. Hypothetically, they can be used for the biotreatment of polluted industrial brines but there are no applications of these prokaryotes in environmental biotechnology at present.

Phototrophic subline of Gracilicutes (group P4-L1p). Cyanobacteria carry out oxygenic photosynthesis with water as the electron donor:

$$CO_2 + H_2O + \text{energy of light} \rightarrow < CH_2O > + O_2$$

where $<CH_2O>$ is the conventional formula of photosynthesized carbohydrates. Cyanobacteria are used in environmental biotechnology for the light-dependant removal of nitrogen and phosphorus from wastewater. However, a main problem of environmental engineering, related to cyanobacteria, is control of their blooming in surface water polluted with ammonia or phosphate. This bloom causes nuisance odours, bad taste of water, and accumulation of allergens and toxins in water. The cyanobacteria are morphologically diverse including unicellular organisms reproduced by binary fission or budding (*Chroococcales*), by multiple fission (*Pleurocapsales*), and filamentous forms without cell differentiation (*Oscillatoriales*) or with cell differentiation (*Nostocales* and *Stigonematales*). In the 16S rRNA classification of cyanobacteria, the cyanobacteria are clustered in 14 sections (16).

Prochlotophytes is a group of aerobic oxygenic phototrophic Gram-negative bacteria that differ from cyanobateria by their set of photosynthetic pigments. Group *Prochloron* is close to cyanobacteria by 16S rRNA phylogeny. This group has no importance for environmental engineering because they have been found only as extracellular symbionts of ascidians, marine animals, in the tropical areas of Pacific and Indian oceans.

Phototrophic subline of Firmicutes (group P4-L2p). There are no currently known obligate aerobic Gram-positive phototrophic bacteria.

5.5. Functions of Eukaryotic Microorganisms

Fungi are used in environmental biotechnology in the composting, soil bioremediation and biodegradation of xenobiotics in the soil. Activated sludge of municipal wastewater treatment has a low content of fungi from the genera *Geotrichum*, *Penicillium*, *Cephalosporium*, *Cladosporium* and *Alternaria*, but the matrix of such microbial aggregates as granules and biofilms can be arranged due to the mycelium of fungi from genera *Fusarium*, *Penicillium*, *Aspergillus*, *Mucor* and *Geotrichum*. The mycelial structure of biofilm facilitates transfer of oxygen and nutrients to the deeper layers of the biofilm. Fungi dominate in the microbial ecosystems with a low pH. Therefore, there may be sludge bulking at a low pH due to the excessive growth of fungi. The hydrolyzing activity of fungi is essential for composting of such organic wastes as municipal refuse, paper, sewage sludge, agricultural and farming wastes, and food-processing waste. The objective of composting is to convert an unstable and unsafe organic waste into a dark-brown, granular, humus-like end-product, with a high content of nutrients, which can be applied as a soil conditioner. Heat produced during composition destroys human pathogens and parasites. Without proper control of composting, the

production of odours of microbial origin (volatile fatty acids, hydrogen sulphide), air-spread spores of fungi, for example, opportunistic pathogen *Aspergillus fumigatus* or *Aspergillus flavus*, producer of cancerogenic aflatoxin, may create safety problems. There are many other species of fungi growing on the surfaces and producing mycotoxins released to aquatic systems and air-spread spores that cause respiratory infection and allergic reactions.

Algae grow on biofilm surfaces exposed to light and used in water or wastewater treatment. They consume nitrogen and phosphate and produce oxygen used by aerobic bacteria and fungi. However, environmental engineers are interested mostly in the control of algae in aquatic systems because some species produce toxins and promote the growth of pathogens in water.

Protozoa are unicellular animals that obtain nutrients by ingesting food particles or microorganisms. Some protozoa can form cysts under adverse environmental conditions. These cysts are resistant to desiccation, starvation, high temperature and disinfection. Changes in the protozoan community reflect the operating conditions of aeration tank and are used for microscopic monitoring of the process in aerobic tanks. Amoeba dominate under a high concentration of organic matter, and ciliates, while flagellates, dominate under lower concentrations. The presence of stalked ciliates attached to the surface of the flocs is a sign of low concentration of organic substances in treated wastewater. Ciliates feed on suspended bacterial cells, thus improving effluent quality. Some functions have rotifers, which are multicellular organisms with size from 50 to 250 μm, attached to the flocs of activated sludge. Predation by protozoa and rotifers can diminish the concentration of pathogens in water during wastewater treatment. From another view, some protozoa are natural hosts of bacterial pathogens, for example, *Legionella spp*. The pathogenic strains of these bacteria grow and survive in cells of protozoa living in warm water. Therefore, the growth of these protozoa must be controlled using biocides or high temperature to prevent outbreaks of Legionnaires disease.

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3
Microbial Systematics

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CONTENTS

Abstract This chapter aims at presenting an overview of different aspects of the classification and nomenclature of the prokaryotes, i.e., the domains *Bacteria* and *Archaea*. Concepts of systematics, taxonomy, classification, nomenclature, and identification are discussed. The number of species of prokaryotes – *Bacteria* and *Archaea* combined – is surprisingly small, 8,226 as of November 2009. It is obvious that the true number of different species much larger: we probably know less than 1% of the types of prokaryotes in Nature. Classification of prokaryotes and description of species based on a polyphasic approach that includes phenotypic as well as genotypic properties. Nomenclature is governed by the rules of the Bacteriological Code (International Code of Nomenclature of Prokaryotes), as determined by the International Committee on Systematics of Prokaryotes.

Key Words Prokaryotes • *Bacteria* • *Archaea* • systematics • taxonomy • nomenclature • bacteriological code • culture collections • small-subunit rRNA.

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1. INTRODUCTION

Environmental biotechnologists and environmental engineers often deal with microorganisms and especially with prokaryotes. These are the organisms responsible for majority of the biodegradative processes of organic carbon in nature, as well as for important reactions in the cycles of the elements including nitrification, denitrification, dissimilatory sulfate reduction, sulfide oxidation, and many others.

It is therefore inevitable that the environmental engineer will encounter many names of microorganisms – prokaryotic as well as eukaryotic – that are involved in the processes studied. The engineer will also be challenged from time to time to identify certain microorganisms when describing the phenomena occurring in the ecosystem under study, as well as to provide information on the organism(s) that causes environmental problems and/or those that provide possible solutions to those problems.

This chapter aims at presenting an overview of different aspects of the classification and nomenclature of the prokaryotes, i.e., the domains *Bacteria* and *Archaea*. These groups are by far the most important as far as their metabolic diversity is concerned. Their classification and nomenclature are governed by the same rules. The classification of the eukaryotic microorganisms in part follows the rules of botanical taxonomy (fungi, yeasts, algae) and in part the zoological taxonomy (protozoa and larger animals). The formal rules used in the nomenclature of these groups are greatly different from those used for the prokaryotes.

The highly diverse prokaryotic world has presented serious challenges to the many microbiologists who have attempted to achieve a satisfactory classification in the past. Many of the problems are yet to be solved, and as a result, the classification schemes have been subject to frequent changes with the increase of our understanding of the physiology and the molecular properties of the different groups of microorganisms. Prokaryote systematics is a highly dynamic science in which the concepts rapidly change in accordance with the development of new techniques and approaches. There is no official classification of prokaryotes, as will be shown in the sections below (this in contrast with prokaryote nomenclature, which is governed by a series of internationally approved rules and regulations). The basic unit of the classification of all living organisms is the species, and it may be surprising to many to read that in the case of the prokaryotes there is no universally recognized definition of the species. Prokaryotic systematics thus lacks a firm theoretical basis. Microbiologists work with a species concept that is much broader than that used in the disciplines of botany and zoology. The number of species of prokaryotes (*Bacteria* and *Archaea* combined, i.e., two out of the three domains of life – *Bacteria*, *Archaea*, and *Eucarya*) that have been described and named may even seem surprisingly small, 8,226 as of November 3, 2009 (1). This number is extremely small compared to the more than a million of insects described, and about 22,000 members of the *Orchidaceae*, a single family of vascular plants. In the following we will explore several aspects of classification, nomenclature, and identification of prokaryotes. We will present evidence for the existence of many more species of bacteria on Earth than the about 8,000 species documented thus far; experimental data suggest the existence of at least two orders of magnitude more species, and possibly even many more. Today, it is generally accepted that less than 1% of the prokaryote species that inhabit our planet have been named.

It is not feasible in a single chapter to provide in-depth information and complete practical guidelines on how to identify, name, and classify prokaryotes. However, environmental engineers and other professionals working in related fields are sometimes faced with the need to identify strains of microorganisms. This chapter intends to summarize some of the current concepts in the field of prokaryote systematics and will refer the reader to more detailed sources of information on the subject.

2. SYSTEMATICS, TAXONOMY, AND NOMENCLATURE OF PROKARYOTES

2.1. General Definitions

Systematics is the scientific study of organisms with the ultimate object of characterizing and arranging them in an orderly manner. The terms "systematics" and "taxonomy" are sometimes used as synonyms. The term "taxonomy" is often defined as the theory and practice of classifying organisms into groups (taxa) on the basis of similarities and relationships. Systematics generally signifies a broader concept that includes the evolutionary and phylogenetic relationships of the organisms studied. Taxonomy can be subdivided into three disciplines: classification, nomenclature, and identification (2). Classification is the orderly arrangement of units into groups. There are many ways in which living organisms can be arranged in groups, and taxonomy is therefore a most subjective branch of science. Classification is purpose-oriented, and there may be different ways that lead toward successful classification (3).

Nomenclature is the assignment of names to the taxonomic groups defined during classification. The rules of nomenclature of living organisms are agreed upon internationally, and they are laid down in three documents: the Botanical Code, the Zoological Code, and the Bacteriological Code, each dealing with a specific group. Here, we will discuss only the International Code of Nomenclature of Bacteria (the Bacteriological Code) (4), renamed as International Code of Nomenclature of Prokaryotes in 2000, and its implications for the nomenclature of prokaryotes. Similar to plants and animals, prokaryotes are given a genus and a species name according to the binomial system introduced by Linnaeus in the middle of the eighteenth century. For example, the genus *Bacillus*, a genus of Gram-positive aerobic endospore-forming bacteria, presently (November 2009) contains about 160 species; examples include *Bacillus subtilis* (a species from which valuable proteolytic enzymes are produced), *Bacillus anthracis* (the anthrax bacillus), and *Bacillus thuringiensis* (a species of which certain strains produce potent substances that kill mosquito larvae) (1).

The nomenclature of prokaryotes is subject to changes, and there are many examples of species that have been renamed, moved to other existing genera, or reclassified in newly established genera in accordance to new insights. For example, the species formerly known as *Bacillus polymyxa* and *Bacillus stearothermophilus* have been renamed *Paenibacillus polymyxa* and *Geobacillus stearothermophilus*, respectively (1), when it became desirable to split up the genus *Bacillus* based on an increased understanding of the phylogenetic relationships within the aerobic Gram-positive endospore-forming bacteria, mainly on the basis of sequence analysis of small-subunit ribosomal RNA (see also Sect. 6.1).

Identification is the practical use of classification schemes and the labeling schemes provided by nomenclature to establish the identity of isolated microorganisms as members of

previously identified species. Identification can be defined as the practical use of classification and nomenclature to determine the identity of an isolate as a member of an established taxon or as a member of a previously unidentified species.

It should be stressed here once more that there is no "official" classification of prokaryotes, but there is an official nomenclature, regulated by internationally agreed-upon rules. The classification provided in *Bergey's Manual of Systematic Bacteriology* (5) (see also Sect. 7.1) is widely used among microbiologists, but was never intended to obtain official status.

The classification used in Bergey's Manual divides the prokaryotes into two domains: the *Bacteria* and the *Archaea*. The domain is therewith the highest taxonomic rank. According to the same classification scheme, all eukaryotic organisms – microorganisms and macroorganisms, plants, and animals – belong to the domain *Eucarya*. The domains, *Bacteria* and *Archaea,* are divided into phyla, which each encompass one or more classes (6, 7). The classes are divided into orders, which are subdivided into families, genera, and species. As stated previously, the species is the basic unit of taxonomy. Sometimes, subdivision of a species into subspecies and infra-subspecific units is necessary.

An important term in bacterial taxonomy and nomenclature is that of *type strain*. For each species, a type strain has been designated, which is the name-bearer of that species and is the reference specimen for the name. Whenever a new prokaryote species is described, the authors are obliged to deposit the type strain of the species in at least two publicly accessible culture collections located in different countries for safekeeping (see Sect. 5), and make subcultures available to any interested scientist for further study. Identification of unknown isolates should use such type strains of recognized species for comparison. The terms "strain" and "isolate" refer to the descendants of a single isolation in pure culture. They are usually made up of a succession of cultures ultimately derived from an initial single colony. For each genus, a type species is defined, and for each family and each order, a type genus is designated.

2.2. *The Definition of the Prokaryote Species*

To the botanist and the zoologist, the definition of the concept "species" presents relatively few problems. In the plant and the animal world, a species is generally defined as a population of individuals that can interbreed under natural conditions, produce fertile offspring, and that is reproductively isolated from other populations. Such a definition is useless in the case of the prokaryotes, as these show no sexual reproduction.

There is no general consensus about the definition of the concept of the species, i.e., the basic taxonomic unit in the prokaryote world (8–11). Definitions found in the literature may for example circumscribe the species as "a distinct group of strains that have certain distinguishing features and that generally bear a close resemblance to one another in the more essential features of organization," or "an assemblage of clonal populations that share a high degree of phenotypic similarity, coupled with an appreciable dissimilarity from other assemblages of the same general kind." Such definitions provide little practical information on how close that resemblance and similarity should be for two strains to be classified in the same species, what features of organization should be considered essential, and what degree of dissimilarity is required for two strains to warrant classification in different species.

The delineation of species according to such definitions is therefore highly subjective. Some definitions found in the literature stress this subjectivity to an even larger extent, such as that of a species as "a group of organisms defined more or less subjectively by the criteria chosen by the taxonomist to show to best advantage as far as possible and putting into practice his individual concept of what a species is" (12).

It is obvious that a simpler, more pragmatic species definition is required to enable the design of classification schemes and the establishment of a nomenclature that can be widely used. Based on the experience of the last 20 years, such a pragmatic definition of the prokaryote species has indeed emerged. This species concept is based on the recommendations published in 1987 by a committee of experts (9). These recommendations were recently confirmed and extended by a new ad-hoc committee (10). The species concept is based on a polyphasic approach (see also Sect. 3.3), which includes description of diagnostic phenotypic features combined with genomic properties. Consistency of phenotypic and genomic characters is required to generate a useful classification system for the prokaryotes (13). It is recommended that a distinct genospecies (i.e., a species discernible only by nucleic acid comparisons) that cannot be differentiated from another genospecies on the basis of any known phenotypic property not be named until some phenotypic differentiating property is found (9). Individually, many of the phenotypic and chemotaxonomic characters used as diagnostic properties are insufficient to delineate species, but together they provide sufficient descriptive information to allow the definition of a species. Description of a species should ideally be based on a comparative study of a large number of isolates to define also the degree of variation of certain properties within the defined boundaries of the species. In practice, however, more than 80% of the new species descriptions that have been published in recent years were based on the study of single isolates. Unfortunately, such species descriptions that are based on one or a few strains only can lead to improper phenotypic circumscription of taxa, making the identification of new isolates as members of a taxon problematic. From time to time, formal proposals have been made to the International Committee for Systematics of Prokaryotes/International Committee for Systematic Bacteriology (see Sect. 4.3) to define a minimum number of isolates necessary as the basis of a description of a new species. However, such proposals have never been formally approved. If they would have, many extremely interesting *Bacteria* and *Archaea* that have been proposed and approved as new species in recent years could not have been described simply because it had not been feasible to obtain more isolates. Our understanding of the prokaryote world would have been much poorer as a result.

The genomic properties to be determined for the delineation of species are based on comparisons of the complete genomes using techniques of DNA similarity determination by DNA–DNA hybridization and/or assessment of the difference in the melting temperature between the homologous and the heterologous DNA hybrids (7, 11). These tests have to be performed under carefully standardized conditions to give reproducible results. The widely accepted criterion defines a prokaryotic species as a group of strains, including the type strain, that share at least 70% total genome DNA–DNA hybridization and have less than $5^{\circ}\mathrm{C}\ \Delta T_{\mathrm{m}}$ (= the difference in the melting temperature between the homologous and the heterologous hybrids formed under standard conditions). The delineation value of 70%, as introduced around 1987 (9), is artificial, but has proven satisfactory in most cases. DNA relatedness

values between 30 and 70% point to a moderate degree of relationship – often parallel to the extent of the genus. There are cases in the literature of species and even genera that share more than 70% DNA–DNA similarity. A well-known case is that of the genera *Escherichia* and *Shigella*, enteric bacteria that share more than 85% similarity. However, for pragmatic reasons, the separation into two species is maintained. At the level of genera or higher taxa, the resolving power of DNA–DNA similarity assays is limited.

It should be stated that DNA–DNA hybridization assays are seldom routinely performed in most laboratories. The protocol includes labeling of the DNA (generally with radioisotopes), shearing, and denaturing the sheared DNA, whereafter the labeled denatured DNA is mixed with excess of unlabeled DNA from the second organism. The mixture is then cooled and allowed to reanneal under carefully controlled conditions. Duplex DNA is then separated from any unhybridized DNA remaining and the amount of bound DNA quantified. A control experiment with homologous DNA is included and its results of the heterologous DNA binding are normalized with respect to the homologous control. Different protocols exist, and nonradioactive methods have also been introduced. The procedure is time-consuming, and allows for pair-wise comparisons only, making comparisons of large numbers of strains cumbersome. A recent reappraisal of the currently used methods in bacterial taxonomy to delineate species concluded that, despite certain drawbacks with respect to reproducibility and workability, DNA–DNA hybridization is still the best criterion for species delineation. Its great advantage is its universal applicability. A disadvantage is that the method gives no indication of which genes contribute to or detract from the similarity. In spite of the usefulness of DNA–DNA similarity determinations, phenotypic properties including chemotaxonomic markers will remain essential to describe new species (10), and such phenotypic properties will always be essential as diagnostic markers to be used when new isolates are to be identified.

Given that there is no clear definition of the prokaryotic species, the guidelines for the delineation of genera or higher taxonomic levels within the prokaryotes are even less clear. The genus may be defined as "a collection of species with many characters in common," but the extent of the shared characters that should exist for species to be classified in a single genus is largely a matter of personal judgment. There is, however, a general consensus that the division into higher taxonomic levels should reflect phylogenetic relationships. As explained in Sect. 6.1, sequence analysis of small-subunit ribosomal RNA (16S rRNA in prokaryotes, 18S rRNA for eukaryotes) has provided a large extent of insight in the phylogenetic relationships among microorganisms. The construction of small subunit rRNA-based phylogenetic trees provides the taxonomist with a powerful technique to determine the phylogenetic position of an isolate to the level of family and genus (14–16). Generally, there is a good correlation between the DNA–DNA similarity and the similarity of the 16S rRNA gene sequence (17). A DNA–DNA similarity of less than 70% generally corresponds with less than 97% 16S rRNA sequence identity. Species classified in a single genus generally share at least 93–95% identity in their 16S rRNA gene sequence. However, at the species level, the 16S rRNA-based methods lack the necessary resolving power, and then DNA–DNA reassociation experiments are still required (18) (see also Sect. 3.3). There are known cases in which two distinctly different species with DNA–DNA similarity of less than 50% have identical 16S rRNA gene sequences.

Compared to the species concept in the plant and the animal world, the bacterial species concept is exceptionally broad. When we would apply the "70% DNA–DNA similarity" criterion to delineate species of higher eukaryotes, the number of recognized species would decrease dramatically. For example, humans would not only belong to the same species as chimpanzees (98.4% DNA–DNA relatedness), gorillas (97.7%), and orangutans (96.5%), but even lemurs (78%) would be classified in the same species! The prokaryotic species, thus, encompasses species that may be highly different on the genetic level (8).

The availability of the methodology to determine complete genome sequences of prokaryotes (generally ranging in length between 2×10^6 and 6×10^6 base pairs) will probably change the rules and concepts used in delineating species in the future. Until now, the sequencing and annotation of bacterial genomes is a time-consuming and costly process, however, the number of complete bacterial and archaeal genome sequences that are available in public data bases is rapidly increasing (952 and 71, respectively, as of November 2009). With the rapid developments in automated genome sequencing technology, the day may come when the publication of the complete genome sequence of the type strain may become obligatory for the description of a new species. In the meantime, the comparison of the existing genome sequences has taught us much important information not only about the structure of the prokaryote genome, but also about the extent of possible lateral gene transfer between prokaryotes, not only at the level of species and genera, but even at the level of phyla and domains (*Archaea–Bacteria*, *Bacteria–Eucarya*, *Archaea–Eucarya*). The question has rightfully been asked whether is still would it be possible to delineate species at all if indeed genes move freely from species to species, even between completely unrelated ones. The conclusion of almost a decade of studies since the first complete bacterial genome sequence was published is, that indeed prokaryotes can "capture" new genes from other organisms, sometimes extremely distantly related. However, each species still appears to have its genetic individuality, and it is surely not so that life is a common gene pool, shared more or less randomly between all organisms that inhabit our planet. There are barriers that prevent a too extensive exchange of genes, and as a result it is still possible to formulate a species concept in the prokaryote world. As stressed above, the DNA–DNA hybridization method is based on the similarities of complete genomes, not on the presence or absence of single genes, so that the result of the test will not be greatly affected by the lateral transfer of a few genes obtained from other organisms.

2.3. The Number of Prokaryotes that Have Been Described

Bacterial nomenclature saw a new beginning in 1980. In view of the confusion that had arisen by that time, with many species of prokaryotes being known under different synonyms, it was decided that the thousands of bacterial names that had been published in the past in the greatly dispersed scientific literature would lose their validity, with the exception of approved lists of about 2,500 species names that were published in that year (19). Since that date, only those names of new prokaryote taxa published in the lists in the *International Journal of Systematic Bacteriology* (from 2000 onwards renamed as the *International Journal of Systematic and Evolutionary Microbiology*, see also Sect. 4.4) obtain standing in the nomenclature of prokaryotes. Such new names can either be published in the form of original articles published

Table 3.1
The Number of Prokaryotes described (*Bacteria* and *Archaea* Combined) with names with standing in Prokaryote nomenclature, as of November 3, 2009. Derived from www.bacterio.cict.fr

Number of phyla[a]	26
Number of classes[b]	70
Number of orders	116
Number of families	253
Number of genera	1,732
Number of species	8,226

[a] The term "Phylum" is not covered by the Bacteriological Code (4).
[b] The term "Class" is not covered by the Bacteriological Code (4).

in that journal, or by including the proposed new names in the "Validation Lists" of species that had before been described ("effectively published") in other scientific journals. This rule has greatly simplified bacterial taxonomy and nomenclature, and it is thus easy to keep track of the number of published species and their names.

Table 3.1 gives an overview of the number of names of taxa (species, genera, families, orders, classes, phyla, and domains) in the prokaryote world, *Bacteria* and *Archaea* combined, that have been validly described by November 3, 2009. These numbers are updated bimonthly with the publication of each new issue of the *International Journal of Systematic and Evolutionary Microbiology*. The "List of Bacterial Names with Standing in Nomenclature" website http://www.bacterio.cict.fr, maintained by Dr. Jean Euzéby (1) provides updated information on the number of name of species (and subspecies) and on the higher taxa of prokaryotes with standing in nomenclature (see also Sect. 4.5).

3. CLASSIFICATION OF PROKARYOTES

Historically, phenotypic properties have dominated the classification schemes of bacteria. In the past, identification was primarily based on properties such as cell morphology, staining properties (the Gram stain; acid-fastness stain), the ability to grow on certain carbon sources, excretion of certain end products, presence or absence of certain enzymatic activities, temperature and pH range of growth, etc. The first genomic property to be included in species descriptions of prokaryotes was the determination of the guanine + cytosine (G + C) percentage in the DNA. This property was introduced in the early 1960s, and has retained its value in bacterial taxonomy ever since, to the extent that no description of a new species is complete without it. Even if the amount of information obtained by the determination of the G + C percentage is limited (it does not provide any information on where in the genome these guanine and cytosine bases are found), it has a distinct advantage that it characterizes the complete genome, not a small part of it that may have a special function. With the technological advancement in DNA sequencing, more genotypic properties were found to be of value in bacterial characterization, classification, and identification. The best known example is of course the sequence determination of the small subunit ribosomal RNA

Table 3.2
Some properties used in identification and polyphasic taxonomy of prokaryotes (20)

Genotypic information
Properties based on the total DNA
Determination of the mol% Guanine + Cytosine
Restriction patterns: Restriction fragment length polymorphism (RFLP), Low frequency restriction fragment analysis (PFGE)
DNA–DNA hybridization
Determination of the genome size
Properties based on DNA segments
Polymerase chain reaction (PCR) based DNA fingerprinting: ribotyping, Amplified rDNA restriction analysis (ARDRA), Randomly amplified polymorphic DNA (RAPD), Amplified fragment length polymorphism (AFLP), Arbitrarily primed PCR, repetitive element sequence-based PCR
DNA sequencing of selected genes
Use of specific probes for detection of selected genes
RNA-based properties
RNA sequencing
Determination of low molecular weight RNA profiles
Phenotypic information
Protein-based properties
Electrophoretic patterns (one- or two-dimensional) of total cellular or cell envelope proteins
Enzyme patterns (multilocus enzyme electrophoresis)
Chemotaxonomic markers
Cellular fatty acids (detected as fatty acid methyl esters)
Detection and characterization of mycolic acids
Polar lipid characterization
Identification of respiratory quinones
Identification of cellular polyamines
Characterization of the cell wall and of extracellular polysaccharides
Phenotypically expressed properties
Cell morphology
Physiological properties such as the range of substrates used
Enzymological tests
Serological characterization using monoclonal or polyclonal antibodies

(for prokaryotes: 16S rRNA). As discussed above (see Sect. 2.2), DNA–DNA hybridization similarity, another genomic property, has obtained special status when it has to be decided whether two strains do or do not belong to the same species. Bacterial taxonomy nowadays can be described as "polyphasic," i.e., involving both phenotypic and genomic traits (13, 20). The sections below will discuss the different methods that are currently employed in this polyphasic approach (summarized in Table 3.2), the kind of information that is obtained using each of the common tests, and the relative merits of the different approaches.

3.1. Genotypic Properties Used in Prokaryote Classification

A look at the upper part of Table 3.2 shows that many genotypic and genomic properties have found their way into modern prokaryote taxonomy. Some of these are properties determined by the complete genome – notably the G + C base ratios and the DNA–DNA hybridization methods. Other methods specifically target a special molecule, which often is 16S rRNA (universally applicable; 18S rRNA for eukaryotes) or specific genes that are present in a certain group of *Bacteria* or *Archaea* only and that provide taxonomically valuable information. Examples of the latter are *amoA* (coding for a subunit of the ammonium monooxygenase that catalyzes the first step in ammonium oxidation during autotrophic nitrification), *nifH* (encoding nitrogenase reductase, one of the enzymes essential for nitrogen fixation), and *dsr*, the gene encoding the dissimilatory bisulfite reductase involved in the dissimilatory reduction of sulfate to sulfide of sulfate reducers.

The G + C base ratio varies over a wide range in the prokaryote world, from approximately 20–80%. While an identical G + C content of two species' DNA does not prove the existence of any relationship (after all, the same overall G + C content can be obtained with many different sequences), a large difference (e.g., of 5 mol% or more) in G + C content unequivocally shows that the isolates cannot be closely related. A number of different techniques are currently in use for the experimental determination of the G + C percentage – in addition of course to complete genome sequencing, which will automatically yield the desired information; there are methods based on thermal denaturation profiles, centrifugation methods that assess the buoyant density of the DNA, and HPLC methods that determine the amount of each nucleotide after hydrolysis of the DNA. The determination of the buoyant density is relatively seldom performed nowadays, as analytical ultracentrifuges are operated in only a few laboratories. The method is based on the principle that the higher the G + C content of the DNA, the higher the buoyant density of the DNA is in a CsCl gradient obtained by means of high-velocity centrifugation. More common is the determination of the thermal denaturation profile of the DNA. As the triple hydrogen bond between G–C pairs is stronger than the double hydrogen bond between A–T, the higher the G + C content of the DNA double helix is, the higher the temperature at which the two stands of the DNA will separate, a phenomenon that can be monitored by the increase in the absorbance at 254 nm that accompanies thermal denaturation. The HPLC method, based on quantification of the fragments obtained after enzymatic hydrolysis of the DNA, has gained much popularity since it was first introduced in 1989. Whatever method is used, different types of reference DNA of known G + C content should be included in the tests for calibration, and information about the method used should be provided (see the example in Table 3.3, showing a recently published description of a new species within the genus *Halorubrum* (Domain *Archaea*, Phylum *Euryarchaeota*, Class *Halobacteria*, Order *Halobacterales* (*Halomebiales*), Family *Halobacteriaceae*) (21).

Determination of 16S rRNA gene sequences has become an essential part of any species description as well. When large numbers of strains should be compared, individual sequencing of the 16S rRNA genes of all isolates is often not feasible. In such cases, shortcuts can be introduced such as ribotyping (see Table 3.2), which is an identification method based on the fragmentation pattern when the genomic DNA is cut by specific restriction enzymes, the

Table 3.3
Example of a description of a new Prokaryote species based on polyphasic taxonomy

	Comments
Description of *Halorubrum terrestre* sp. nov.	sp. nov. = species nova = new species
Halorubrum terrestre (ter.res'tre. L. neut. adj. *terrestre* of the soil, from which the strains were isolated)	The etymology of the specific epithet proposed, in this case a Latin adjective
Cells are pleomorphic, flat and disc-shaped, 1.0–1.5 × 1.5–2.5 μm in size. Motile. Gas vacuoles not produced	The morphological properties of the species, as observed microscopically
Colonies are orange-red	Description of special properties such as pigmentation, as observed in colonies on agar plates or in liquid culture
Growth occurs in media that contain 15–30% NaCl, with optimum growth at 25% NaCl. Growth occurs between 28 and 50°C (optimum at 37–45°C) and pH 5–9 (optimum, 7.5)	The physical and chemical conditions required for growth: salt concentration, temperature, pH
Chemo-organotrophic; aerobic; oxidase- and catalase-positive	The mode of metabolism and the relation of the species to molecular oxygen
Acid is produced from glycerol, but not from arabinose, fructose, galactose, glucose, lactose, maltose, sucrose, or trehalose. Nitrate is not reduced to nitrite. Indole is not produced from tryptophan. Voges–Proskauer test is negative. Starch, gelatin, and casein are not hydrolyzed. H_2S is not produced. Arginine dihydrolase, lysine decarboxylase, and ornithine decarboxylase are not produced. The following compounds are not used as sole carbon and energy sources: arabinose, cellobiose, aesculin, fructose, fucose, gluconolactone, glucose, glucosamine, inulin, mannose, melibiose, raffinose, rhamnose, ribose, sucrose, trehalose, xylose, adonitol, dulcitol, erythritol, ethanol, glycerol, mannitol, *meso*-inositol, propanol, sorbitol, α-aminovalerate, butyrate, caprylate, citrate, fumarate, glutamate, glycerate, 2-oxoglutarate, malate, malonate, oxalate, propionate, saccharate, and tartrate. The following compounds are not used as sole carbon, nitrogen or energy sources: L-alanine, L-arginine, L-asparagine, betaine, creatine, L-glutamine, glycine, L-histidine, L-lysine, L-methionine, L-ornithine, L-proline, putrescine, sarcosine, L-serine, L-threonine, and L-valine	Description of the substrates on which the species can grow and those that do not support growth, as well as the result of selected enzymatic tests that provide information on the metabolic abilities of the species
Susceptible to anisomycin, bacitracin, and novobiocin; resistant to ampicillin, chloramphenicol, kanamycin, nalidixic acid, penicillin G, polymyxin, streptomycin, and tetracycline	Information on the sensitivity of the species to different antibiotics and other antibacterial compounds

(*Continued*)

Table 3.3
(Continued)

	Comments
Polar lipids are $C_{20}C_{20}$ derivatives of phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester, phosphatidylglycerol sulfate and a sulfated diglycosyl diether	Chemotaxonomic information on the types of lipids present, with emphasis on those lipids that are diagnostic for different genera within the *Halobacteriaceae*
DNA G + C content is 64.2–64.9 mol% (T_m method)	The range of Guanine + Cytosine content of the genomic DNA of the isolates of the new species, including information on the methodology used for its determination (thermal denaturation)
The type strain is $4p^T$ (=VKM B-1739^T = JCM 10247^T)	The designated type strain (indicated with a superscript capital T) and its accession number in two public culture collections (VKM = All-Russian Collection of Microorganisms, Russian Academy of Sciences, Pushchino, Russia; JCM = Japanese Collection of Microorganisms, Saitama, Japan)
DNA G + C content of this strain is 64.4 mol% (T_m method)	The Guanine + Cytosine content of the genomic DNA of the type strain of the new species, including information on the methodology used for its determination (thermal denaturation)
Isolated from saline soils	The habitat from which the strains investigated were isolated
The GenBank/EMBL/DDBJ accession number for 16S rRNA gene sequence of *Halorubrum terrestre* strain VKM B-1739^T is AB09016	The accession number of the 16S rRNA gene nucleotide sequence of the type strain, as deposited in the public gene sequences databases

fragments separated by electrophoresis, and then hybridized with a probe specific for 16S rRNA genes. Each species has its unique characteristic restriction pattern, and computerized databases exist in which the restriction patterns of many species are stored. The method is much more rapid than full sequencing of the 16S rRNA gene, and is very specific. There are other current DNA fingerprinting methods, listed in Table 3.2, such as randomly amplified polymorphic DNA (RAPD) strain typing (22).

3.2. Phenotypic Properties Used in Prokaryote Classification

A large number of phenotypic properties have been found useful in the description, classification, and identification of bacteria. Table 3.2 shows some of the most important ones that

are generally applicable. There are many other properties that are relevant for specific groups of microorganisms.

Among the phenotypic properties valuable for the description of bacterial isolates and of value in bacterial systematics and taxonomy, one can name:

- Cell shape, cell size, motility, mode of flagellation
- Colonial morphology
- Gram-staining behavior, the ultrastructure, and the chemical structure of the cell wall (presence of peptidoglycan, type of peptidoglycan, especially the nature of the peptides linking the polysaccharide chains), presence of an outer membrane, presence of hopanoids and teichoic acids in the cell wall
- The presence of exopolysaccharides and their structure
- Formation of endospores
- Presence of cellular inclusions such as gas vesicles, storage materials, etc., and other ultrastructural characteristics
- Pigmentation and characterization of the chemical nature of the pigments present (bacteriochlorophylls, chlorophylls, phycobiliproteins, etc. in phototrophic *Bacteria*, carotenoids both in phototrophic and in nonphototrophic prokaryotes, other classes of pigments)
- The nature of the membrane lipids (ether-linked in *Archaea*, ester-linked in *Bacteria*), and the types of fatty acids present (generally determined as fatty acid methyl esters by gas chromatography). Some fatty acids are very valuable as diagnostic markers. Rigorous standardization of the methodology used is essential, and cultures to be compared should be grown under identical conditions, as growth conditions may have a strong influence on the fatty acids pattern
- For respiratory organisms – the chemical nature of the respiratory quinones
- The types of polyamines present in the cell
- Temperature, pH, and salinity range and optimum for growth
- The types of metabolism performed by the cells: aerobic, anaerobic, chemoorganotrophic, phototrophic, chemolithotrophic, etc., with information on the range of energy sources, electron donors, electron acceptors, and carbon sources used. Miniaturized standardized tests, such as those provided by the BIOLOG^{R} system, are often helpful. The latter consists of microtiter plates with wells that contain potential growth substrates and a redox indicator. Utilization of the carbon source will cause a color change of the indicator, enabling the simultaneous determination of the utilization of 95 different substrates within a short time. The procedure can also easily be automatized. Other commercially available test kits (such as, e.g., the API system for identification of enteric bacteria, which consists of miniaturized test tubes with different media and reagents) enable the rapid determination of other physiological properties such as the production of certain enzymes, growth and acid formation on specific carbon sources, and others.
- Special nutritional requirements
- Excretion of exoenzymes (amylases, proteases, lipases, etc.)
- Presence of special diagnostic enzymes
- Susceptibility to a range of antibiotics and other antibacterial substances; susceptibility to attack by specific bacteriophages
- Immunological properties (reactions with specific antibodies, etc.)

It must be stressed that the results of individual phenotypic tests are insufficient to provide information on the identity of bacterial isolates and on the genetic relatedness of strains. However, integration of the results of a large number of phenotypic tests provides reliable descriptive information enabling to recognize prokaryote taxa. This is illustrated in Table 3.3,

which provides an example of a recently published species description that includes a large number of phenotypic tests of diagnostic value. It is clear that description of an isolate as a member of a new species requires extensive documentation of its properties, morphological as well as physiological, 16S rRNA sequence determination, assessment of the base composition of the DNA, determination of DNA–DNA similarity with its closest relatives, and documentation of as many other characteristics as possible.

A special type of taxonomy that is based on the comparison of many bacterial strains for a large number of mainly phenotypic properties is the so-called numerical taxonomy (23), also sometimes referred to as phenetic or Adansonian taxonomy. In this type of taxonomic characterization, developed in the 1950s based on multivariate analysis, the strains are divided into clusters on the basis of large numbers of tests followed by computer-assisted evaluation of the results. These tests should belong to different categories (morphology, physiology, biochemistry, serology, etc.) and should be independent of each other. An important principle of this type of classification is that it is based on equal weighting of each of the characters determined, so that each of the many tests (100–200 tests are ideally included in such studies, and at least 50–60 are required to yield relevant results) has an equal importance in the final outcome, rather than stressing the importance of certain traits. Following data collection of the different tests, the results are coded as positive or negative, and the resemblance between the strains is expressed in a similarity matrix. This matrix is then analyzed for taxonomic structure, and the strains are arranged in groups (so-called phenons, equivalent to species in many cases). On the basis of the delineation of these phenons, the diagnostic characters of each group can be identified, and these can then later be used for the preparation of identification schemes that will enable to place additional strains within the established framework.

3.3. The Polyphasic Approach Toward Prokaryote Classification

As stated in Sect. 2.2, there are no generally agreed-upon rules to delineate genera, except the notion that genera should reflect phylogenetic relationships. Also, the delineation of species is still problematic in the prokaryote world. Nowadays, there is a broad consensus among microbial taxonomists that phylogenetic data are of superior value for the delineation of genera and species, but that "polyphasic" definition of the taxa is required to describe and define taxa at the genus and the species level and to differentiate them from their neighbors (13, 20).

The polyphasic approach to taxonomy uses a combination of a variety of different phenotypic and genetic properties to establish a classification of microorganisms. It is the most obvious strategy to collect a maximum amount of direct and indirect information about the total genome. The term "polyphasic" was introduced in 1970 to describe taxonomy that assembles and accumulates multiple sources of information, based on genetic–phylogenetic as well as on phenotypic data and ecological properties. Nowadays, polyphasic taxonomy refers to a consensus type of taxonomy and aims to utilize all the available data in delineating consensus groups. The more properties are included in the descriptions (see Table 3.2), the more robust and stable the resulting classification schemes will be. Different properties have different resolving power; some are species-specific, while others are valuable to discriminate genera, families, and orders. Descriptions of species using the polyphasic approach should

reflect the phylogenetic relationships of the species with other related taxa, include total genome DNA–DNA hybridization to determine the genomic relationships with related taxa, and also provide further descriptive genomic, phenotypic, and chemotaxonomic information.

4. NAMING OF PROKARYOTES

4.1. The Binomial System of Naming Prokaryotes

Similar to the eukaryotic organisms, the species of prokaryotes have generic names and specific epithets derived from Latin, latinized Greek or latinized modern words or names (24, 25). The binomial system that was introduced by Linnaeus for the plant world in the 1750s is used for the domains *Bacteria* and *Archaea* as well. In many cases, do these names give some information about the properties of the organism such as shape, color, habitat, or physiology. Others have been named in honor of famous microbiologists in the past and present. Three examples of bacteria and their etymology: the name *Streptococcus pneumoniae* is derived from the Greek adjective streptos – στρεπτος, twisted, flexible, the Greek noun kokkos – κοκκος designating a seed, a berry, and the Greek noun pneuma – πνευμα – breath, from where the neo-Latin pneumonia. The nitrifying, nitrite-oxidizing bacterium *Nitrobacter winogradskyi* derives its name from the Latin noun nitrum, nitrate, and bacter, being an equivalent for the Greek noun bakterion – βακτεριον, rod, staff; the species was named in honor of Sergei Winogradsky, who in the 1880s discovered the nitrifying bacteria and formulated the concept of chemolithotrophy. Finally, the name *Acidithiobacillus ferrooxidans* provides considerable information on the physiology of the organism: the Latin adjective acidus means acidic; thion – θιον is Greek for sulfur, bacillus is derived from the Latin diminutive noun bacillum, a small rod, ferrum is the Latin form of iron, and the neo-Latin verb oxidare means to oxidize. The name, thus, signifies that the organism is a rod-shaped bacterium that lives in acidic environments, is involved in sulfur transformation, and oxidizes iron compounds.

Each description of a new species (see also Table 3.3) should include a proposal for its name and explain the etymology of that name. The naming of bacteria is subject to many rules and recommendations, both derived from linguistic constraints and from scientific considerations. Practical recommendations on how to name a new prokaryote can be found in a number of treatises on the subject (24, 25).

The phylum *Cyanobacteria* presents special nomenclature problems, as the group is also included by the botanists under the rules of the Botanical Code as *Cyanophyta* or blue-green algae. This group consists of organisms with a prokaryotic cell structure that display oxygenic photosynthesis, i.e., physiologically they resemble the eukaryotic algae and the higher plants. The rules of botanical nomenclature are very different from those of the Bacteriological Code, as the botanical types are not axenic live cultures such as are required under the Bacteriological Code, but descriptions and material preserved in herbaria. The result is a highly confusing and unsatisfactory situation in which many "species" appear under different names in the literature. The 1980 approved lists of bacterial names (19) did not contain any names of *Cyanobacteria*, and only very few names of species have since been validly published in the *International Journal of Systematic Bacteriology/International Journal of Systematic and*

Evolutionary Microbiology. The latest edition of Bergey's Manual (5) (see also Sect. 7.1) does not divide the phylum into classes, orders, and families, but instead provides a provisional division of the group into subsections, each subsection consisting of several "form-genera." It is to be expected that the nomenclature problems of this group will not very soon find a solution that will satisfy bacteriologists and botanists alike.

4.2. The Bacteriological Code

The nomenclature of the prokaryotes, *Bacteria* as well as *Archaea*, is regulated by the rules of the Bacteriological Code – The International Code of Nomenclature of Bacteria, as approved at the Ninth International Congress of Microbiology, Moscow, 1966, revised in 1990 (4), and amended at the meetings of the International Committee for Systematics of Prokaryotes (before 2000: International Committee of Systematic Bacteriology (see Sect. 4.3). A new revised version of the Code is presently in preparation – to be renamed International Code of Nomenclature of Prokaryotes since 2000). The Bacteriological Code presents the formal framework according to which prokaryotes are named and according to which existing names can be changed or rejected. It covers the rules for the naming of species (and subspecies), genera, families, and orders of prokaryotes. No provisions are made by the Code for the naming of the higher taxa: class, phylum, and domain.

One aspect in which the Bacteriological Code differs from the Botanical Code and the Zoological Code, the latter two being similar documents that regulate nomenclature in the plant and the animal world, is the rule (Rule no. 24a) according to which a new start was made in prokaryote nomenclature with the publication of the 1980 approved lists of names (19), therewith abolishing the need to search the older, and often very confusing literature (see also Sect. 2.3). In contrast, botanists who want to establish whether a certain plant has been recorded before in the literature often have to search the scientific journals and books as far back as 1753, the year in which Linnaeus published his "Species Plantarum." Zoologists face a similar problem when they must decide whether an animal they discovered may be described as a new species.

4.3. The International Committee on Systematics of Prokaryotes

The rules that regulate the nomenclature of prokaryotes, as published in the Bacteriological Code, are set by the International Committee on Systematics of Prokaryotes (ICSP) (before 2000: the International Committee of Systematic Bacteriology). This committee is a constituent part of the International Union of Microbiological Societies (IUMS). The committee meets at least once every 3 years – at the time of the IUMS congresses. The committee discusses nomenclatural problems that have arisen in different groups of prokaryotes and proposes changes and amendments to the rules of the Bacteriological Code. The minutes of the committee's meetings are published in the *International Journal of Systematic and Evolutionary Microbiology* (see Sect. 4.4). Information on the committee, its members, and its different subcommittees can be found in its Web site: http://www.the-icsp.org.

The ICSP has established several subcommittees. One important subcommittee is the Judicial Commission, a committee that deals with problematic cases in bacterial nomenclature and renders judicial decisions in instances of controversy about the validity of a name, identity

of type strains, and cases of emerging problems with the interpretation of the rules of the Bacteriological Code. It may propose amendments to the Code and consider exceptions that may be needed to certain rules. The decisions of the Judicial Commission need to obtain approval of the ICSP to obtain standing. Problems can be brought to the attention of the Judicial Commission by submission of a "Request for an Opinion" to be published in the *International Journal of Systematic and Evolutionary Microbiology*.

The ICSP has established a number of taxonomic subcommittees that discuss nomenclatural problems of specific groups of prokaryotes. Currently, there are 28 such subcommittees, dealing with groups such as *Bacillus* and related organisms, phototrophic bacteria, methanogenic *Archaea*, Gram-negative anaerobic rods, *Bifidobacterium*, *Lactobacillus* and related organisms, the genus *Mycobacterium*, staphylococci and streptococci, and others. Details about the membership and about the taxa covered by these subcommittees can be found in the web site of the ICSP. The minutes of the meetings of these subcommittees are published as well in the *International Journal of Systematic and Evolutionary Microbiology*.

4.4. The International Journal of Systematic and Evolutionary Microbiology

The *International Journal of Systematic and Evolutionary Microbiology* (prior to 2000: *International Journal of Systematic Bacteriology*) is the journal in which new names of taxa of prokaryotes must be published in order to obtain standing in the nomenclature; since 1976 validation is only possible by publication in this journal. It is also possible to describe the new species or other taxa in another scientific journal ("effective publication"), but the new name will not obtain standing in the nomenclature until it has been included in the "Validation Lists" of names first published elsewhere. Such validation lists are included regularly in the issues of the journal, which presently appears monthly.

In addition to being the platform for describing new prokaryote taxa, the *International Journal of Systematic and Evolutionary Microbiology* publishes articles that address taxonomy, phylogeny and evolution of prokaryotes (as well as of fungi and some other groups of eukaryotic protists). In addition, it contains the minutes of the meetings of the International Committee of Systematics of Prokaryotes, its Judicial Commission and its taxonomic subcommittees, as well as correspondence relating to bacterial nomenclature such as "Request for an Opinion" documents with queries to the Judicial Commission (see Sect. 4.3).

4.5. Information on Nomenclature of Prokaryotes on the Internet

The web site www.bacterio.cict.fr, maintained by Dr. Jean Euzéby of the University of Toulouse, France (1), contains a wealth of information on all names of prokaryotes that have standing in the nomenclature. The site is updated monthly with the publication of the latest issue of the *International Journal of Systematic and Evolutionary Microbiology*. It provides important information on the type strains of each species and on any name changes and current or past nomenclature problems. In addition, the site contains a great deal of additional information relevant to prokaryote nomenclature. For example, the information on the total number of species with standing in the nomenclature as given in Table 3.1 was derived from this web site.

In addition, much information on specific groups of microorganisms can be found in the Web pages of several of the International Committee on Systematics of Prokaryotes taxonomic subcommittees (www.the-icsp.org; see also Sect. 4.3).

5. CULTURE COLLECTIONS OF PROKARYOTES AND THEIR IMPORTANCE IN TAXONOMY AND IDENTIFICATION

As explained in Sect. 2.1, the type strain of each newly described species must be deposited in at least two publicly accessible culture collections located in different countries, so that the strain will be preserved and made available for further study by any interested scientist. Such culture collections are extremely important for the preservation of microbial biodiversity, and their importance for microbial taxonomy cannot be overestimated when it comes, for example, to referencing strains that should be used when comparing new isolates (26). Culture collections generally preserve bacterial strains either in dry, lyophilized form, to be revived by wetting and suspension in suitable growth medium, or frozen in liquid nitrogen. In addition to storing and distributing publicly available strains, culture collections may provide safekeeping facilities for patented strains of microorganisms. Many also provide characterization and identification facilities.

A list of culture collections that maintain cultures of prokaryotes can be found in the www.bacterio.cict.fr Web site (see Sect. 4.5), and a representative list is found in Vol. 1 of the second edition of *Bergey's Manual of Systematic Bacteriology* (26) (see also Sect. 7.1). Some of these culture collections maintain Web sites that, in addition to the strain catalogs and technical details about depositing and ordering strains, provide extensive information about the history and the nomenclature of the strains, recipes for media in which the isolates can be grown, and much more. The Web sites of the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany – www.dsmz.de) and the American Type Culture Collection (ATCC, Manassas, VA, USA – www.atcc.org) are especially useful in this respect.

6. SMALL-SUBUNIT rRNA-BASED CLASSIFICATION OF PROKARYOTES

Before the late 1970s, no methods were available that would enable linking bacterial systematics and taxonomy with bacterial phylogeny. Thereafter, molecular methods have been introduced, based on sequencing of genes that can be used as "evolutionary clocks," i.e., that provide information on evolution in the prokaryote world. The principle of molecular taxonomy and of phylogenetic tree reconstruction is based on the concept that biological macromolecules can be used as evolutionary chronometers that measure evolutionary change. Mutations that have occurred during the course of time have become fixed in the populations, resulting in diversity in sequences of nucleotides in genes and of amino acids in proteins. Evolutionary distances can thus be measured by differences in nucleotide or amino acid sequence of monomers in homologous macromolecules. As explained in Sect. 3.3, one of the goals of polyphasic taxonomy is that the classification of the genera should reflect phylogenetic relationships. Thanks to the availability of the appropriate techniques, notably the sequencing

of the genes encoding the small-subunit (16S) ribosomal RNA, the fulfillment of this goal is now within reach.

6.1. 16S rRNA as a Phylogenetic Marker

When Carl Woese started to compare nucleotide sequences of small-subunit ribosomal RNA (16S rRNA in prokaryotes, 18S rRNA in eukaryotes) at the University of Illinois in the late 1970s, few people realized that the approach used and the results obtained would within one to two decades revolutionize our views not only about prokaryote evolution, but also about classification of prokaryotes (27). It was to Woese's credit that he realized early on that the ribosomal RNA molecules are the best suited to serve as molecular chronometers to track down the course of prokaryote phylogeny (14–16). As each cell has ribosomes, ribosomal RNA is universally distributed and functionally homologous, and the rate of change in these molecules has proven sufficiently slow to be useful to reconstruct the phylogeny of organisms that exist on Earth for three and a half billion years at least. Common ancestry, genetic stability, appropriate size, and the presence of independently evolving domains within the molecules are other properties that make the 16S and 18S rRNAs ideally suited to serve as phylogenetic markers.

Prokaryote ribosomes contain three molecules of RNA, 5S rRNA, 16S rRNA, and 23S rRNA, with about 120, 1,540, and 2,900 nucleotides, respectively. The latter two are large enough to contain sufficient information for the purpose of phylogenetic tree reconstruction. Technically, the sequencing of the 16S rRNA (or the gene that codes for it) is easier than sequencing of the much longer 23S rRNA. The results obtained from analysis of the 23S rRNA gene sequencing generally confirm those obtained with the 16S rRNA gene. Accordingly, 16S rRNA has become the molecule of choice for general use in phylogenetic studies of prokaryotes.

Determination of phylogenetic relationships, based on 16S and to a lesser extent on 23S rRNA sequence similarities, has become routine procedure in bacterial taxonomy. Sequencing of the genes generally follows their prior amplification by PCR. No characterization of new species of prokaryotes is nowadays complete without presentation of the 16S rRNA gene sequence and deposition of this sequence in a public database, such as the GenBank, to make it available to the scientific world (see also Table 3.2). There is also a specialized database, the Ribosomal Data Base Project (http://rdp.msu.edu), which presently (November 10, 2009) contains 1,235,044 16S rRNA gene sequences, both of cultured organisms and of sequences recovered from DNA isolated from the mixed community present in the environment (see Sect. 9). New sequences can be compared by aligning them with those present in the database, and phylogenetic trees can then be computed using statistical methods. Figure 3.1 shows an example of the phylogenetic tree of the prokaryotes obtained on the basis of 16S rRNA gene sequence comparisons (based on sequences derived from cultured species only; a few phyla of *Bacteria* that are currently represented by only very few cultured species are not shown), and Table 3.4 summarizes the properties of the most important phylogenetic groups that have emerged from these comparisons. It should be noted that the exact topology of such trees may to some extent depend on the computational procedure followed. A discussion of the algorithms used for the calculation of the tree topologies is outside the scope of this chapter.

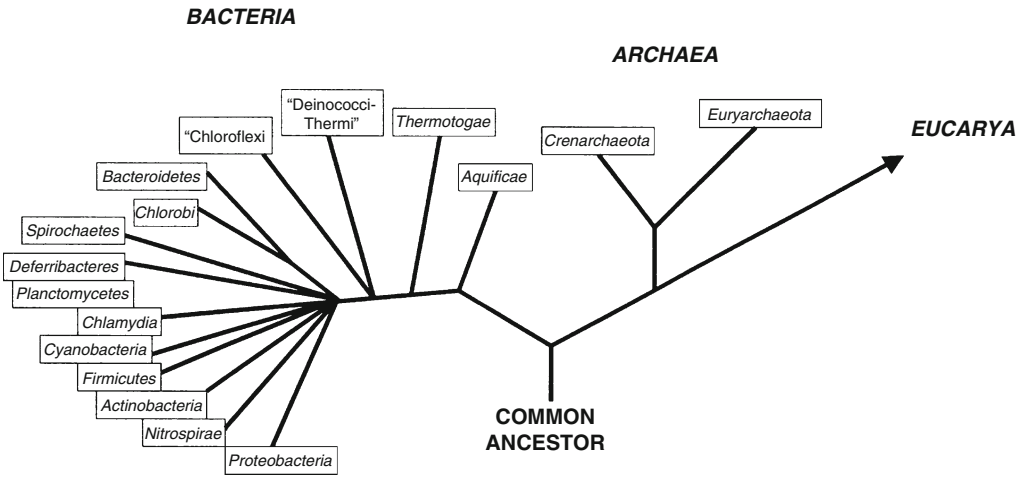

Fig. 3.1. Schematic rooted phylogenetic tree, based on small subunit ribosomal RNA comparisons, showing the most important phyla of *Bacteria* and *Archaea*.

Overall, it can be stated that the resulting phylogenetic trees are rather robust constructs. Their topology is largely confirmed by comparative analyses of other conserved molecules (elongation factors, the β-subunit of ATP synthase, and other proteins).

The existence of this extensive and ever-growing database enables us to rapidly place any newly isolated prokaryote within the phylogenetic classification scheme at least to the level of the genus. As stressed earlier (see Sect. 2.2), the resolving power of 16S rRNA gene as a taxonomic marker is insufficient to allow identification to the species level.

One of the most important concepts that emerged from 16S rRNA and 18S rRNA sequence comparisons, already from the very beginning of these studies in Carl Woese's laboratory in the 1970s, is that the prokaryote world is not phylogenetically homogeneous. On the basis of the small subunit ribosomal RNA sequences, the fundamental division of the forms of life that inhabit planet Earth should be not into two groups (prokaryotes and eukaryotes), as was customary at the time, but into three groups, the eukaryotes and two groups ("primary kingdoms," now called domains) of prokaryotes, which among each other show as little phylogenetic relationship as each of these domains with the eukaryotes. Woese originally named these prokaryotes, *Eubacteria* and *Archaebacteria*. The names currently used are *Bacteria* and *Archaea* for the prokaryotic domains, and *Eucarya* for the eukaryotic domain (see also Fig. 3.1) (14–16). The following sections explore the differences between the *Bacteria* and the *Archaea*, and provide an overview of the properties of the different groups (at least at the level of phyla) within each of these two basic domains of prokaryote life. Phylogenetic analyses based on conserved protein sequences generally support a closer relationship between the *Archaea* and the *Eucarya* than between the *Archaea* and the *Bacteria*, such as is also suggested by the tree topology shown in Fig. 3.1. The concept that the prokaryotes are phylogenetically heterogeneous was only slowly accepted by the scientific world. However, nowadays, the basic division of life into three domains, separating the *Archaea* from the *Bacteria* is now accepted

Table 3.4
The major divisions of the Prokaryote world – the domains, phyla, and classes as given in the classification proposed in the second edition of Bergey's manual of systematic bacteriology (5)

Domain	Phylum	Class	Main representatives and further information
Archaea			
	Crenarchaeota		
		Thermoprotei	All cultured representatives are extremely thermophilic, mostly anaerobic isolates from hydrothermal vents, hot springs, and other thermal environments. Some well-known species: *Pyrodictium*, *Sulfolobus*
	Euryarchaeota		
		Methanobacteria	Strictly anaerobic methanogenic prokaryotes (e.g., *Methanobacterium*, *Methanobrevibacter*), including a few thermophiles (*Methanothermus*)
		Methanococci	Strictly anaerobic methanogenic prokaryotes (e.g., *Methanococcus, Methanospirillum*, *Methanosarcina*), including a few thermophiles (*Methanocaldococcus*)
		Halobacteria	Extremely halophilic aerobes or facultative anaerobes such as *Halobacterium* and *Haloarcula*; some are alkaliphilic (e.g., *Natronobacterium*)
		Thermoplasmata	Thermophilic organisms such as *Thermoplasma*, and thermoacidophiles (*Picrophilus*)
		Archaeoglobi	Anaerobic thermophiles with chemoautotrophic or heterotrophic metabolism, using sulfate (*Archaeoglobus*) or nitrate (*Ferroglobus*) as electron acceptor
		Methanopyri	A separate lineage of thermophilic methanogens (*Methanopyrus*)
Bacteria			
	Aquificae		
		Aquificae	Moderately thermophilic to hyperthermophilic chemolithotrophs and chemoorganotrophs (*Aquifex*, *Hydrogenobacter*)
	Thermotogae		
		Thermotogae	Extreme thermophilic anaerobes with an outer sheath-like envelope (*Thermotoga*, *Geotoga*)

(Continued)

Table 3.4
(Continued)

Domain	Phylum	Class	Main representatives and further information
	Thermodesulfo-bacteria		
		Thermodesulfo-bacteria	Currently represented by a single, deep-branching genus *Thermodesulfobacterium*, of sulfate-reducing anaerobes
	"Deinococcus-Thermus"		
		Deinococci	A group of chemoorganotrophic aerobes that includes *Deinococcus*, a genus of radiation-resistant cocci, and *Thermus*, a genus of thermophilic bacteria from hot springs
	Chrysiogenetes		
		Chrysiogenetes	A deep lineage within the *Bacteria*, currently represented by a single genus *Chrysiogenes* with a single species that grows anaerobically by oxidizing acetate with arsenate as electron acceptor
	Chloroflexi		
		"Chloroflexi"	Gram-negative bacteria showing gliding motility. Some (*Chloroflexus*) are anoxygenic phototrophs, others (*Herpetosiphon*) are aerobic heterotrophs
	Thermomicrobia		
		Thermomicrobia	A phylum currently represented by a single genus *Thermomicrobium* with a single species of hydrocarbon-utilizing thermophiles
	Nitrospirae		
		"Nitrospira"	A heterogeneous group that includes nitrite-oxidizing autotrophs (*Nitrospira*), iron-oxidizers (*Leptospirillum*), and thermophilic sulfate reducers (*Thermodesulfovibrio*)
	Deferribacteres		
		Deferribacteres	Heterotrophic anaerobes (*Deferribacter*, *Geovibrio*), which use oxidized iron, manganese, or nitrate as electron acceptor

Cyanobacteria		
	Cyanobacteria	Aerobic oxygenic phototrophs, unicellular as well as filamentous, including many types that fix molecular nitrogen. Different classification schemes exist, mostly based on the Botanical Code. Well-known species include the unicellular *Synechococcus* and *Microcystis* and filamentous types such as *Oscillatoria, Phormidium, Anabaena*, and *Nostoc*. Few species have yet been named under the Bacteriological Code
Chlorobi		
	"Chlorobia"	Anaerobic oxygenic phototrophs that oxidize reduced sulfur compounds (*Chlorobium*, *Prosthecochloris*, and others)
Proteobacteria		
	"Alphaproteo-bacteria"	A large and very heterogeneous group of Gram-negative bacteria, including anoxygenic photoheterotrophs (*Rhodospirillum*, *Rhodobacter*), aerobic heterotrophs (*Paracoccus*, *Caulobacter*, *Acetobacter*), parasites/pathogens (*Rickettsia*), symbionts (*Rhizobium*), chemoautotrophs (*Nitrobacter*), and many others
	"Betaproteo-bacteria"	Another large and very heterogeneous group of Gram-negative bacteria, which includes heterotrophs (*Spirillum*, *Zoogloea*) chemoautotrophs (*Thiobacillus*, *Nitrosomonas*), and anoxygenic photoheterotrophs (*Rhodoferax*)
	"Gammaproteo-bacteria"	The third subgroup of the Proteobacteria, no less heterogeneous than the first two. It has anoxygenic photoautotrophic representatives (e.g., *Chromatium*), chemolithotrophic ammonium oxidizers (e.g., *Nitrosococcus*), chemolithotrophic sulfur oxidizers (*Beggiatoa*), heterotrophic aerobes (*Pseudomonas*, *Azotobacter*, *Vibrio*), fermentative anaerobes (*Ruminobacter*), the enteric bacteria (*Escherichia*, *Salmonella*, *Proteus*), and many others
	"Deltaproteo-bacteria"	Another physiologically heterogeneous group that includes most dissimilatory sulfate-reducing bacteria (*Desulfovibrio*, *Desulfobacter*), certain nitrifying chemoautotrophs (*Nitrospina*), iron-reducing bacteria (*Geobacter*), anaerobic obligatory syntrophic bacteria (*Syntrophobacter*), predatory bacteria (*Bdellovibrio*), and others

(Continued)

Table 3.4
(Continued)

Domain	Phylum	Class	Main representatives and further information
		"Epsilon-proteobacteria"	A small subgroup of the *Proteobacteria* that contains a number of interesting organisms such as *Helicobacter*, the causative agent of gastric ulcers, *Thiovulum*, a sulfur-oxidizing bacterium, and *Campylobacter*, a genus of intestinal bacteria
	Firmicutes		
		"Clostridia"	Anaerobic fermentative Gram-positive bacteria, some forming endospores, including genera such as *Clostridium*, *Ruminococcus*, *Peptostreptococcus*, endospore-forming dissimilatory sulfate reducers of the genus *Desulfotomaculum*, and anoxygenic phototrophs (*Heliobacterium*)
		Mollicutes	A group of mostly wall-less pathogens such as *Mycoplasma, Acholeplasma*, and *Erysipelothrix*
		"Bacilli"	This group of Gram-positive bacteria includes the aerobic endospore-forming organisms (*Bacillus* and related species), and the lactic acid bacteria (*Lactobacillus*, *Leuconostoc*, *Streptococcus*)
	Actinobacteria		
		Actinobacteria	A large group of mostly aerobic heterotrophic microorganisms, many of them growing in the form of mycelia (*Steptomyces*, *Actinomyces*), pathogens such as *Corynebacterium* and *Mycobacterium*, fermentative anaerobes (*Propionibacterium*), symbiotic nitrogen fixers (*Frankia*), and others
	Planctomycetes		
		"Planctomycetacia"	A small group of mostly aerobic heterotrophic bacteria of unusual cellular structure (*Plancomyces*, *Pirellula*). It includes the anaerobic chemoautotrophic organisms that oxidize ammonium ions with nitrite as electron acceptor
	Chlamydiae		
		"Chlamydiae"	A group of intracellular parasites and pathogens (*Chlamydia*)
	Spirochaetes		
		"Spirochaetes"	Aerobic and anaerobic organisms with an unusual mode of motility. The group includes free-living species (*Spirochaeta*), symbionts (*Cristispira*), as well as pathogens (*Treponema*, *Borrelia*)

Fibrobacteres		
	"Fibrobacteres"	A deep lineage within the *Bacteria*, presently consisting of a single genus, *Fibrobacter* – anaerobic fermentative organisms found in the rumen of ruminant animals
Acidobacteria		
	"Acidobacteria"	A small group of acidophilic organotrophs (*Acidobacterium*) and iron reducers (*Geothrix*)
Bacteroidetes		
	"Bacteroides"	Anaerobic fermentative bacteria (*Bacteroides* and others)
	"Flavobacteria"	Generally aerobic, often pigmented heterotrophs such as *Flavobacterium*
	"Sphingobacteria"	Heterotrophic aerobic bacteria involved in aerobic degradation, often of polymeric substrates (*Cytophaga*, *Flexibacter*, and the thermophilic *Rhodothermus*)
Fusobacteria		
	"Fusobacteria"	A small group of fermentative anaerobes of different metabolic types (*Fusobacterium*, *Propionigenium*)
Verrucomicrobia		
	Verrucomicrobiae	A small group of prosthecate bacteria, including *Verrucomicrobium* and *Prosthecobacter*, forming a deep linage within the phylogenetic tree of the *Bacteria*
Dictyoglomi		
	"Dictyoglomi"	A linage of *Bacteria* thus far represented only by *Dictyoglomus*, an anaerobic thermophilic organotroph

Names of taxa in italic type have been formally described in the literature and have standing in the bacteriological nomenclature. It should be noted that the terms "Domain", "Phylum," and "Class" are not covered by the Bacteriological Code (4). Names printed in roman type in quotation marks are newly proposed names yet without standing in the nomenclature.

by nearly all bacteriologists. Alternative views are, however, proposed from time to time (28). A discussion of these alternative models of prokaryote evolution is outside the scope of this chapter.

Phylogenetic trees, such as the one shown in Fig. 3.1, do not provide an answer to the question of the nature of the universal ancestor of all life forms on Earth. One can also not infer from these trees whether the *Archaea* are a more ancient group than the *Bacteria*, or whether the prokaryotes formed an ancestral stage that led to the development of the primitive eukaryotic cell. It is, however, well established that the mitochondria found in most eukaryotic cells have an ancestry that can be traced back to the *Proteobacteria*, one of the phyla within the domain *Bacteria*. Similarly, the origin of the chloroplasts, the organelles responsible for light harvesting, and autotrophic fixation of carbon dioxide in algae and higher plants, is within the *Cyanobacteria* phylum of the *Bacteria*. These organelles are the size of prokaryotes, contain their own DNA, and have ribosomes that resemble in size and properties those of the *Bacteria* rather than those of the eukaryotic cell. Genome sequence analyses of representatives of the three domains have made it clear that phylogenetically speaking, the eukaryotic cell is thus a chimera, which includes components derived from the *Eucarya*, the *Bacteria*, and also the *Archaea*.

6.2. The Differences Between Bacteria and Archaea

If indeed the *Archaea* are phylogenetically so distant from the *Bacteria* as it appears from the 16S rRNA gene sequence-based trees (Fig. 3.1), it should be expected that this great evolutionary distance would also find its expression in a large number of other properties, including phenotypic ones. Morphologically, the *Archaea* and the *Bacteria* are very similar, and also at the level of the cell ultrastructure, there are no obvious differences between typical representatives of both groups. Because of this apparent morphological and structural similarity of the members of the two domains, the three-domain model of Woese met with much skepticism in the first years. However, when more in-depth comparative studies were made of the representatives of the two domains, it became clear that indeed there are far-reaching differences between *Bacteria* and *Archaea*, not only in the nucleotide sequences of ribosomal RNAs, but also in many fundamental properties of the cell, including the structure of the cell wall, the type of lipids in the membrane, the properties of the transcription mechanism of DNA to form RNA, the details of operation of the protein synthesis machinery of the ribosome, sensitivity to different antibiotics, and others. Table 3.5 summarizes the most important of those differences that define the two prokaryotic domains.

One of the most prominent differences between the members of the archaeal and the bacterial domains is the structure of the cell wall. With a few exceptions, the cell wall of the *Bacteria* contains peptidoglycan, either in a thin layer and accompanied by other cell wall and outer membrane layers in the Gram-negative members, or a thick layer in the Gram-positives. Peptidoglycan is altogether absent in the archaeal domain. Accordingly, the archaeal cell wall is not lysed by the enzyme lysozyme, and none of the *Archaea* is susceptible to penicillin and other β-lactam antibiotics that inhibit the cross-linking of the polysaccharide chains by peptide chains in peptidoglycan. Another striking difference between the domains is the structure of the membrane lipids. The lipids of the *Bacteria* closely resemble the lipids

Table 3.5
The major differences between the *Archaea* and the *Bacteria* (29)

Property	*Archaea*	*Bacteria*	Comments
Presence of peptidoglycan in the cell wall	Absent	Present in most	Peptidoglycan is absent in the eukaryote world as well
Sensitivity to penicillin and other [β]-lactam antibiotics	Resistant	Mostly sensitive	β-Lactam antibiotics inhibit the cross-linking of the polysaccharide chains during the formation of peptidoglycan, a component absent in the *Archaea*
Nature of the membrane lipids	Diphytanyl (C_{20}) glycerol diethers or dibiphytanyl (C_{40}) diglycerol tetraethers (branched long-chain alcohols – phytanols – ether linked to glycerol)	Glycerol esters of aliphatic fatty acids (generally straight-chain, C_{16} and C_{18}-dominated)	The *Bacteria* have the same types of lipids as the eukaryotic micro- and macroorganisms. This type of lipids is missing altogether in the *Archaea*. Ether-linked fatty acids are only rarely found in the domain *Bacteria*
Antibiotic sensitivity of the protein-synthesizing machinery of the ribosome	Chloramphenicol- and kanamycin-resistant, often anisomycin-sensitive	Chloramphenicol- and kanamycin-sensitive, anisomycin resistant	The eucaryal protein synthesis resembles that of the *Archaea* in the sensitivity to the named antibiotics. However, *Archaea* are not inhibited by cycloheximide, a potent inhibitor of protein synthesis by the eukaryote ribosome

(Continued)

Table 3.5
(Continued)

Property	*Archaea*	*Bacteria*	Comments
First amino acid to initiate a polypeptide chain during protein synthesis	Methionine	*N*-formylmethionine	Methionine
Sensitivity of the ADP-ribosylation of the peptide elongation factor EF-2 to diphtheria toxin	Sensitive	Insensitive	The eukaryotic ribosome is susceptible to inhibition by diphtheria toxin as well
Structure of the promoter for initiation of RNA synthesis	TATA box	−10 and −35 sequences (Pribnow box)	The eukaryotic promoters contain the TATA box, similar to the *Archaea*
Properties of the DNA-dependent RNA polymerases	Multicomponent enzymes, containing 8–12 polypeptides; not inhibited by rifampicin and streptolydigin	$a_2\beta\beta'\sigma$ type; inhibited by rifampicin and streptolydigin	In the eukaryotes, multicomponent enzymes (12–14 polypeptides) are found
Special properties of the tRNAs	The "common arm" of the tRNA usually contains ribothymidine	The "common arm" of the tRNA usually contains pseudouridine or 1-methylpseudouridine	

of the *Eucarya*, with generally straight-chain aliphatic fatty acids bound to glycerol by ester bonds. The *Archaea*, on the other hand, contain lipids in which the chemical bond between the glycerol moieties and the hydrophobic chains is an ether instead of an ester bond. Moreover, the hydrophobic chains are not straight-chain 16- or 18-carbon fatty acids, but isoprenoid branched chains with generally 20 carbons. Many *Archaea*, notably the hyperthermophilic species, have instead of a lipid bilayer a lipid monolayer membrane in which two glycerol moieties are linked by 40-carbon isoprenoid (biphytanyl) chains, providing a highly stable membrane with covalent bonds spanning over its whole width.

There are additional differences between *Archaea* and *Bacteria*, as listed in Table 3.5. Some of these are connected with the protein synthesis machinery, and they result in different sensitivities to antibiotics. Others are located in the mechanism of transcription.

6.3. An Overview of the Bacteria

The classification scheme of the prokaryotes into two domains, *Bacteria* and *Archaea*, as given in the latest edition of Bergey's Manual (5) (see Sect. 7.1) can be conveniently used as a framework to provide an overview of the different groups of prokaryotic organisms (Table 3.5). It must be again stressed more that this classification should not be considered as an "official" classification of the prokaryotes, as such an official classification does not exist (see Sect. 2.1).

Volume 1 of the latest edition of Bergey's Manual (2001) divides the domain *Bacteria* into 23 phyla and 31 classes. Some of these phyla contain as yet a few species only, which have obtained their special status on the basis of their highly divergent 16S rRNA gene sequences. Other phyla contain many hundreds of species – examples are the *Proteobacteria* and the *Actinobacteria*. Some phyla and classes consist of physiologically and/or morphologically similar groups of microorganisms. Well-known examples are the *Thermotogae* and the *Aquificae*, which consist entirely of thermophiles, the *Chlorobi*, which are all anoxygenic phototrophic prokaryotes, the *Spirochaetes*, spiral-shaped cells with a characteristic mode of motility due to the unusual way the flagella are inserted, and the *Cyanobacteria*, which are all oxygenic phototrophs. It cannot be excluded that these phyla may prove more diverse when more representatives of the groups will be isolated in the future. Other phyla and classes are very heterogeneous from the aspect of the physiology of their members. Most of the five classes of the phylum *Proteobacteria* contain obligatory and facultative aerobic, obligatory anaerobic, photoautotrophic, photoheterotrophic, and chemolithotrophic organisms of highly diverse morphology, range of substrates uses as carbon and energy sources, etc. The only reason why these had been brought together in one phylum or class is the similarity in 16S rRNA gene sequence, on which this particular classification scheme is heavily based. Also, the phyla, *Firmicutes* and *Bacteroidetes,* contain species that are differ greatly in their physiological and other phenotypic properties.

Most of the Gram-positive bacteria are clustered in the phylum *Firmicutes*, but this phylum also contains species that show a negative Gram stain reaction, such as the wall-less *Mollicutes* (*Mycoplasma* and relatives). Gram-negative organisms are found all over the phylogenetic tree of the *Bacteria*. The Gram stain is thus of little value to assess the position of a bacterial isolate in the phylogenetically based classification scheme of Bergey's Manual. Possession of

chlorophyll or bacteriochlorophyll derivatives and a phototrophic life style alone is also insufficient to place any isolate in its proper place in the system. Chlorophyll-based photosynthesis has not yet been encountered in the domain *Archaea*; however, the property is widespread in the bacterial domain, no less than five of the phyla contain phototrophs. Chlorophyll *a*-based oxygenic photosynthesis is found in the *Cyanobacteria*, and bacteriochlorophyll-based anoxygenic photosynthesis is found in all known representatives of the *Chlorobi*, in most species of the *Chloroflexi*, in many representatives of the *Proteobacteria* (within the classes "Alphaproteobacteria," the "Betaproteobacteria," and the "Gammaproteobacteria" – classes that all contain very heterogeneous assemblages of species as far as their physiology is concerned), and in a few genera of the family "Heliobacteriaceae" within the phylum *Firmicutes*.

6.4. An Overview of the Archaea

The Bergey's Manual classification scheme (5), the domain *Archaea* is subdivided into two phyla – the *Crenarchaeota* with one class, and the *Euryarchaeota* with seven classes. With the exception of most methanogens (*Euryarchaeota*), all cultured representatives of the domain *Archaea* are extremophiles that inhabit environments with extremely high temperatures (these include many methanogens as well), often combined with growth at low pH, or environments characterized by a high salt concentration, in many cases combined with high pH values. Physiologically, the group is very heterogeneous. One property that is notably absent from the archaeal domain is that of chlorophyll-based photosynthesis, a feature so widespread in the bacterial domain. Use of light energy is, however, possible in some extremely halophilic representatives of the family *Halobacteriaceae* (*Euryarchaeota*), based on light absorption by bacteriorhodopsin with the formation of a transmembrane proton gradient. Until recently, this type of light utilization was even considered unique to the archaeal domain. However, with the discovery of proteorhodopsin – a similar pigment that is found in the membrane of certain (yet to be cultured) representatives of the *Proteobacteria* – this claim can no longer be maintained.

All cultured members of the *Crenarchaeota* (class *Thermoprotei*, orders *Thermoproteales*, *Sulfolobales*, and *Desulfurococcales*) are thermophilic. The group contains aerobes as well as anaerobes. Many representatives obtain their energy by oxidizing hydrogen or organic compounds while using elemental sulfur as terminal electron acceptor in respiration; others reduce sulfur compounds. Some are chemoautotrophs; others require organic carbon sources.

In recent years, it has become clear that the *Crenarchaeota* phylum contains nonextremophilic representatives as well, and these appear to be widespread. Sequencing of 16S rRNA genes isolated from marine bacterioplankton showed that a substantial fraction of the prokaryotic community in the open sea consists of *Archaea*, *Crenarchaeota* as well as *Euryarchaeota* (30). A marine crenarchaeote named ("*Candidatus* Nitrosopumilus maritimus") has recently been isolated; it is an autotrophic ammonia-oxidizing organism. Little can yet be said about the physiology and the function of other groups of Archaea in the marine environment.

In the phylum *Euryarchaeota,* a greater phenotypical diversity is encountered than in the *Crenarchaeota*. Three out of the seven classes presently recognized (the *Methanobacteria*, the *Methanococci*, and the *Methanopyri*) consist of methanogenic anaerobes. The *Halobacteria*

generally have an aerobic life style, and their habitat is restricted to hypersaline environments, typically from 150 g/L salt up to saturation. The three remaining classes (*Thermoplasmata*, *Thermococci*, and *Archaeoglobi*) are all thermophilic, and most live as anaerobic or facultative aerobic heterotrophs. The *Archaeoglobi* are heterotrophs or chemoautotrophs, and perform anaerobic respiration with sulfate or nitrate as terminal electron acceptors. As stated earlier, the open sea contains large communities of *Euryarchaeota* as well. These have not yet been brought into culture, and their mode of metabolism is as yet unknown.

7. SOURCES OF INFORMATION ON PROKARYOTE SYSTEMATICS

Except for the general textbooks of microbiology (29), there are two major sources of valuable information on classification of prokaryotes and on the properties of each group: *Bergey's Manual of Systematic Bacteriology* and *The Prokaryotes*.

7.1. Bergey's Manual of Systematic Bacteriology

Bergey's Manual of Systematic Bacteriology (5) and its predecessor, *Bergey's Manual of Determinative Bacteriology,* have served microbiologists since 1923. Eight editions of *Bergey's Manual of Determinative Bacteriology* have been published between 1923 and 1974. The first edition of *Bergey's Manual of Systematic Bacteriology* was published in four volumes between 1984 and 1990. The first volume of the second edition (see also http://www.bergeys.com), which covers the *Archaea* and the deep branching and phototrophic *Bacteria*, was released in 2001 (5). The second volume, covering the *Proteobacteria* was released in 2005 and the third volume on the *Firmicutes* in 2009; two more volumes are scheduled to follow in the coming years.

Bergey's Manual provides formal descriptions of all prokaryote taxa described to date, including both phenotypic and genetic information – all in accordance with the polyphasic approach outlined in Sect. 3.3. It also supplies much useful information that enables the microbiologist to identify his isolates. The older editions (*Bergey's Manual of Determinative Bacteriology*) provided extensive keys for the identification of bacterial isolates, resembling the dichotomous identification keys found in plant identification manuals. This approach has largely been abandoned in the two editions of *Bergey's Manual of Systematic Bacteriology*. Here, bacterial and archaeal classification is primarily based on 16S rRNA gene sequence comparisons (5, 31). The classification scheme shown in Table 3.5 is based on the latest edition of the manual. Although this classification scheme does not represent an "official" classification of prokaryotes, it has been adopted by the scientific community more or less as a "consensus" framework to classify prokaryotes. Tables for differentiation of the various taxa are included in each chapter to make the information accessible for identification purposes too.

7.2. The Prokaryotes

The Prokaryotes (32) is another extremely valuable resource of information on bacterial diversity and systematics. This handbook is now in its third edition. The first edition (1981) was published in two volumes, the second (1992) in four. The third edition was first published online only, but a printed edition in 7 volumes, encompassing 259 chapters on more than

7,500 pages, was published in 2006. These chapters cover all aspects of the biology of the prokaryotes, provide useful practical suggestions for growing and handling them, and include recipes of growth media for the cultivation of each group. The wealth of information supplied is also very useful as an aid toward identification of new isolates.

8. IDENTIFICATION OF PROKARYOTE ISOLATES

Environmental microbiologists are often faced with the need to identify bacteria isolated from the environment. From the earlier sections in this chapter, it should be apparent that identification of prokaryotes is not a simple procedure. There are no straightforward identification tables such as those existing for the higher plants. The older editions of *Bergey's Manual* indeed contained such dichotomous identification keys, but nowadays such keys are no longer satisfactory. Characterization of species, and therefore identification as well, is now based on the polyphasic approach to taxonomy (13, 20, 33) (see Sect. 3.3). The old identification tables are, however, still of considerable use for specialized groups of bacteria such as pathogens in clinical microbiology and potential pathogens in public health microbiology. Such identification schemes should not be considered as classification schemes, and they serve for practical purposes only (22). In such identification schemes, key phenotypic characteristics should be chosen for testing so that they can be easily determined by most microbiology laboratories. It is important that the identification depend on a pattern of several properties, not merely one or a few characteristics. It is also desirable that the determination of those characteristics chosen for an identification scheme be relatively inexpensive, and that the tests to be performed will give results in a short time.

In most cases, identification of a prokaryote begins at the level of domain, to descend to the level of phyla, classes, orders, and families, to finally narrow down to the level of the genus and the species. Different kinds of information are necessary in each step, as exemplified in Table 3.6. This table shows the place of a single bacterium, *Streptococcus pneumoniae*, in the taxonomic hierarchy, and includes information on the criteria on the basis of which the species can be classified in each of the taxonomic ranks. It may be noted that 16S rRNA gene sequence information is of great value to place an organism within the higher ranks, but less so at the species level.

In accordance with the polyphasic approach (13, 20, 33), there are many properties that should be investigated to obtain a proper identification. These include morphological characters (cell shape and size, the Gram-reaction, cell inclusions, presence and nature of the surface layers, including extracellular capsules), information on motility (presence of flagella, their number and the way they are inserted into the cell, gliding movement), the mode of nutrition (assimilatory metabolism) and energy generation (dissimilatory metabolism), the cells' relationship to molecular oxygen, temperature, pH, tolerance toward and requirement for salt, and many others. Miniaturized tests, such as the BIOLOG® and the API system, are often very useful. Additional tests of value toward the identification of the isolate may be its sensitivity toward different antibiotics, as well as immunological properties. Genotypic information is often essential. Notably, the 16S rRNA gene sequence is a very valuable tool for placing any isolate in the proper place in the classification scheme, at least down to the

Table 3.6
The species *Streptococcus pneumoniae* in the taxonomic hierarchy

Taxonomic division	Name	Properties	Confirmed by
Domain	*Bacteria*	Prokaryotic cell structure; Ribosomal RNA sequences typical of the *Bacteria*	Microscopy; 16S rRNA gene sequence; Presence of peptidoglycan in the cell wall; Lipids containing straight-chain fatty acids bound by ester bonds to glycerol
Phylum	*Firmicutes*	Gram-positive cell wall structure; Ribosomal RNA sequences typical of the *Firmicutes*	Gram stain and analysis of the cell wall structure; 16S rRNA gene sequence
Class	"Bacilli"	Aerobic or anaerobic – aerotolerant life style; Ribosomal RNA sequences typical of the "Bacilli"	16S rRNA gene sequence
Order	"Lactobacillales"	Fermentative metabolism with aerotolerant growth; Ribosomal RNA sequences typical of the "Lactobacillales"	Analysis of products formed during growth under aerobic and anaerobic conditions; 16S rRNA gene sequence
Family	*Streptococcaceae*	Spherical cells in pairs or chains or tetrads; Fermentative metabolism with fastidious nutritional demands; Ribosomal RNA sequences typical of the family *Streptococcaceae*	Microscopy; Analysis of fermentation products; Tests for growth on simple and complex media; 16S rRNA gene sequence
Genus	*Streptococcus*	Cell division in one plane resulting in pairs and chains; Homolactic type of fermentation; Morphological properties; Ribosomal RNA sequences typical of the genus *Streptococcus*	Microscopy; Analysis of fermentation products; Microscopy; 16S rRNA gene sequence; G + C content of DNA
Species	*Streptococcus pneumoniae*	Cells typically in pairs; Distal ends of each pair of cells tend to be pointed or lance-shaped; Cells often surrounded by a polysaccharide capsule; Sodium hippurate not hydrolyzed; Inulin not fermented; Characteristic reaction on blood agar; Inhabits the respiratory tract of man and animals	Microscopy; Fermentation tests on different substrates; DNA–DNA hybridization; Serological properties

family and genus level. For final identification of the species, DNA–DNA hybridization tests are the ultimate tool to decide whether two isolates should be classified in the same species. More specific tests, such as serotyping of phage typing, may be necessary for certain groups of microorganisms to obtain a reliable identification.

It is essential to ensure that pure cultures be used when the above tests are performed, otherwise contamination by other microorganisms makes the results of any of these tests meaningless. Another important rule is that the test methods should be carefully standardized, and known "positive" and "negative" controls should be included, so that the result obtained with the unknown isolate can be compared with the behavior of known organisms. For identification to the species level, it is essential that the unknown be compared with the type strains of the related species (see Sect. 2.1).

9. THE NUMBER OF DIFFERENT SPECIES OF PROKARYOTES IN NATURE

All the information provided in the preceding sections, and all classification and identification schemes for the prokaryotes, including those given in *Bergey's Manual*, are based on those 8,226 species of prokaryotes that have been isolated, characterized, and whose names have been validly published. New species are constantly being discovered. Thus, 593, 631, and 598 new species names have been validated in 2006, 2007, and 2008, respectively.

Nowadays, it is well established that the over 8,000 prokaryote species cultured and described form only a small part of the true number of species extant in nature. It is known for a long time that our cultivation techniques enable growth of only a small fraction of the microorganisms present in any natural sample. Comparison of the number of bacterial colonies that appear on agar plates or the number of bacteria determined with other growth-dependent methods with the number of bacteria that can be observed by direct examination of the sample by microscopic techniques invariably shows that only a small fraction of those organisms present can be cultured. The discrepancy between the viable counts and the total microscopic counts is in many cases a difference of several orders of magnitude. This observation, known as "the great plate count anomaly," shows that our cultivation methods are inappropriate for growing all prokaryotes (8). It also suggests that among those many organisms that do not form colonies on agar plates or cannot be cultured with any other available technique may be many novel species with unknown characteristics. Nature can cultivate all microorganisms, but the microbiologists still have much to learn about the proper methods to bring even the numerically dominant *Bacteria* and *Archaea* into culture. Our isolation and cultivation methods, which to a large extent are based on the procedures introduced by Robert Koch and his coworkers in the 1880s, are obviously not suitable for many prokaryotes.

Introduction of 16S rRNA gene sequencing methods into environmental microbiological studies has confirmed that we know only a small fraction of the number of species of prokaryotes. Moreover, it can now be ascertained that, in most cases, we even do not know the identity of those microorganisms that are numerically dominant in common environments such as soil, seawater, rivers, lakes, etc. Characterization of the microbial community in complex ecosystems nowadays often includes the sequencing of 16S rRNA genes present in DNA extracted from the natural community. In a typical experiment, DNA is isolated from the

biomass present in the sample. The genes encoding 16S rRNA are then amplified by use of the polymerase chain reaction (PCR), either using universal primers (i.e., primers that will enable amplification of all 16S rRNA genes, from all prokaryotes and even eukaryotes, or primers that target specific groups such as the domain *Bacteria*, the class "Betaproteobacteria,") or a certain family or genus within that class. The products obtained are then separated using electrophoretic techniques, purified, and sequenced, often following an additional cloning step to further increase the amount of material and to improve its purity (34). It must be stressed here that there are many potential problems with the methodology that can distort the result of the analysis, and the technology still has many limitations that should be recognized (35).

Comparison of the sequences obtained with the known 16S rRNA gene sequences of the bacterial and archaeal species, as present in the GenBank and in the Ribosomal Data Base (http://rdp.cme.msu.edu, see Sect. 6.1), almost invariably shows that the sequences obtained differ from those of the type strains of the established species. In most cases, the differences are so substantial that the organism that harbors the 16S rRNA gene sequence characterized would deserve classification in a new genus and often in a new order, a new class or sometimes even a new phylum, if only the organism that harbors this 16S rRNA gene could be isolated in culture, characterized, deposited in culture collections, and named in accordance with the rules of the Bacteriological Code. The Ribosomal Database is full of such "environmental 16S rRNA gene sequences" recovered from natural samples that represent yet unknown species. It only seldom occurs that a complete match is found between a small subunit rRNA gene sequence isolated from nature and any of the type strains of the over 8,000 described and named species of prokaryotes. This shows that indeed the numerically dominant organisms in the environment studied are not those species that have been named and are available from culture collections. Based on such studies, it can be estimated that the number of prokaryote species described and named thus far is at most 1–2% of the true number of bacterial species extant, as based on the current species concept. The true fraction may even be much lower than that (34).

A completely different line of evidence that enables an estimate of the true diversity of the microbial communities in water, soil, and other ecosystems comes from measurements of the renaturation kinetics of DNA extracted from the community following thermal denaturation (36). When thermally denatured DNA derived from different organisms is mixed and cooled down, the average time needed for a single DNA strand to find its homolog depends on the frequency in which that homolog occurs within the mixture, and thus on the number of other genomes present. Based on the results of such studies, Dykhuizen (37) calculated that there may be at least 10^9 different bacterial species on Earth that differ from each other sufficiently to meet the species delineation criteria explained in Sect. 2.2. According to his estimations, there may be more than half a million different bacterial species in a single 30 g sample of forest soil.

The extensive database of 16S rRNA gene sequences allows for the design of specific probes that enable the detection of specific groups of prokaryotes in the natural environment. The popular technique of fluorescence in situ hybridization ("FISH") is based on the design of 16S rRNA-targeted probes that are labeled with a fluorescent marker. The cells are fixed so that they become permeable to the probes. After reaction with the probes, the excess nonfixed

probe is washed off, and the samples are then examined in the fluorescence microscope. Probes of different specificity can be designed, from general, domain- or class-specific probes to probes that allow discriminating between members of the same genus or species, provided that the stringency of the washing procedure is sufficient to differentiate between very similar sequences. The technique allows obtaining information on the spatial distribution of different types of microorganisms within complex ecosystems (38, 39).

These culture-independent small-subunit rRNA-based techniques have shown that there are major groups of microorganisms in nature that are present in very large numbers in the most common ecosystems, but of which we do not have a single representative in culture (40, 41). As outlined in Sect. 6.4, the *Archaea* are presently classified in two phyla, the *Euryarchaeota*, which are either obligatory anaerobic methanogens, extreme halophiles, or thermophiles/thermoacidophiles, and the *Crenarchaeota*, all known representatives of which are extreme thermophiles. It is now well documented that the world's oceans contain large amounts of *Archaea* of both phyla. *Archaea*-related 16S rRNA gene sequences belonging to different groups, and different from the sequences of the cultured *Archaea*, are consistently being amplified from DNA extracted from marine picoplankton. Moreover, fluorescent in situ hybridization using *Archaea*- and *Bacteria*-specific probes has shown that about 30% of all prokaryotes in the oceans belong to the archaeal domain (30). The domain *Archaea* thus consists not only of extremophiles and methanogens. However, we have very little information on the physiology of these extremely abundant marine *Archaea*, and only recently has the first representative of marine *Crenarchaeota* been isolated: the ammonia-oxidizing autotroph "*Candidatus* Nitrosopumilus maritimus. More archaeal rRNA gene sequences unrelated to any of the cultured groups have been recovered from other environments. A lineage designated "Korarchaeota" is present in certain hot springs. Based on the 16S rRNA gene sequences, the group is sufficiently different from the *Crenarchaeota* and the *Euryarchaeota* to obtain the status of a new phylum. No members of this group have yet been cultured. Similarly, many groups of *Bacteria*, differing from the cultured ones at the phylum level, have been recognized on the basis of environmental 16S rRNA gene sequences, and are awaiting to be isolated (40).

Unfortunately, the 16S rRNA gene sequence alone does not provide any information on the physiological properties of all these yet uncultured prokaryotes. Isolation and characterization of these abundant microorganisms, which thus far have eluded all attempts toward their cultivation, continues to be a major challenge to the microbiologist who wants to know those prokaryotes that to a large extent determine the properties of the ecosystem studied.

10. CONCLUSIONS

The description of the present status of prokaryote taxonomy and classification, as given in the preceding sections, shows that prokaryote classification is not at all straightforward. First of all, there is no clear species concept for the prokaryotes; all classification systems have to be based on some kind of a consensus of what a prokaryote species is and how to discriminate it from all other species. We have further seen that the number of species of *Archaea* and *Bacteria* together that have been described and whose names have obtained standing in the nomenclature under the Bacteriological Code is relatively small – little over 8,200.

Comparison of the properties of new isolates with these species described in the literature enables their identification as members of a recognized species or, if different from all other species, as members of a new, yet to be described species. A polyphasic approach, including determination of both genetic and phenotypic properties, to compare strains is essential to properly assign them to genera and species within the existing classification schemes.

Even the identification of all species of microorganisms that can be isolated from any ecosystem using the currently available techniques will not provide a reliable picture of the microbial diversity in that ecosystem. The applications of molecular biology techniques, especially those that target 16S rRNAs or the genes encoding them, have unequivocally shown that those microorganisms that have been cultured thus far form only a small fraction of the true prokaryotic diversity in any ecosystem. Generally, the numerically dominant types belong to species that are still waiting to be isolated. Those 8,226 described and named species probably represent no more than 1–2% of the true number of bacterial species, possibly even much less.

Finally, it must be stressed that classification of microorganisms is a dynamic process, and that our views of how the prokaryotes can best be classified are constantly changing with the advancement of our knowledge. As stated before, there is no "official" classification of prokaryotes, and "consensus" classifications such as those given in Table 3.4 are constantly subject to change.

NOMENCLATURE

DNA = Deoxyribonucleic acid
RNA = Ribonucleic acid
rDNA = DNA coding for ribosomal RNA
rRNA = Ribosomal RNA
S = Svedberg unit of sedimentation, equal to 10^{-13} s

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4
Microbial Ecology

Nicolai S. Panikov

CONTENTS

Abstract Ecology is the science that specifically examines the relationship between microorganisms and their biotic and abiotic environment. Like plant, animal and human ecology, the microbial ecology applies the general ecological principles to explain life functions of microorganisms in situ, i.e., directly in their natural environment rather than simulated under artificial laboratory conditions ex situ or in vitro. In this chapter "Microbial Ecology," we will focus on specific aspects of this extensive scientific discipline, which seem to be essential for biotechnological developments. Assuming that the reader is not a professional ecologist, in the first part of the chapter we summarize the major theoretical concepts and "laws" of macroecology needed to understand the language in this esoteric area. The second part deals with the modern instruments and tools of microbial ecology. The final and third part of the chapter surveys the major types of the Earth's ecosystems with special emphasis on quantitative analysis of the diversity of natural environments and microbial inhabitants as well as biotechnological applications associated with the respective natural ecosystems.

Key Words Ecology • microorganisms • natural ecosystems.

1. INTRODUCTION

The word *ecology* was coined by the German zoologist Ernst Haeckel, who applied the term *oekologie* to the "relation of the animal both to its organic as well as its inorganic environment." The word comes from the Greek *oikos*, meaning "household, home, or place

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to live." Thus, ecology deals with the organism and its environment. The word *environment* includes both other organisms and physical surroundings. It involves relationships between individuals within a population and between individuals of different populations.

Ecology draws upon numerous fields, including climatology, oceanography, soil science, chemistry, geology, animal behavior, taxonomy, and mathematics. Ecology is often confused with environmental science. It contributes to the study of environmental problems, but it is a distinct scientific discipline (note that environmental science is broader and combines the power of ecology with many other natural and social sciences for a better understanding and management of the local and global environment).

Some definitions stress the point that ecology, as a part of life science, studies living matter at levels above an organism: populations, communities, ecosystems, and biosphere.

Microbial ecology is the science that specifically examines the relationship between microorganisms and their biotic and abiotic environment. Like plant, animal, and human ecology, microbial ecology applies the general ecological principles to explain life functions of microorganisms in situ, i.e., directly in their natural environment rather than simulated under artificial laboratory conditions ex situ or in vitro. Although the in situ microbial processes are the ultimate goal in the majority of ecological studies, it does not exclude laboratory experiments and mathematical modeling as efficient research tools at intermediate stages aimed at the elucidation of underlying mechanisms and testing hypothesis.

The biotechnological importance of microbial ecology is obvious first of all for the development of *environmental biotechnologies* aimed at in situ activation or release of the beneficial microbial populations such as ice-nucleation bacteria, producers of plant hormones, nitrogen-fixing bacteria, antagonists of soil pathogens, pollutant's degraders etc into the environment. Cleaning of soils and ocean from pollutants, waste water treatment, pest control and many other modern environmental technologies do require understanding of microbial ecology. However, even conventional branches of biotechnology distinct from environmental science can greatly benefit from the close cross-link with microbial ecology. The reasons are that:

- The natural environment is the ongoing source of new microorganisms, which carry novel functions to be exploited in various technological applications. Microbial ecology provides the guiding principles and helps to optimize the search of new organisms with desirable technological qualities.
- The natural microbial community has been evolved for billions of years and shaped by "merciless" natural selection. That is the way in which natural communities could be often considered as optimally designed systems, having remarkably high efficiency and parsimony and therefore desirable for modern biotechnology. The knowledge of nature's optimal design can efficiently help in optimizing the man-made technological systems.
- The natural systems are not only older, but also more complicated, e.g., have higher number of links with other systems. Sometimes, the behavior of such systems can be counter-intuitive with sudden twists and unpredicted sideeffects. From this point of view, microbial and general ecology is a valuable source of instructive examples teaching the art of balance and wisdom in any kind of biotechnological development.

In this chapter, we focus on specific aspects of this extensive scientific discipline, which seem to be essential for biotechnological development. The first part of the chapter summarizes the

major theoretical concepts and "laws" of macroecology needed to understand the language in this esoteric area. The second part deals with the modern instruments and tools of microbial ecology. The final part of the chapter surveys Earth's major ecosystems with a special emphasis on a quantitative analysis of the diversity of natural environments and microbial inhabitants as well as biotechnological applications associated with the respective natural ecosystems.

2. THE MAJOR TERMS, PRINCIPLES, AND CONCEPTS OF GENERAL AND MICROBIAL ECOLOGY

Most ecological principles and "laws" do not belong to the category of experimentally confirmed facts or mathematically derived statements, as is the case in physics, chemistry, or molecular biology. Rather, they are reasonable assumptions or empirical generalization based on numerous observations on how plants and animals establish themselves in various natural environments. No doubt, general ecology was developed mainly from the studies of higher forms of life, the microbial world being mostly neglected. The major advantage of macro- vs. microorganisms in ecological studies stems from the fact that plant and animal communities are much better visualized, enumerated, and identified, and with greater precision and less cost. The "golden age" in microbial taxonomy has started only recently because of the remarkably quick progress in molecular biology and molecular ecology. In the last decade, we have found a way to bring an order to bacterial taxonomy and develop reliable methods of assessing microbial diversity on the basis of phospholipid analysis and nucleic acids sequencing. On the other hand, the great advantages of microbial ecology over ecology of macroorganisms are: (a) essentially deeper understanding of molecular, chemical, and physical mechanisms behind life functions in situ, (b) much quicker development of microbial communities (e.g., days and months vs. years and centuries for plants communities), and (c) wider possibilities for experimental simulation, and testing of theoretical hypotheses. Therefore, in microbial ecology we are closer to realizing the full understanding, prediction, and control of the natural systems on the basis of solid quantitative knowledge rather than wealth of practical/empirical experience. Probably in the nearest future, the conceptual framework of general ecology will be experimentally tested and improved on the basis of studies of microbial populations in situ and their interactions with macroorganisms. In the following line, we give a short summary of the current concepts in general ecology and introduce the reader to the specific language in this area which often looks deceptively simple.

2.1. From Molecule to Biosphere: The Hierarchy of Organizational Levels in Biology

Figure 4.1 shows separate hierarchies for higher forms of life (plants-animals) and for microorganisms. The complexity and multitude of internal links increases in the following order: molecules < macromolecular complexes < cell organelles < cell < tissue < whole organism < population < community < ecosystem < biosphere. Ecology focuses only on the top levels, starting from *organism* and *population* level up to *ecosystem* and *biosphere* levels. Note that although the majority of microbes (bacteria, archaea and yeasts) formally belong to the category of *unicellular organisms,* the functional analog of macroorganism is

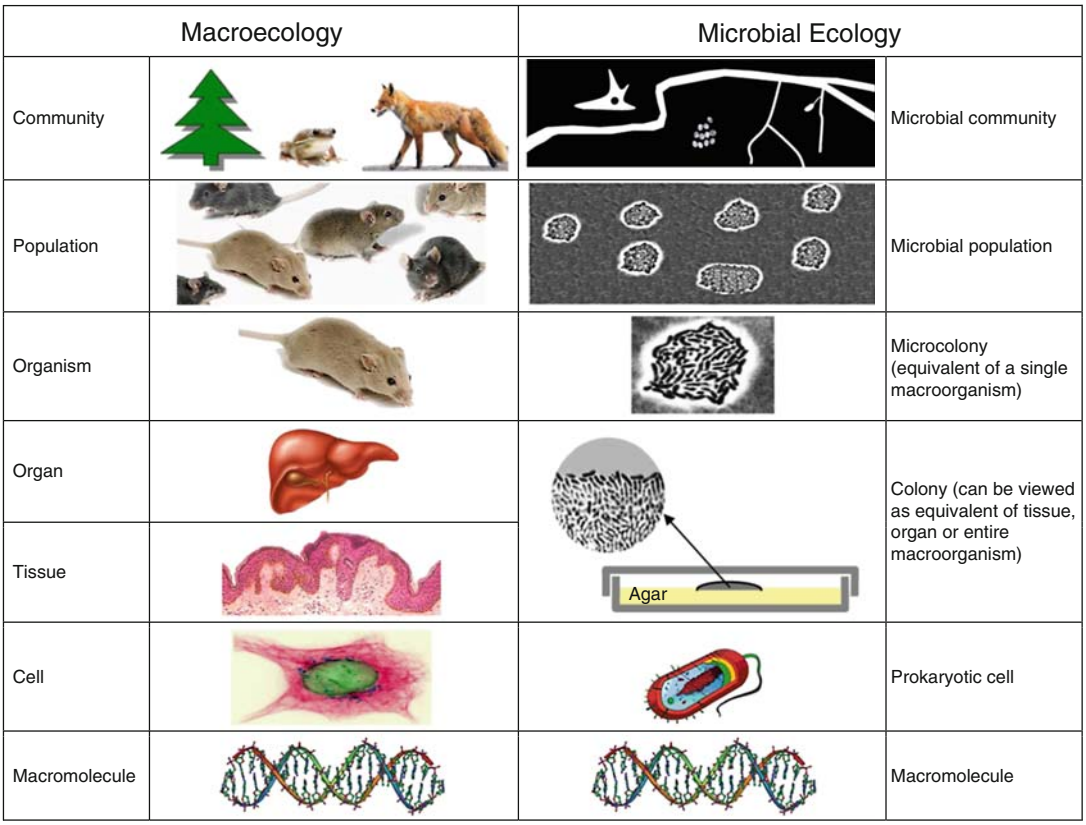


Fig. 4.1. Levels of biological organization. The ecosystem level incorporates the interactions among organisms and their abiotic environment. The left column shows the conventional definitions accepted in general ecology (1), right column is modified to include microbial components.

not a single cell, but microbial colony, flock, biofilm and other cell congeries. In spite of morphological simplicity and uniformity, the bacterial cells within a colony are differentiated in a way similar to the cells and tissue of plants and animals (2). The morphologically differentiated microbial prokaryotes and eukaryotes, such as *Mixococcus* and *Dictyostelium* as well as numerous spore- and rhizome forming fungi, produce structures similar to tissues of plants and animals and are called *pseudotissues*. Finally, prokaryotes have signal metabolites resembling primitive endocrine system of animals: some cells in the bacterial population produce hormone-like compounds which are delivered to other members of cell population

and "order" them to turn on or off several essential life functions: dimorphic transition "cells-mycelium," attachment to or detachment from solid surface, biofilm formation, transition to virulent state or sporulation. Thus, modern molecular data indicate that unicellular organisms are not as primitive as we believed 10–20 years ago. The hierarchy structure for microbial prokaryotes and eukaryotes should be appended with "tissue" and "multicellular organism" levels similar (although not identical) to plants and animals.

The term *population* refers to a group of individual organisms that belong to one species or one functional type and occurs in a specified habitat. In microbial ecology, we can speak of, for example, the population of *Arthrobacter globiformis* in tundra soil or populations of free-living and symbiotrophic N_2-fixing prokaryotes in the soil under a clover field. There could be many other categories of microbial populations, taxonomically homogeneous or mixed, but combined by identical physiological function: denitrifying, nitrifying, photosynthetic, methanogenic, sulfate-reducing, H_2-oxidizing, PCB-degrading microorganisms, etc.

Community (sometimes called *biotic community*) includes all populations occupying the given habitat. As a rule we speak of *microbial community* occupying sediment, lake or soil which includes all the diverse microbial world of specified habitat. However, the full term *community* includes all biotic components: microorganisms, plants and animals which are found within the boundary of the habitat and interact with each other in various degrees (see discussion below). The community interacts also with *abiotic* environment; they tightly couple together to form the *ecosystem:*

$$\text{Ecosystem} = \text{Biotic community} + \text{Abiotic environment}$$

Many European and especially Russian ecologists use the terms "biogeocenosis" and *beocenosis* instead of : "ecosystem" and "community" respectively. Although there are some subtle differences in the content of these terms, it is advisable to take them as full equivalents and use the terms ecosystem/community as a preferential and shorter option. All terrestrial and aquatic (freshwater and marine) ecosystems are combined into a *biosphere* or *ecosphere*, which includes all organisms on Earth interacting with abiotic components supporting life.

None of the known ecosystem is devoid of the microbial component. At the same time, some ecosystems are fully microbial: hyperthermal, ultra cold (permafrost), hypersaline and other ecosystems of the so-called extreme type, which is discussed below.

2.2. The Ecosystem Concept

The ecosystem concept, introduced by Arthur Tansley in 1935, is central to modern ecology; it provides a framework for understanding the flows of energy and elements between organisms and their abiotic surroundings. The concept of *food chains* (introduced in the 1920s by Charles Elton) specifies the direction of energy flows between several trophic levels (Fig. 4.2).

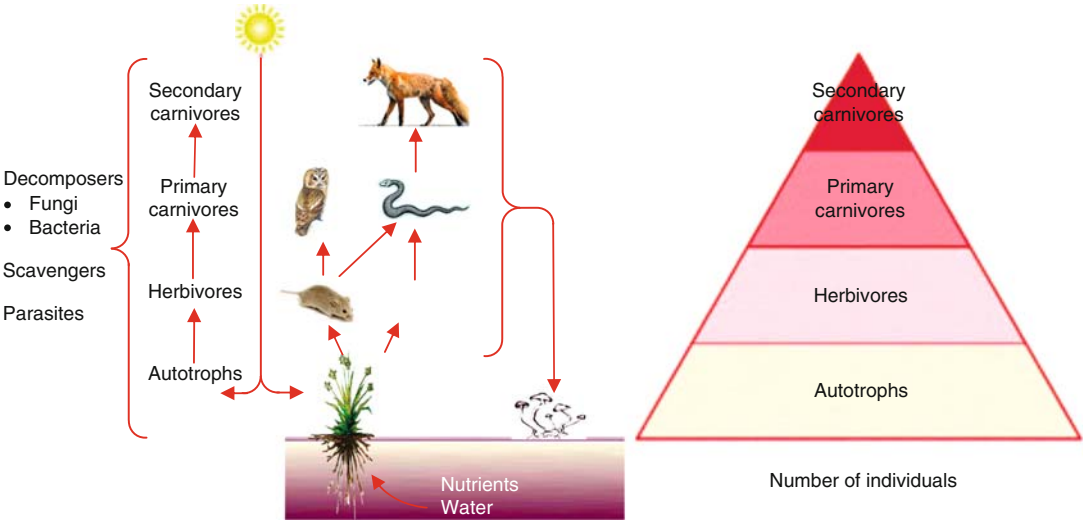


Fig. 4.2. (**a**) A generalized diagram of an ecosystem showing trophic interactions. (**b**) Charles Elton's pyramid of numbers. The number of individuals in each trophic level is represented by the size of the bar. Both of Elton's findings are evident in this figure: The number of individuals decreases moving up the food chain, and food chains are rarely longer than four to five levels. With permission from Wiley, Nature Encyclopedia of Life Sciences.

All organisms are grouped into several discrete categories:

1. *Producers*, the autotrophic organisms (photosynthetic plants as well as photo- and chemosynthetic bacteria) constructing their bodies from CO_2 and other inorganic compounds. These organisms form the base of the food chain.
2. *Herbivores* are animals that consume plants.
3. *Primary carnivores* are meat-eating animals that consume herbivores.
4. S*econdary carnivores* that consume other animals (in some ecosystems we can find also *tertiary carnivores* feeding on the secondary ones).
5. *Decomposers*. The majority of microorganisms (bacteria, archaea, and fungi) as well as small animals utilize the dead organic matter (plant litter and animals residues) as a source of energy and building blocks for their bodies. As a result of decomposition, they release (mobilize) inorganic elements from dead bodies and make them available for plants to keep the primary production going.

Groups 2–5 are also called *heterotrophs;* contrary to *autotrophs* they require organic compounds as nutrients. Herbivores and carnivores belong to the *consumers* category *(holozoic* type of nutrition characteristic for all animals using jaws and tooth or equivalents for intake of food), while in Group 5, decomposers are organisms with *osmotrophic* type of nutrition (transporting soluble nutrients through cellular membrane). Insoluble substrates (e.g., lignocellulose and other insoluble organic matter, oil and sulfur droplets, etc) should be converted to soluble forms with extracellular enzymes, surfactants or chelating agents. We can subdivide organisms also as *biophages* (eating other living organisms) and *saprophages* or *detritophages* (consuming dead organic matter). Microbial biophages include (a) parasites (*Bdellovibrio*)

which invade host cells and multiply inside causing cell lysis, (b) predators attacking other cell with extracellular lytic enzymes (mixobacteria, nematode-trapping fungi), and (c) symbiotic heterotrophic microorganisms closely associated with autotrophic macroscopic partners (mycorrhiza, rhizobia, mycobiont in lichen, etc). The majority of soil and aquatic microbes belong to the category of saprophages or saprotrophs using dead organic matter as a source of nutrient and energy.

Generally, food chains are rarely longer than four to five trophic levels, and lower trophic levels contain more individuals (higher number of species and bigger biomass) than higher trophic levels. The latter pattern came to be known as Elton's "pyramid of numbers" (Fig. 4.2). The progressive reduction in the size of each trophic level is explained by the fact that only approximately 10% of the total energy in a trophic level is passed along to the next trophic level, with the rest being lost as indigestible material and heat from metabolic respiration. Purely microbial food chain is generally more efficient, e.g., grazing of bacterial prey by protozoa can be characterized by conversion of at least 20–40% of consumed bacterial mass into cell mass of protozoa. An even higher efficiency of conversion is observed for decomposers growing on easily available organic substrates.

2.2.1. Food Chain and Metabolic Network

Microbial populations either in situ or ex situ (in laboratory culture) produce a significant amount of extracellular metabolites. In natural habitats, these compounds form a pool of C-compounds which encourage both competition for common substrates and cooperation through the so-called *metabiotic interactions*, in which the product of one species is utilized by other species. Several simple compounds often participate in such *interspecific exchange* of mass and energy that are called *central metabolites* or *centrobolite*. Examples include molecular hydrogen, acetate, methane, etc. For instance, H_2 is produced by cyanobacteria and by microbes with active nitrogenase as well as by fermenting bacteria and fungi; it is consumed by methanogens, acetogens, sulfate-reducers and aerobic H_2-oxidizing bacteria. Removal of H_2 by methanogens is essential to sustain anaerobic degradation of plant residues; otherwise, equilibrium is shifted toward the formation of toxic fatty acids:

$$CH_3CH_2COOH + H_2O \overset{\text{Syntrophic bacteria}}{\longleftrightarrow} CH_3COOH + CO_2 + H_2 \uparrow$$

Interestingly, the functional group of *synthrophic bacteria* can catalyze this reaction in both directions depending on the activity of complementing microbial population, e.g., methanogens or acetogens (the synthrophy stands for the cross-feeding that occurs when two organisms mutually complement each other in terms of nutritional factors or catabolic enzymes related to substrate utilization).

The metabolic network (see example in Fig. 4.3) and food chain have one common feature: both provide flows of energy and matter between organisms and abiotic environment. The difference is that metabolic interspecific exchange occurs within the same trophic level of osmotrophic organisms, while the food chain or food web (the highly branched chain) assumes the flow of energy between different trophic levels. The efficiency of energy conversion by osmotrophic organisms is analyzed by a scientific discipline called growth stoichiometry.

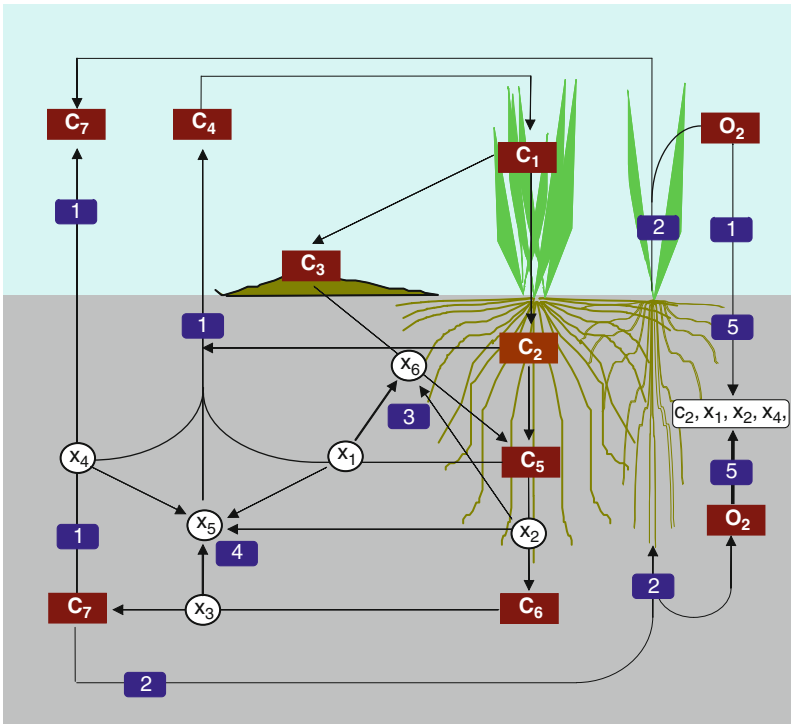


Fig. 4.3. Example of metabolic network functioning in submerged soils and wetlands (3). *Carbon*-reservoirs**:** c_1, green phytomass, c_2, below-ground phytomass (roots and rhizoid), c_3, plant litter, c_4, CO_2, c_5, low molecular weight C-compounds, c_6, volatile fatty acids, c_7, CH_4. *Biocatalysts:* x_1, aerobic soil microorganisms, x_2, fermenting microorganisms, x_3, methanogens, x_4, methanotrophs, x_5, protozoa (microscopic animals feeding on microbial cells), x_6, hydrolytic enzymes. The *arrows* indicate the following basic processes: (*Plant*-mediated) $c_4 \rightarrow c_1$, plant photosynthesis, $c_1 \rightarrow c_2$, transport of C-compounds (photosynthates) from leaves to roots, $c_1 \rightarrow c_3$, plant litter formation, $c_2 \rightarrow c_5$, root exudation, $c_2 \rightarrow c_4$, root respiration. (*Microbial*) $c_3 \rightarrow c_5$, depolymerization of plant litter, $c_5 \rightarrow c_6$, fermentation (anaerobic conversion of sugars to acetate and other volatile compounds), $c_6 \rightarrow c_7$, CH_4 formation, $c_5 \rightarrow c_4$, total microbial respiration, $c_7 \rightarrow c_4$, CH_4 consumption/oxidation. (*General*) 1 gas molecular diffusion, 2 gas vascular transport, 3 biosynthesis of hydrolytic enzymes, 4 protozoan grazing, 5 oxygen uptake for respiration.

2.2.2. The Basics of Microbial Stoichiometry

Two groups of chemical species serve as substrates for microbial growth both in situ and ex situ: (a) *catabolic substrates,* which are sources of energy, and (b) *anabolic or conserved substrates,* which are sources of biogenic elements forming cellular material. Examples of catabolic substrates are H_2 for lithotrophic hydrogen bacteria, NH_4^+ and NO_2^- for nitrifying bacteria, oxidizable or fermentable organic substances for heterotrophic species, etc. Their consumption is accompanied by oxidation and dissipation of chemical substances into waste products which are no longer reusable as an energy source (H_2O, NO_3^-, SO_4^{2-}, CO_2, etc.).

Fermentation products (acetate, ethanol, butyrate, H_2 etc.) seem to be the exception as they do contain reusable oxidation potential, but reutilization can take place only by other organisms or after dramatic changes in environmental conditions, e.g., after transition from anaerobic conditions supervising fermentation to aerobic conditions switching to respiratory catabolism.

The anabolic substrates after uptake are incorporated into de novo synthesized cell components, which are conserved in biomass (that is why they are called sometimes *conserved substrates*). Unlike catabolic substrates, they can be reabsorbed after excretion or cell lysis. The conserved substrates include nearly all the noncarbon sources of biogenic elements (N, P, K, Mg, Fe, and trace elements), CO_2 for autotrophs, as well as the indispensable amino acids and growth factors.

Historically, microbial ecologists dealing with the marine environment focused mainly on conserved substrates that seem to limit growth of phytoplankton (Fe, Co, P, vitamin B12), while terrestrial studies focused on energy sources (available organic compounds in soil solution, CH_4, NH_4^+, etc).

The stoichiometric parameters *growth yield* is defined as:

$$Y = -\mathrm{d}s/\mathrm{d}t \sim -\Delta x/\Delta s \tag{1}$$

Where, Δx is the increase in microbial biomass consequent on utilization of the amount Δs of substrate. Dividing both parts of Eq. (1) by $x\mathrm{d}t$, gives the relationship between growth rate and substrate consumption:

$$Y = -\frac{\mathrm{d}x}{\mathrm{d}s} = \frac{\mathrm{d}x}{x\mathrm{d}t} : \frac{\mathrm{d}s}{x\mathrm{d}t} = -\frac{\mu}{q} \tag{2}$$

where μ is specific growth rate and q is specific rate of substrate consumption.

The reason for Y variation is different for catabolic and anabolic substrates. In the case of energy sources, some fraction of the total substrate flux is diverted from growth per se to meet the so-called *maintenance functions* including:

- Resynthesis of self-degrading cell proteins, nucleic acids, and other macromolecules
- Osmotic work to keep the concentration gradient between cell interior and environment
- Cell motility

$$\begin{array}{c} \text{total energy source uptake} = \text{consumption for growth} + \text{consumption for maintenance} \\ q \qquad\qquad\qquad\qquad \mu/Y^{\max} \qquad\qquad\qquad\qquad m \end{array} \tag{3}$$

where m is the maintenance coefficient, the specific rate of catabolic substrate consumption by non-growing cells (i.e., $m = q$ when $\mu = 0$).

With some rare exceptions (fungal exospores and bacterial cysts), microbial cells are not stable at $\mu = 0$ and either grow ($\mu > 0$) or lyse ($\mu < 0$). Therefore, the maintenance coefficient is found by linear extrapolation of a series of $q(\mu)$-measurements to the point where μ is zero. Under chronic starvation, the maintenance coefficient m decreases as compared with intensive growth; as a result, when $\mu \to 0$, the growth yield Y tends to some low limit $Y^{\min} > 0$ rather than to zero.

There is also *wasteful oxidation* of substrate under at least three specific circumstances: (a) when growth is nutrient-limited and energy-sufficient, (b) when starving cells are brought to rich nutrient medium (famine-to-feast transition) and (c) under effect of some uncoupling inhibitors. In all listed cases, the cell catabolic machinery produces more energy that can be used for ATP generation. Such wasteful catabolism frequently occurs in natural environment under transition from one trophic regime to another (e.g., spring bloom after winter starvation) as well as ex situ when ecologists try to cultivate natural microbial populations on rich artificial media (famine-to-feast transition occurring with conventional plating). The wasteful catabolism should be differentiated from the maintenance per se.

Cell yield on anabolic substrates varies mainly as a result of alterations in biomass chemical composition expressed by parameter σ_s, the intracellular content of deficient element or *cell quota*. The variation in N content in bacteria from 5 to 15% gives the σ_s diapason 0.05–0.15 g N (g cell mass)$^{-1}$. For most known cases, the quota σ_s increases parallel to growth acceleration because the higher growth rate requires higher *intracellular content* of proteins and RNA (contain N, P, S) as well as K^+, Mg^{2+} and vitamins participating in all primary metabolic reactions. The yield and cell quota are inversely related to each other, e.g., the low N-content $\sigma_N = 0.05$ g N/g corresponds to the high cell yield $Y_N = 1/\sigma_N = 20$ g cell/g N utilized. The high N-content in rapidly growing cells can be attained only with low cell yield $Y_N = 1/0.15 = 6.67$ g cell/g N.

2.2.3. Microbial Loop

The concept of a microbial loop was first introduced in marine ecology (4). In essence, it postulates that part of the primary production reaches grazers as soluble organic matter (SOM) instead of being channeled directly to them. The concentration of SOM is very low and only bacteria are able to absorb SOM for their growth. Finally, the particulate bacterial cell mass which is essentially more concentrated food than SOM is grazed by protozoa and other animals. Similar microbial loop functions in terrestrial habitats (Fig. 4.4): plants produce not only phytomass per se, but also significant amount of root and shoot exudates (at least up to 30% of gross photosynthesis) providing C-source for microbes in *rhizosphere* and *phyllosphere* respectively (see below Sect. 3). The microbial loop in soil and water greatly accelerates the cycling of carbon and other elements, mainly due to the fact that exudation products of plants and other phototrophic organisms are much more available than dead organic matter in marine or terrestrial detritus.

Usually in general ecology, the autotrophic and heterotrophic processes are considered spatially separated, and food chains are believed to vary between two extremes called *pastoral* and *detrital* food chains: in the pastoral type, plants are directly consumed/grazed by phytophages, while in the second type, there is significant accumulation of dead organic matter (detritus). The microbial loop uniformly and widely spread across most of known ecosystems should form the third type of food chain.

2.2.4. Homeostasis

Ecosystems possess the remarkable ability for *homeostatic* self-regulation; they are able to resist perturbations and preserve stability in a changeable environment. The homeostatic

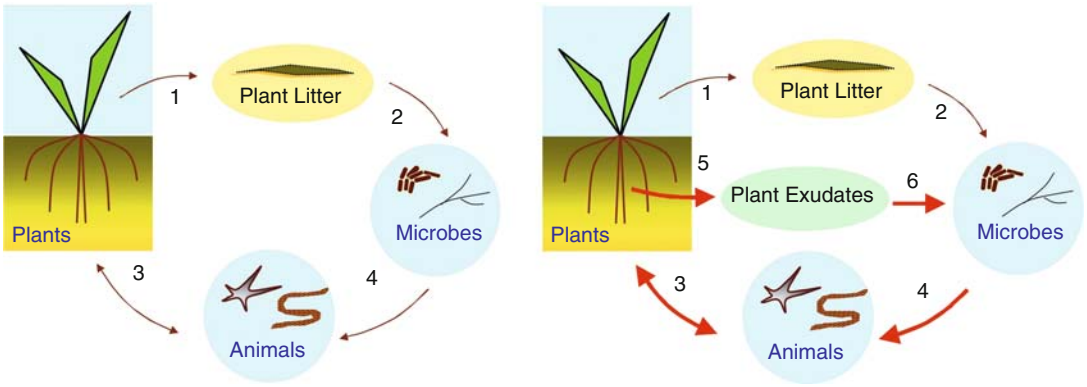


Fig. 4.4. Simplified illustration of microbial loop concept as applied to soil community. Left: soil C-cycle without microbial loop. Right: C-cycle with microbial loop initiated by root exudation (red arros).

mechanisms include various *negative feedbacks* that result when a perturbation induces a response from a biotic component of the ecosystem, decreasing the size of perturbation. The *positive feedbacks* generally play a destabilizing role (although they are needed for development of organisms). One example of destabilizing positive feedbacks is the greenhouse effect exerted by radiative gases CO_2 and CH_4: their accumulation in atmosphere causes warming, while soil warming activates more production than consumption of these gases via methanogenesis and aerobic decomposition of dead organic matter.

2.2.5. Ecosystem Productivity

The primary productivity of ecosystem shows the rate of photosynthetic production, the conversion of the solar energy into phytomass. Gross primary production (GPP) is the sum of net primary production (NPP) and plant respiration (R), which is the reverse process of photosynthesis, the oxidation of phytomass to CO_2. The secondary productivity (SP) of ecosystem is the rate of biomass formation by heterotrophic components of ecosystem, consumers and decomposers. All terms of ecosystem's energy balance are *rates*, and should not be confused with instant biomass of producers, consumers and decomposers which is characterized as a *standing crop*. If we draw an analogy with terms of chemical and microbiological kinetics, then we will see that a standing crop is equivalent to concentration (current or instant concentration) of microbial cell mass (x), e.g., mg cell/L or g cell mass/m^2. The secondary microbial productivity is equivalent to microbial growth rate, which is a product of $\mu \cdot x$ of the true specific growth rate μ [see Eq. (2)] and cell mass x with dimension g cell mass/day/m^2. Finally, the seasonal production Δx is integral:

$$\Delta x = \int_0^{150} \mu(t)x(t)\mathrm{d}t$$

where 150 is a typical mid-latitude duration of season in days; note that both μ and x are time dependent variables. It is very important to distinguish true (μ) and apparent (μ_{app}) growth rates:

$$\mu_{app} = \mu - a$$

where the term "a" is an integral measure of elimination (washout, grazing, lysis, etc). If we observe the dynamics of $x(t)$, then time-derivative dx/dt gives us only an apparent value of the growth rate, the true value being hidden by cell mass elimination (see sections below for a review of experimental approaches to assess the true growth rate of microbial populations in situ).

2.3. *Environmental Factors*

In this section, we will only touch on the effects of environmental factors on natural microbial populations. Interested readers can find detailed descriptions of specific factors (temperature, pressure, nutrient concentration, pH, tonicity, radiation, toxic compound and inhibitors, aeration etc) in comprehensive survey and books on microbial ecology (5–7); here, we will consider only the most general approaches.

2.3.1. Liebig's "Law of Minimum"

Justus von Liebig in 1842 came to the conclusion that the growth of crop plants was held in check by the most limiting mineral nutrient. Later, Cambridge botanist Blackman (8) gave a mathematical formulation to this law:

$$\mu = \min\{k_i s_i\} \tag{4}$$

where $s_1, s_2, \ldots, s_n$ are quantitative expressions for various environmental factors affecting growth of plants or other organisms and k_i is respective first order kinetic constant. Therefore, only one factor from many potential environmental variables happens to be limiting and controls the activity and growth of given population. For example, phytoplankton in the ocean are most likely to be controlled by availability of Fe (9), while heterotrophic bacteria in most of aquatic and terrestrial habitats are tended to be limited by organic substrates.

In precise laboratory experiments with chemostat (Fig. 4.5), Liebig's Law of Minimum was shown to stop working in the domain of so-called dual or multiple growth limitations where not one, but several factors (e.g., two nutrients) simultaneously affect activity of population. Thus, Liebig's law is no more than an approximation to the reality if we neglect the interaction between several nutritional factors. Another common failure of Liebig's law is observed when community is not stable but moving from one steady state to another; in this case, the effects exerted by various environmental (external) and metabolic (internal) factors can transiently change in a rather complicated way, which does not fit into a simplistic Liebig formula. For example, a transient process can start from microbial population limited with C-source by an abrupt increase in its availability; the next most probable bottleneck should be intracellular concentration of ribosomal particles (the biggest metabolic inertia) and after growth acceleration, the availability of oxygen can be the most probable limiting factor in the case of aerobic population. Finally, one should remember that Liebig's law is applicable only

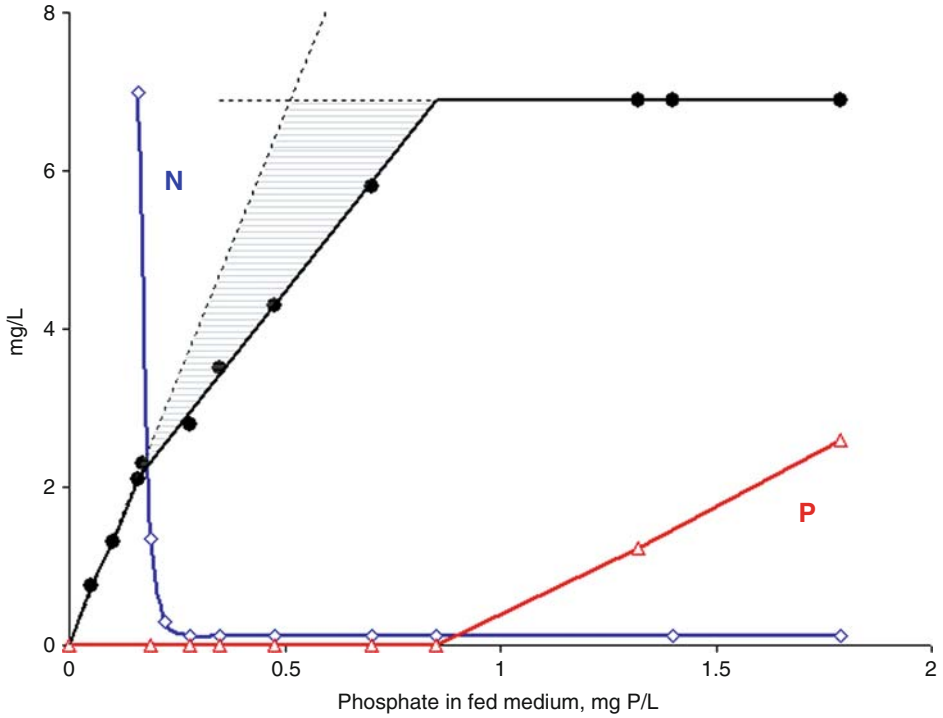


Fig. 4.5. Violation of the Liebig's Law: control of microbial growth by two factors simultaneously (10).

to such environmental factors which belong to the category of resources (e.g., concentration of nutrients or dissolved O_2, water content, etc), while other factors characterizing the state of environment (temperature, pH, Eh, soil texture, etc) do not follow this law and are to be considered within the "tolerance" law, which is discussed below.

2.3.2. Shelford's Tolerance "Law"

The lack of possibility to exist and flourish in natural environment for a particular species is determined by both deficiency and excess in the expression of any environmental factors. This law is much more universal and can be applied to practically all abiotic factors: nutrients (at high concentration any nutrient can be toxic), temperature, pH, light, etc. In each case, the effect of environmental factor on life function appears as bell-shape curve (it can be symmetrical or asymmetrical) between ecological minimum and maximum. Several factors can interact, shifting the tolerance range to either direction, for example, with an ample supply of nutrients, microbes can remain active under colder and hotter climates than starving populations. On the other hand, starving nongrowing and half-dormant microorganisms display better survival capability as compared with actively growing cells.

Several conclusions derived from Shelford's law are as follows:

1. Organisms can have wide tolerance to one factor and narrow one for another factor.
2. Organisms with wider tolerance to many factors are generally ubiquitous.

3. Under unfavorable conditions in respect to one environmental factor, the tolerance to other factors also can be significantly reduced.
4. Under natural conditions, most organisms occur far from the environmental optimum found in laboratory or field experiments due to competition with other populations.

The tolerance range for microbial populations can be determined by two major approaches: (a) varying the factor intensity in laboratory or field experiments and follow the respective response of a studied population (growth rate, metabolic activity); and (b) long-term observation of population abundance in situ with simultaneous recording of environmental factor in question with subsequent use of statistical (e.g., linear or nonlinear regression) analysis).

Both approaches are subject to errors due to: competition with other populations (decline in response can be caused by competitive exclusion rather than inadequacy of environment), effects of other environmental factors (error especially high with second approach), restricted size of population in question (in laboratory experiments we can use isolates with a lower tolerance range as compared with community in situ).

Ecotone is a transitional zone between two communities containing the characteristic species of each, e.g., tundra-forest, meadow-forest, or soft–hard ground transition in marine ecosystems. There is a trend to increase the populational density and species diversity at ecotones, this phenomenon is called the *border effect.*

The gradient of environmental (ecotopic) factors is often observed in nature as progressive continuous changes from one level of pH, light intensity, salinity, dissolved oxygen, redox potential, nutrient content, temperature, and other characteristics. Various organisms having different tolerance limits occupy their own unique position along the gradient minimizing competition for life resources (Fig. 4.6). The ecological minimum, L defines the low boundary of habitat colonization below which life is no longer supported (we will use this notion below to describe specialized life strategy of extremophiles).

2.4. Population Dynamics, Succession and Life Strategy Concept

In this section, we will summarize studies on dynamics and evolution of (microbial) ecosystems. The major challenge of such research is to attain such a level of understanding of the particular ecosystem which allows us predict its dynamics including species abundance (population dynamics) and the replacement of one set of populations by another (succession).

2.4.1. Population Dynamics and Fluctuations

Usually, population density is expressed as a number of organisms per unit area or per unit volume of habitat (N). The rate of changes in N is determined by the relationship between birth rate (r) and mortality rate (a), which is described by the empirical logistic equation:

$$\mathrm{d}N / \mathrm{d}t = rN - aN^2 = rN(1 - N/K), \quad K = r/a \tag{5}$$

If growth is started at some low values of $N << K$, then growth is almost exponential ($\mathrm{d}N/\mathrm{d}t \sim rN$). Afterward, the growth rate progressively declines because the birth is proportional to N, while mortality is proportional to N^2. As soon as the term rN is larger than aN^2, the derivative $\mathrm{d}N/\mathrm{d}t > 0$ and population grows, approaching the upper asymptotic value K, called the carrying capacity of respective ecosystem. The logistic equation is fully

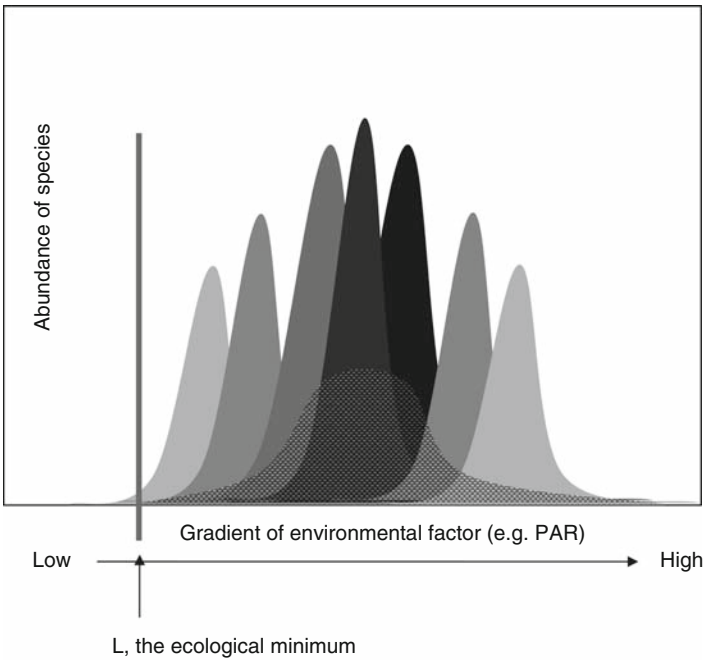


Fig. 4.6. Species continuum across environmental gradient.

empirical, but has a surprisingly wide area of application for the numerous observation data on population/community transient dynamics. Typically, these data are the time series of population dynamics after some kind of perturbation of the natural steady state ecosystem, e.g., forest fire, volcano eruption, soil tillage or fumigation, irrigation, drainage, etc. (see below section on succession). In all known cases, we have one common phenomenon, the temporal relief from competition between various populations for common limiting substrate and temporal excess of free nutrient reserves which allows the population to grow with the rate close to r-value of the logistic equation. As soon as the population density approaches the carrying capacity K, the environmental space is getting fully occupied with organisms, competition increases.

Population at a density of about K as a rule displays *fluctuations* and *cyclic oscillations*. It is important to distinguish (a) seasonal fluctuations which are controlled mainly by environmental factors such as temperature, radiation and precipitation, and (b) changes which have both longer and shorter than one year characteristic time and generally are related to some internal controlling factor at genetic or phenotypic levels. A classical example of the latter cyclic oscillations is 9–10 years of oscillations in populations of lynx and white hare in Hudson Bay (11) or 5–7 days of oscillations in numbers of soil bacteria and microbial activity (12). It is not known for certain what is the main inducer of the observed oscillations: genetic program, cosmic factors such as periodic changes in the nature of solar radiation, or mobile signal metabolites (H_2, ethylene oxide) playing the role of "community hormone."

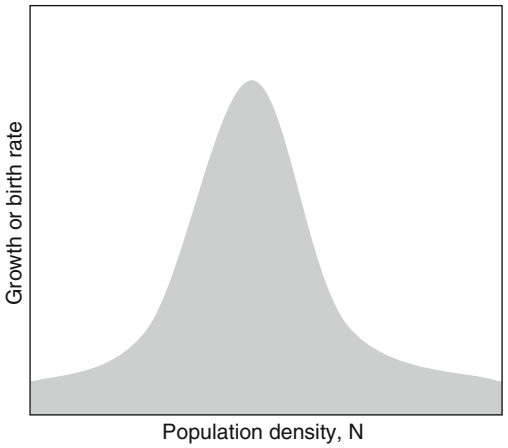


Fig. 4.7. Illustration of the Alle-principle.

Less mysterious is the so-called "Alle principle," which states that overcrowding of environment as well as a too low density tends to restrict population growth: the plot of growth rate versus N is usually bell-shaped with a maximum at "optimal" density of individual organisms (Fig. 4.7). That is why sparse populations resist being evenly distributed and instead aggregate into colonies of various sizes and shapes. The molecular mechanisms of both positive and negative interactions between individual organisms within single population are combined now under general term "quorum sensing."

Bacteria and other unicellular organisms show group behavior: for example, in living biofilms, individual cells at different locations in the biofilm may have different activities. The molecular mechanism of quorum sensing is used to monitor the bacterial population density. This process relies on the production of a low-molecular-mass signal molecule (often called "autoinducer" or recently quormon), the extracellular concentration of which is related to the population density of the producing organism. Cells can sense the signal molecule allowing the whole population to initiate a concerted action once a critical concentration (corresponding to a particular population density) has been reached. Gram-negative and gram-positive bacteria use different signal molecules to measure their population density (Fig. 4.8). Gram-negative bacteria have the cell–cell communication based on *N*-acyl-homoserine lactone (AHL) signals. The first example and the paradigm of gram-negative quorum signaling is the luxI–luxR quorum sensing system of *Vibrio fischeri*, involved in population density-dependent regulation of bioluminescence. *V. fischeri* is a free-living marine bacterium that also occupies the light organ of the squid *Euprymna scolopes*. The high population density required for bioluminescence is reached only in the microenvironment of the light organ.

The AHL signaling system of *V. fischeri* involves two major components: luxI is the AHL synthase gene that is part of the bioluminescence operon luxICDABEG and luxR codes for the transcriptional activator. At low population density, the transcription of luxICDABEG is weak. The AHL quorum sensing signal molecule produced by LuxI at a basal level, 3O,C6-HSL

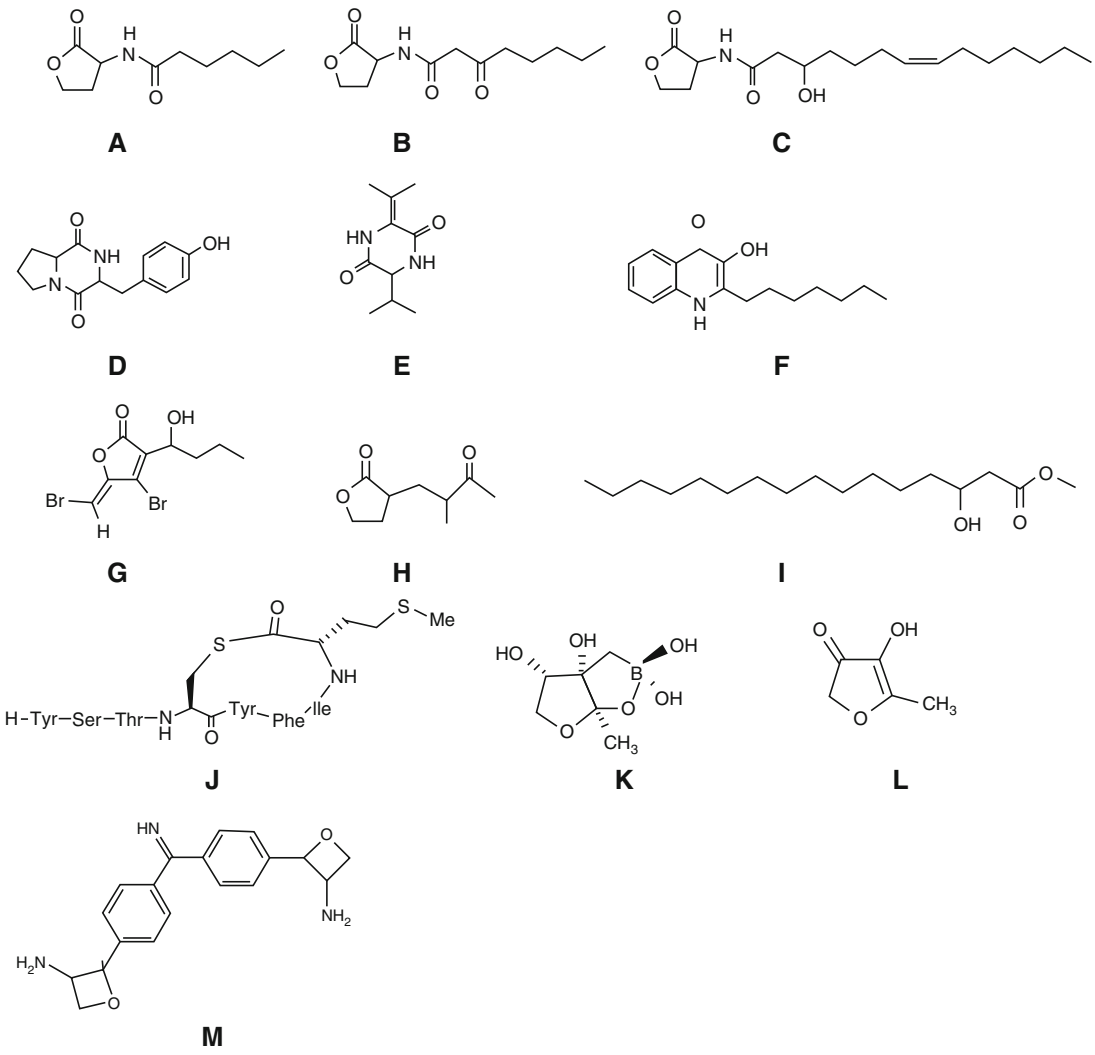


Fig. 4.8. Different quorum sensing signal molecules (13). (**A–C**) Examples of microbial AHLs without substitution on the C3, or with an oxy or hydroxyl group. (**A**) *N*-hexanoyl-L-homoserine lactone or C6-HSL. (**B**) *N*-(3-oxooctanoyl)-L-homoserine lactone or 3O,C8-HSL. (**C**) *N*-(3R-hydroxy-7-cis-tetradecenoyl)-L-homoserine lactone or 3OH,C14:1-HSL. (**D, E**) Microbial diketopiperazines. (**D**) cyclo(L-Pro-L-Tyr). (**E**) cyclo(D-Ala-L-Val). (**F**) 2-Heptyl-3-hydroxy-4-quinolone (PQS) produced by *Pseudomonas aeruginosa*. (**G**) 4-Bromo-5- (bromomethylene)-3-(10-hydroxybutyl)-2(5H)-furanone of *D. pulchra*. (**H**) c-butyrolactone produced by *Xanthomonas campestris*. (**I**) 3-Hydroxypalmitic acid methyl ester of *Ralstonia solanacearum*. (**J**) Group IV cyclic thiolactone from *Staphylococcus aureus*. (**K**) Putative structure for Vibrio harveyi AI-2. (**L**) It is also possible that this compound and 4-hydroxy-5-methyl-3(2H)furanone (MHF) are interconvertable. (**M**) bradyoxetin, a four-membered oxetane ring, from *Bradyrhizobium japonicum*. (With permission from Elsevier).

Table 4.1
Effect of ecological interactions on population growth

Type of interaction	Effect on population A	Effect on population B
Neutralism	0	0
Amensalism	0	−
Commensalism	0	+
Mutualism	+	+
Predation/parasitism	+	−
Competition	−	−

(see below), diffuses through the membrane. The LuxR transcriptional activator is inactive at this moment. With increasing population density, the AHL concentration increases. When a threshold concentration is reached, the signal molecule binds to the LuxR transcriptional activator. This complex is active and binds to the promoter region of the bioluminescence operon luxICDABEG. This leads to a rapid amplification of the AHL signal 3O,C6-HSL and consequently induces bioluminescence.

Types of interactions between organisms are summarized in Table 4.1. There are many examples of each of the listed types of interactions (5). Only competitive interactions have been studied in a precise experiment with two and more populations of protozoa cultured in the same flask (14). One of the competing species was always completely eliminated. On the basis of such experiments, the principle of competitive exclusion was formulated, which states that a particular ecological niche can be occupied by only one species. Below, we will clarify why natural habitats always contain coexisting species.

2.4.2. Development and Evolution of Ecosystems

The development of ecosystems is usually called *ecological succession.* Questions related to the notions of ecological succession or evolution of ecosystems include: What are the limits for community stability after perturbation/disturbance of environment? What are the driving forces for community dynamics? Can we predict it based on environmental data? Is there relationship between composition of biotic community and ecosystem's functions?

The English word "succession" and scientific term "ecological succession" are not identical. The second term is defined in many ways, starting from the simplistic version "the replacement of populations by other populations better adapted to fill the ecological niche" (5) to a descriptive inclusive one: "The *gradual and orderly process* of ecosystem development brought about by changes in community composition and the production of a climax characteristic of a particular geographic region" (15). We can observe changes in community composition in seasonal or multiyear dynamics because of fluctuation. But contrary to fluctuations and seasonal dynamics which are *cyclic or random*, the ecological succession proceeds as an *orderly, unidirectional and irreversible* process. Succession is usually initiated by dramatic changes in the state of abiotic environment: climatic warming or cooling, flooding or desertification, fire, volcano eruption with lava-stream, etc. We can distinguish between

autotrophic and heterotrophic succession. The former assumes the development of plants or other autotrophic community on the initially bare land (e.g., on the magma rocks). Heterotrophic succession takes place after heavy deposition of organic matter, e.g., amendment of poor soil with manure. Succession is called *primary* if the development of ecosystem starts from zero: on the suddenly released rocks, lava-stream or sand dune. The *secondary* succession is much quicker and takes place, say, as reforestation of abandoned arable field or after forest fire or clear cutting.

Succession in microbial community takes place concurrently with the evolution of an entire ecosystem because the gradual and orderly replacement of plant and animal species affects microbial microenvironment. We can also observe purely microbial succession in the laboratory or field experiments with microcosms (microecosystems). Figure 4.9 shows the growth dynamics of consecutive replacement of one microbial group by another after soil amendment with glucose or cellulose.

The mechanisms of succession are viewed entirely differently by ecologists supporting one of the two competing paradigms: holistic or meristic.

According to the holistic concept (syn: *organisms*), the biotic component of ecosystem is a kind of superorganisms. It has stable structure and strong deterministic interactions based on differentiation of econiches similar to interactions between specialized tissues and cells within multicellular organism. Succession is analogous to ontogenetic development of individual differentiated organisms. It can be accurately predicted and is driven by changes in the physical state of habitat caused by community: the early populations modify the physical state of habitat providing better growth conditions for the next stage organisms; such replacement continues until the equilibrium is attained between the biotic and abiotic components in climax community.

The meristic approach (syn: continualism) assumes that various species have a relatively high degree of freedom. Although there are some biotic interactions between species, they can enter and leave a community through immigration and emigration. The replacement of species during succession is also not strictly deterministic and has clearly expressed stochastic nature. The replacement occurs mainly as a result of competition between organisms occupying the same econiche. One cannot accurately predict the temporal profile of the transient community (i.e., the list of species and schedule of replacement) due to significant effects of chance, local conditions and past history. However, there is a well-expressed trend in consecutive changes in the community structure from predominantly r-selected to predominantly K-selected species.

2.4.3. The Concept of Life Strategy

The most essential element of the second approach is the concept of *life strategy* and *continuum.* Life strategy is defined as "a combination of adaptive reactions which provides the possibility for a given population to coexist with other organisms and occupy some part of niche hyperspace" (16). Usually, the strategy is characterized by the so-called "survival triad": (a) the ability to compete with other populations, (b) to recover after perturbations, and (c) to survive stresses. In this manner, one may distinguish three types of natural selection:

1. K-selection operates in climax ecosystems under stable and predictable conditions without frequent perturbation and stresses. The habitats of this type are overcrowded, thus the main

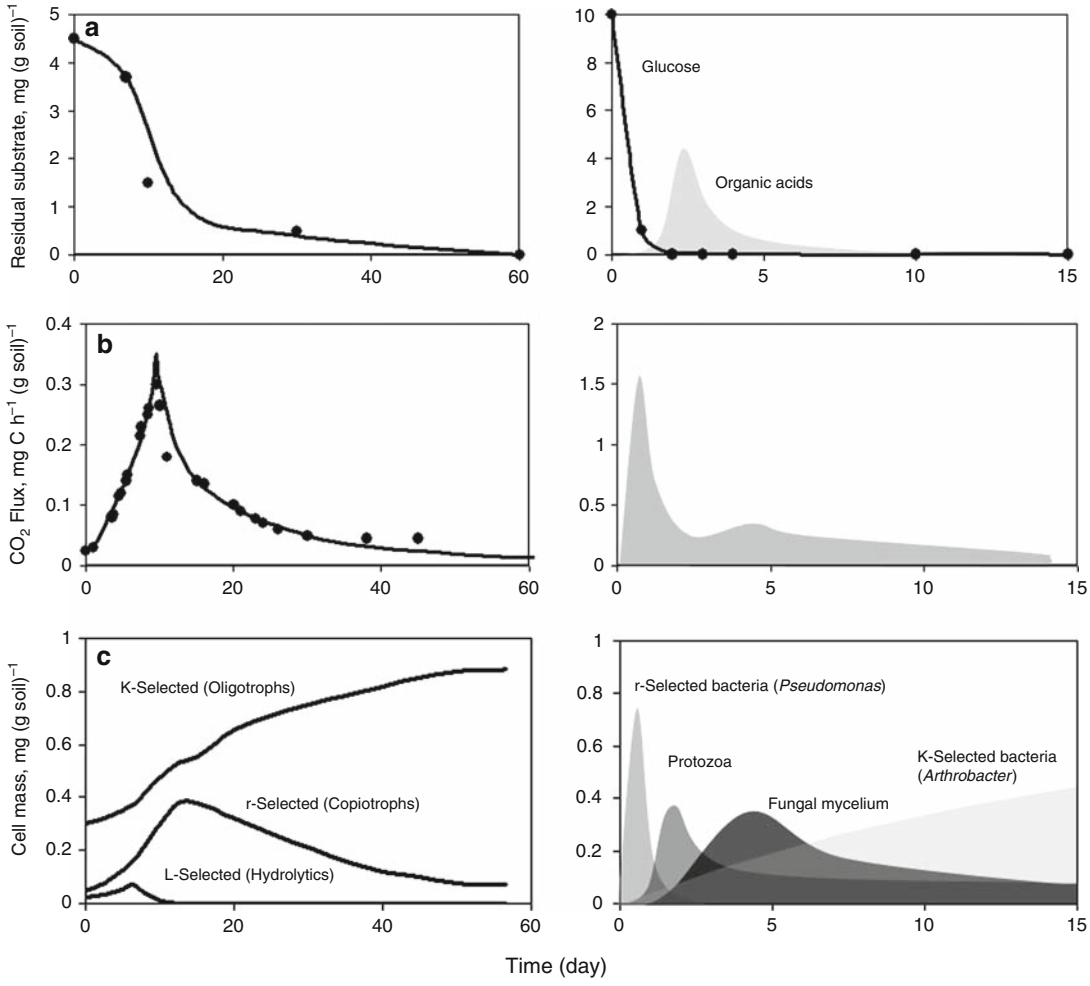


Fig. 4.9. Examples of microbial successions induced by soil amendment with cellulose (*left column*) and glucose (*right column*); after (7). Decomposition was recorded as dynamics of residual substrate (**a**) and CO_2 evolution rate (**b**). Abundance of various microbial groups (**c**) was evaluated on the basis of microscopic observations with UV microscope and simulation with SCM.

feature of *K*-selected species should be a high competitive ability ("lions" type of strategy). Their generation time is relatively long and they have few progeny, but nevertheless these species maintain high population densities.

2. *r*-Selection operates on the pioneer stages of succession initiated by some perturbation of a climax ecosystem i.e., a sudden change of environmental conditions (not necessarily adverse), a flash of nutrients, a cataclysmic elimination of competitors. The main result of perturbation is temporary relief from the pressure of severe competition for nutrient resources. *r*-Selected species survive in ephemeral, unpredictable habitats because of mobility and high reproduction rates (opportunistic

or a "jackal" type of strategy). They are not good competitors and are always ready to leave the resources once they become depleted or overcrowded.

3. *L*-selection operates under adverse environmental conditions caused by various stresses. Stress factors could be abiotic (nonoptimal salt concentration, temperature, pH, water content, etc.) or biotic (antagonism, starvation caused by the depletion of substrate by more successful competitors). The products of *L*-selection are the patient species resistant to a particular stress factor ("camel" type of strategy).

The *r* and *K* notations is derived from logistic equation: *K* stands for carrying capacity and state of community close to climax with maximal competition, while *r* is the maximal growth/birth rate observed at the origin of logistic curve and corresponding to pioneer stages of succession. *L* stands for ecological minimum on the environmental gradient or the minimal density of population under unfavorable environmental conditions allowing positive birth rate (Figs. 4.6 and 4.7).

The concept of *rKL*-selection is not absolute, being meaningful only in the comparison of several organisms. The best way to identify the life strategy of some studied organisms would be to locate them in one common *rKL-continuum*. The more prosperous a particular species is under the conditions (1), (2) or (3), the closer it is placed to the *K*-, *r*- or *L*-pole of this continuum. An example of such an ordination is shown in Fig. 4.10.

The differences between two competitive paradigms are summarized in Table 4.2 and flowchart diagram. The first concept of a superorganism tends to overemphasize the strength of biotic and in particular symbiotic interactions and underestimates the competition between

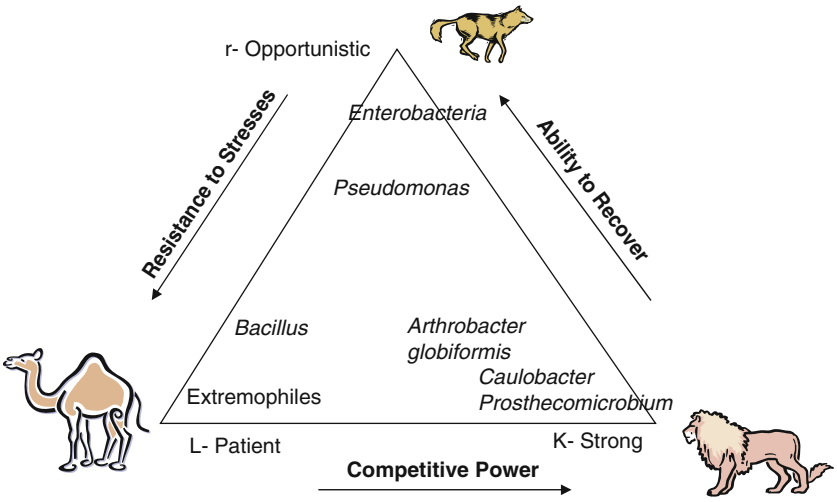
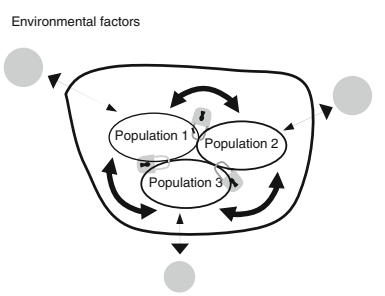
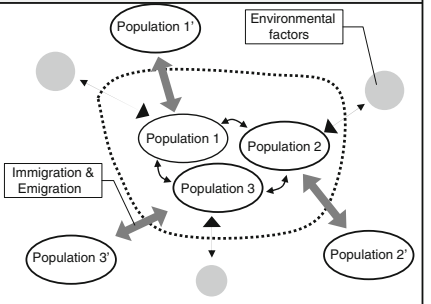


Fig. 4.10. The illustration of the concept of life strategies. All natural microorganisms are located along three axes characterizing survival triad: the ability to compete for resources (K-axis), recover after stresses (r-axis) and resist unfavorable environment (L-axis). Respectively, one can distinguish the following three types of natural selection which correspond to three types of life strategy.

Table 4.2
Comparison of holistic and meristic paradigms explaining the driving forces of succession and evolution of community

Holistic (superorganism) paradigm	Meristic (continuum) paradigm
	
Community structure	
Constant, precisely determined by strong interspecific links	Flexible, depends on immigration & emigration, past history and local conditions
Boundary of ecosystem	
Clearly expressed	Not clearly expressed, peripheral gradient
Prediction possibility	
High	Low
Pioneer stages of succession	
Abiotic environment is not appropriate for life. The first colonizers are stress-resistant species which improve environment for other organisms	Ecological vacuum: environment is not saturated by organisms and the first colonizers are opportunistic species, no competition, no sever stress
Transient community: effects of organisms on abiotic environment	
Abiotic environment is getting better and better due to 'edification'; the productivity of community increases. The reverse effect of environment on organisms is usually not emphasized although not rejected.	Strong selective effects of environment on organisms, progressive increase of competition for resources which are getting more and more limited. The 'edification' effect of organisms on environment is not emphasized although not rejected.
Climax community	
Stabilized community with maximal symbiotic interactions, biomass and information content per unit of available energy flux	Stabilized community with maximum of competitive interactions between biotic components occupying similar econiches

biotic components. The second paradigm appears more realistic (stochastic nature of ecosystem's evolution, importance of competition and selection pressure from environment), but probably underestimates the significance of gradual modification of environment by organisms (such as soil forming processes) as essential component of long-term succession.

In microbial ecology, the superorganismal paradigm is intuitively more attractive for ecologists focusing on metabolic networks within microbial community (Fig. 4.3). Such networking assumes the existence of strong interactions between different members of community and is more naturally associated with deterministic approach and the holistic view of community as a superorganism. At least, the organismal paradigm is appropriate at initial theoretical studies aimed at characterization of the most essential key functional features of the studied natural ecosystem. The following comparison of diverse ecosystems and tracing their evolution probably would greatly benefit from the second more realistic continuum paradigm and concept of life strategy. However, before we can discuss the microbiological interpretation of a life strategy concept, we must touch on the basics of microbial growth kinetics.

2.4.4. Growth Kinetics of Microorganisms with Different Life Strategy

Under favorable growth conditions (temporary excess of nutrient substrates, absence of inhibition), the bacterial growth rate should be proportional to the instant cell mass, x, the quotient μ remaining constant:

$$\mathrm{d}x/\mathrm{d}t = \mu x \tag{6}$$

The integration of Eq. (6) at initial condition, $x = x_0$ at time $t = 0$, gives the exponential equation:

$$x = x_0 e^{\mu t} \text{ or } \ln x = \ln x_0 + \mu t \tag{7}$$

However, the specific growth rate μ remains constant only for limited time and narrow environmental conditions. According to the popular Monod model (17), the μ value is controlled by concentration of *limiting substrate* and the biomass formation is linked to substrate uptake by mass-conservation condition [Eq. (2)], then:

$$\begin{aligned} \frac{\mathrm{d}x}{\mathrm{d}t} &= \mu(s)x; \quad \mu(s) = \mu_\mathrm{m}\frac{s}{K_\mathrm{s}+s} - a \\ \frac{\mathrm{d}s}{\mathrm{d}t} &= -\frac{1}{Y}\frac{\mathrm{d}x}{\mathrm{d}t} \end{aligned} \tag{8}$$

Equation set (8) contains four parameters: yield Y, maximal specific growth rate μ_m, saturation constant K_s (substrate concentration at which $\mu = 0.5\,\mu_\mathrm{m}$), and specific maintenance rate which is related to maintenance coefficient $a = Y^{\max}m$ [see Eq. (2.2.2)]. The set of these four parameters can be thought of as "ID" for particular organisms and used to predict their growth dynamics. Remarkably, this model was used to develop a *chemostat theory* before actual experiments with continuous culture were undertaken – a very rare event in the history of mathematical biology! The model predicts a number of counter-intuitive features of chemostat, e.g., that specific growth rate μ can be set up by experimentalist by changing

the medium flow at any values between 0 and μ_m (before exponential growth was believed to occur only at $\mu = \mu_m$) and that μ-values do not depend on the feed-substrate concentration and is governed solely by the *residual* substrate concentration in the culture.

However, the Monod model fails to explain a number of essential growth phenomena observed experimentally: lag-phase, death of starving cells, product formation and any kind of adaptive changes in microbial population, such as induction-repression of enzymes, yield variation, changes in the cell RNA content etc. These gaps were filled in by so-called structured models.

Structured models explicitly describe variations in cell composition. They usually include mass balance equations not only for *external* substrate(s), but also several *intracellular* components, C_1, C_2, ..., C_n. For each variable C_i, a differential equation is written which takes into account all sources, r_+, and sinks, r_-, as well as its dilution due to cell mass expansion (growth),

$$\frac{dC_i}{dt} = r_+(s, C_1, \ldots, C_n) - r_-(s, C_1, \ldots, C_n) - \mu C_i \tag{9}$$

The earliest structured models accounted for no more than three to five cell constituents, e.g., the total cell proteins, RNA and DNA, reserved polysaccharides, ATP-pool, etc. The modern meticulous models contain up to hundreds and even thousands of internal variables borrowed directly from available genomic data bases. The recent challenge was to develop a *virtual cell,* to construct a biological system in silico without essential reductionistic compromise. However, the predictive capability of these intricate models are rather modest: they are still a "caricature parody" of the real cell, but already too complex to be studied mathematically (stability analysis, parameters identification, etc.) or to improve understanding of the biosystem. The best choice of a mathematical model lies, apparently, midway between unstructured and highly structured models outlined here. One of the best known examples is *synthetic chemostat model* (SCM).

According to SCM (7), the microbial growth occurs as a conversion of exosubstrate S into a number of cell macromolecules X′ via a pool of intermediates L part of which are respired to CO_2 (Fig. 4.11):

Macromolecular cell components are susceptible to degradation (turnover), and intermediates L can leak out. The array $\mathbf{X}'$, the cell composition is not fixed and varies in response

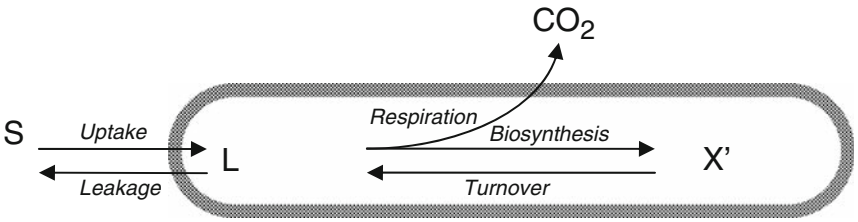


Fig. 4.11. Chart-flow diagram describing cell growth according to SCM.

to a changing environment. The heart of the SCM is the solution of the problem; how to characterize these variations without going to extreme intricacy.

For this purpose, all the macromolecular cell constituents are divided into two groups:

1. **P**rimary cell constituents necessary for intensive growth (P-components).
2. Components needed for cell s**U**rvival under any kind of growth restriction (U-components).

The content of P-components (ribosomes and all enzymes of the primary metabolic pathways) increases parallel to growth acceleration. The contribution of U-components under good growth conditions decreases (to comply with conservation conditions $\mathbf{P} + \mathbf{U} = \text{const}$), and attains the maximum under chronic environmental stress to improve cell resistance. The typical U-components are enzymes of the secondary metabolism, protective pigments, reserved substances, transport systems of high affinity. An interdependent variation of individual P- and U-components is approximated by a linear function of some *master variable* r^*:

$$\mathbf{P} + \mathbf{U} = \frac{P_\mathrm{l}}{P_\mathrm{n}} + \frac{U_\mathrm{l}}{U_\mathrm{m}} = \frac{P_\mathrm{l}^{\min}}{P_\mathrm{n}^{\min}} + \frac{U_\mathrm{l}^{\min}}{U_\mathrm{m}^{\min}} + r^* \frac{P_\mathrm{l}^{\max} - P_\mathrm{l}^{\min}}{P_\mathrm{n}^{\max} - P_\mathrm{n}^{\min}} + (1 - r^*) \frac{U_\mathrm{l}^{\max} - U_\mathrm{l}^{\min}}{U_\mathrm{m}^{\max} - U_\mathrm{m}^{\min}} \quad (10)$$

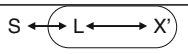
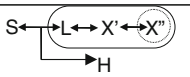
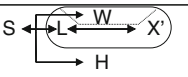
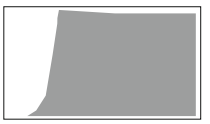
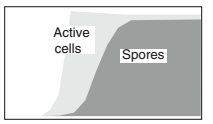
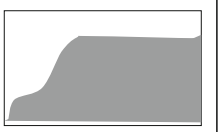
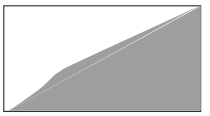
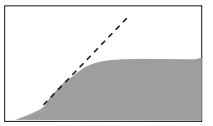
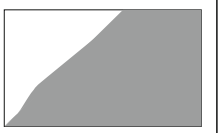
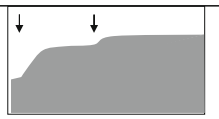
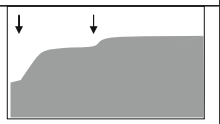
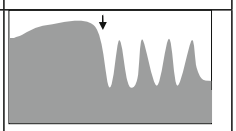
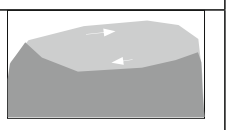
Where, $P^{\max}$, $U^{\max}$ and $P^{\min}$, $U^{\min}$ are respectively upper and low boundaries for **P** and **U** adaptive variations, and r is the *scalar* function, not *array!* The r^*-value depends directly on environmental factors, e.g., on the limiting substrate concentration and r^*-variable is participating in all kinetic expressions (q_s, m, μ) to simulate the combined effects of the *current* environmental factor(s) and cellular physiological state determined by the growth conditions *in the past*.

The simulative capabilities of structured models like SCM are high enough to mimic and explain the majority of available experimental data on various microbial cultures (steady-state and transient, continuous and batch from lag- to decline phases). What is important for ecological applications, the SCM realistically describes and predicts not only growth per se, but also many other dynamic phenomena: survival dynamics under starvation, formation of dwarf cells under growth restriction, the adaptive adjustment in cell maintenance requirements, variation of growth potential and affinity to substrate, utilization of substrate mixture, etc.

Going back to the concept of life strategy, we can now use kinetic data to describe quantitatively, why the variation in the pressure of natural selection (K-, r- and L-types) resulted in diversity of dynamic growth patterns of various microbes isolated from natural habitats. Table 4.3 summarizes the results of kinetic studies of the typical microbial r-, K- and L-selected species chosen on the basis of field observations (how frequently respective microbial species were found in climax or pioneer communities or in unfavorable habitats) as well as on complementary laboratory experiments with cultivation under conditions simulating respective natural environment (7).

Enterobacteria, pseudomonas, baker and fodder yeast are mainly products of r-selection. Their dynamic behavior is erroneously considered to be typical for *all* microbes: rapid and balanced growth, short lag-periods and smooth transitory processes. They dominate in those natural habitats which are frequently "rejuvenated" to the pioneer succession stage: hot spots of substrate amendment, animals gut and feces, rhizosphere with diurnal fluctuations in exudation rate and perpetual changes in "addresses" of exudation loci due to apical extension

Table 4.3
Diversity of growth patterns in soil bacteria stemming from difference in the life strategies (18)

Type	*Pseudomonas-Enterobacteria*	*Bacillus-Streptomyces*	*Arthrobacter-Caulobacter*
			
Scheme of metabolic flow			
Batch: cell growth and starvation decline			
Dialysis culture: cell mass dynamics			
Chemostat transients: dynamics of cell mass after abrupt change in D			
C-limited chemostat culture: steady-state biomass versus D			

Diversity of growth patterns displayed by soil bacteria of various life strategies. Upper row shows typical morphology of selected microbial groups, next row demonstrates cell growth chart and the main state variables of the respective modification of SCM used for simulation (X″ is prospore compartment, H is autoinhibitor and W is poly-β-oxybutyrate (reserved compounds), S, L and X′ and explained in the text. Black arrows indicate the time of switching from one dilution rate, D, to another in chemostat culture, white arrow shows direction of sequential D-changes, either from low D to high or reverse, in chemostat culture displaying multistability.

of root hair. Petri dish with reach medium like LB or yeasts extract is good simulation of such hot spots; that is why *r*-selected species are easily isolated from soil.

K-selected bacterial species are much less amenable to isolation and cultivation. Probably, most unculturable microbial species belong to this type of life strategy. When cultivated

under artificial laboratory conditions, they are fastidious and unpredictable. The best option for their cultivation is continuous culture with cell retention: fed-batch, dialysis culture or batch culture with C-substrate delivered via gas phase (volatile C-substrates, such as ethanol or VFA). Under these cultivation conditions, the K-selected species display high yield and almost 100% viability even at extremely slow cell division (generation time up to months). In batch culture, their growth is slow with the "false diauxie," biphasic growth on the single substrate. In chemostat and turbidostat, they display oscillations and multiple steady states. To simulate the described abnormal behavior of *Arthrobacter* and other oligotrophic species, the SCM was elaborated to include intermediates with autoinhibition functions (e.g., peroxides as respiratory by-products) and the possibility of direct incorporation of deficient C-substrate to pool of reserved compounds (Table 4.3). The main feature of the growth control in oligotrophic species is the relative independence of transport, catabolic and anabolic reactions which does not allow rapid balanced growth, but gives great advantage in consumption of highly dilute substrates and survival prolonged starvation.

Many *L*-selected microorganisms (bacilli, actinomycetes, some fungi) share the following common features: spore formation, production of antibiotics, and synthesis of hydrolytic enzymes. All these features help them survive even if they fail in direct combat with competitors for deficient nutrients. Kinetic studies allow us to understand why they are weak competitors. The most striking feature was observed in *Bacillus* dialysis culture: the bacteria stopped growth after 2–3 weeks when the residual glucose level dropped below the threshold value of 20–50 μg/L. In rich environments even with intermittent supply of nutrients (feast-to-famine transitions simulated in batch culture), these bacteria perform well. They rapidly deplete the available substrates, which triggers sporulation and transition to a dormant state, preserving the bacteria from extinction. However, the chronic starvation typical for most oligotrophic environments is the "trap" for bacilli; they are provoked to sporulate but are not able to finish it in a normal way. The slow feed via the dialysis membrane provides glucose levels which are too high to allow termination of normal sporulation and too low for growth because of the the uncoupling action of metabolite H and acceleration of turnover rate (see Table 4.3).

3. METHODS OF MICROBIAL ECOLOGY

As in any other biospheric and ecological sciences, there are three main approaches in microbial ecology:

1. In situ (field) observations with minimal disturbance of the studied processes and communities
2. Laboratory and field experiments with deliberate modification of the natural object aimed at revealing of unknown functional relationships
3. Mathematical and conceptual modeling aimed at generation of new theoretical knowledge, testing hypotheses and comparison of theoretical concepts

Microbial ecology has its own "sore spot": a relatively weak development of theoretical concept (mathematical modeling is not as popular and appreciated as in other ecological disciplines) is associated with the ongoing problem of inadequate laboratory surrogates for natural populations. Contrary to macroecology, microbial ecology has long been developing

as an experimental science with a doubtful and elusive research subject. Just imagine the frustration of an animal ecologist who is confined in his supposedly comprehensive study with only a domesticated cow, goat and donkey! In microbial ecology, we have had to deal with a limited range of cultivated microbes for a detailed study of their possible functions in situ. This is because many subtle features of microbial behavior can be only disclosed in accurate laboratory studies with pure cultures. This leads the researcher to ask: Is such extrapolation really justified? Are axenic laboratory cultures sufficiently representative of their natural progenitors?

3.1. Natural Microbial Populations and "Laboratory Artifacts"

Different opinions have been expressed in respect of this ongoing problem. On the one hand, "pure cultures could certainly be regarded as a physiological artifact" (Kluyver) and so "a clear demarcation line should be drawn between data obtained under abnormal experimental conditions, which invoke microorganisms to reveal some new features, and data from observations on ecological factors in nature" (19, pp. 25–47). On the other hand, "many properties of pure laboratory cultures are also exhibited by microbial populations under natural conditions" (20), and "unless there are indications to the contrary, it is justifiable, and operationally necessary, to assume that in most characteristics pertinent to the habitat, pure cultures do resemble their progenitors in nature" (21, pp. 100–101).

Properties of microbes in pure laboratory cultures may differ from those of their ancestors in natural habitats because of the following factors: (a) the lack of metabolic interaction with other organisms normally present in situ; (b) autoselection of mutants in the long-term course of isolation, purification, and maintenance of cell culture; (c) phenotypic changes in the physiological state of microbial cells in response to a changed environment (different with respect to the availability and spectrum of substrates and modifiers, temperature, humidity, etc.). It was primarily the third factor that Winogradsky was referring to when he wrote of "invoking" laboratory forms to grow abnormally. We also regard this as a fundamental factor. The first factor is not decisive since, in soil, subsoils and sediments, microbial growth is confined to microsites where practically pure cell clones develop. In homogeneous natural habitats, such as waters, there are negative and positive interactions via metabolites. However, antagonists are not able to sustain co-existence and, in the case of positive cooperative effects, it is in fact, microbial associations that are isolated from the natural habitat rather than pure cultures. Factors (b) and (c) are almost indistinguishable in practical terms. They are also fairly similar in principle because both the selection and phenotypic variations are not random, but tend toward a better adjustment of the population to the given growth conditions.

In terms of quantitative microbiology, factor (c) may be interpreted as a difference in the vector of physiological state of a laboratory culture as compared with a population in situ. At Winogradsky's time, two major cultivation techniques were available, plating on solid agar media and liquid batch culture with nutrient broth. Microbial cells grown under such conditions do have a peculiar physiological state which is indeed dissimilar from that of in situ soil microbes. Today, we have a much wider assortment of cultivation techniques. Consequently, we have the improved ability to control the physiological state of a laboratory culture and may intentionally shape it by cultivation conditions. Particular challenging is to

use (a) continuously starving batch culture with spend/exhausted nutrients and dialysis culture to maintain deeply limited and very slowly growing cell populations; (b) nonsteady state cultures with deliberate fluctuation of cultivation conditions simulating natural rhythms; (c) careful design and selection of chemical composition of nutrient media resembling the most essential features of the natural habitat; special efficient approach is to use dialysis membrane separating cultivation chamber with almost intact natural community producing the whole spectrum of metabolic products needed for growth of indigenous populations. With these approaches, microbial ecologists have made significant progress in their attempts to increase the number of cultured microorganisms.

3.2. "Great Plate Count Anomaly"

It was discovered as early as the nineteenth century that plating on nutrient agar and serial dilutions fails to encourage growth of the most abundant in situ microbial species. The first explanation to this phenomenon was given by S. Winogradsky and only recently via environmental gene retrieval (extraction of total soil DNA, amplification of, say, 16S rRNA gene and following sequencing) it was confirmed explicitly that cultured forms are only minor components of the entire natural community. This inability to recover the most numerous organisms from natural habitats by using cultural approaches has been called the "enumeration anomaly" or the "Great Plate Count Anomaly" (22). For example, Hugenholz et al. (23) reported the discovery of 36 major phylogenetic groups of eubacteria in natural communities, which is about triple the number of those that have been cultivated in pure culture. The relative proportion of uncultured forms varies in different habitats. Sometimes, environmental gene retrieval and plating give identical results indicating that ALL microbes can grow on artificial laboratory media. For instance, the plating and MPN enumeration of psychrophilic bacteria in summertime Arctic pack ice from the Chukchi Sea gave up to 62% of culturability as compared with direct microscopy (24). However, most of the complex natural habitats have as low as 0.1–1% of the total amount of phylotypes able to grow on artificial media. A similar proportion is normally reported by comparison of plate count with direct microscopy: the last one gives $\sim 1,000$ times higher number than the first one.

What is the reason for discrepancy between plate count and direct microscopy? Let us consider the following equation:

$$\text{Direct Count} = M_1 + X_1 + A(\text{CFU} + X_2 + X_3 + X_4 + X_5) \tag{11}$$

In this equation, Direct Count stands for the total amount of cells seen under microscope, M_1 is the number of microscopic errors, i.e., spherical or rod-shaped abiotic particles erroneously taken as cells.

CFU is the number of Colony Forming Units, the actual result of plating shows the number of cultured cells. A is the average number of cells in aggregates in the droplet of suspension added to the plate. The higher A is, the more significant is the underestimation of the real number of culturable cells in natural habitat. The A-value is higher for soils and sediments than aquatic habitats (large amount of solids catalyze aggregation) and for filamentous and slimy cells as compared with small cells without capsule.

X_1 is the number of cells/aggregates which do not grow on selected media; it is obvious that there are no universal media adequate for all physiological groups of microorganisms, there are no common cultivation conditions, say temperature, pH, Eh, CO_2 and O_2 partial pressure in the head space to satisfy all the multitude of the growth requirements (for example, methanogenic bacteria cannot be grown on an aerobically incubated Petri dish with yeast extract agar).

X_2 is the number of cells or cell aggregates attached to a pipette during the preparation of serial dilution. We can minimize this number by using hydrophobic plastic tips, but can never completely eliminate this error.

X_3 is the number of stressed or viable-and-unculturable cells/cell aggregates. The reason for stress is not fully understood, but we have several experimental methods to reproduce such metabolic stress as "substrate-accelerated death" by prolonged incubation of the "normal" soil bacteria on media with excess of catabolites (e.g., glucose) and deficient in nitrogen (7). It was also found that hormone-like signal metabolites were inhibiting cell division.

X_4 is the number of microcolonies which stopped their development because of any kind of competition (lack of available space on the agar plate, inhibition by antibiotics produced by other colonies, etc); contribution of this factor is especially high when analyzed microbial suspension is too dense giving more than 50 CFU already the first 2 days.

Finally, X_5 are those K-selected microbes which grow too slowly. We know from in situ measurements that a generation time of more than one month is quite a probable event, implying up to one year period for development of a visible colony. However, 1–3 weeks is too long to await plating results; also, agar layers tend to be dried or contaminated. Fortunately, we can estimate roughly the number of slow-growing microbes by occasionally recording the dynamics of CFU on a single Petri dish during several months, for example. The plot of CFU versus time usually gives several waves (25), each of which can be approximated by the first-order rate equation (Fig. 4.12):

$$N = \sum_{i=1}^{n} N_i^{\infty}(1 - e^{-ki(t-t_i)}), \quad i = 1, 2, \ldots, n \tag{12}$$

where n is the number of waves (usually $1 < n < 4$), and $N^{\infty}{}_i$, k_i, and t_i are empiric constants.

Now, let us assign to all terms numerical values which we have in some "typical" top soil: "Direct Count"$= 5 \times 10^9$ cell/g, CFU $= 0.5 \times 10^7$ CFU/g (3 days aerobic incubation, YEA – yeast extract agar), M_1 was assumed to be 20% of the total count (fair assumption even for experienced microscopist!), A $= 10$, $X_2 = X_4 = 10^5$, $X_3 = 0.2 \times$ CFU $= 10^6$, and $X_5 =$ $10 \times$ CFU. To comply with mass balance, the main unknown variable X_1 should be equal to approximately 85%, which seems to be a reasonable estimate. Thus, the main reason for lack of agreement between direct microscopy and plating is the immense metabolic diversity of the majority of natural habitats.

Thus, about 15% of the total unicellular objects revealed by direct microscopy should be considered known and potentially culturable aerobically on standard YEA medium if all technical errors of plating are eliminated (cell aggregation and adhesion, stress, nonoptimized

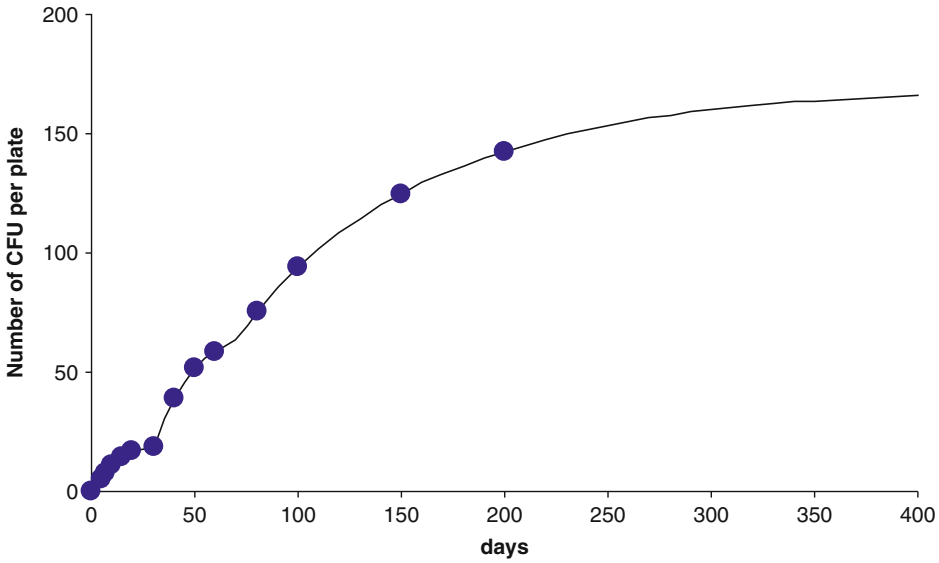


Fig. 4.12. Dynamics of colonies formation on Petri dish with YE agar. The continuous curve calculated from Eq. (12) to find out an amount of slowly growing microbes.

dilution and too short incubation). The remaining 85% of the soil community is missed because they are not aerobes or do not like yeast extract. Application of the full range of available cultivation techniques would at least double the number of culturable organisms, implying that we should know more than 30% of the entire soil community.

The molecular techniques based on sequencing of SSU rRNA usually give less optimistic results: no more than 10% of known phylotypes. The reassociation kinetics of the total soil DNA (26) displays a high degree of heterogeneity of microbial DNA in the majority of habitats. Calculations based on empirical relationships between reassociation kinetic constants and DNA heterogeneity expressed as conditional genome numbers reveals also a huge gap between known and total microbial diversity.

3.3. Estimation of the Microbial Numbers and Biomass in Soils and Water

There are five classes of analytical techniques suitable for determining microbial biomass:

1. Ex situ germs enumeration (plating and MPN),
2. Direct microscopy,
3. Kinetic methods (biomass of specific microbial group is calculated from kinetic data on instant response of natural samples to added substrate).
4. Biochemical methods (detection of specific microbial metabolites – ATP, DNA, muramic acid, chitin, phospholipids profile or fumigation flux)
5. Methods based on DNA sequencing (FISH – Fluorescence In Situ Hybridization)

Table 4.4 outlines the specific advantages and shortcomings of these approaches (for details, see discussion in a numerous experimental papers and reviews, e.g., (27–30)). Obviously, the

Table 4.4
Comparison of different method for soil biomass determination

Methods	What is measured	Conversion factor	Shortcomings	Advantages
Plating and MPN	Number of CFU (colony forming units) or highest positive dilution	Absent or extremely unreliable	Low recovery resulted from cell adhesion and clumping, and unculturability; variability of CFU sizes	Isolation and identification of microbes, differentiation of physiological and taxonomic groups, detection of individual populations carrying genetic markers
Direct microscopy	Bacterial number and mycelia length	Derived from measured cells biovolume and assumed dry matter content and cells bulk density. Not reliable	Subjective procedure, unreliable differentiation of cells from soil particles and plant debris	Characterization of bio-morphological structure of community across space; detection of individual populations in combination with DNA probes and immunofluorescence
Biochemical	Content of unique cell constituents: ATP, DNA, membrane lipids, or OM release after fumigation	Estimated from (1) analysis of isolates, (2) mass balance in lab incubations, (3) comparison with direct microscopy	Occurrence of analyzed chemical species in plant roots, animals and in extracellular milieu, wide variation of conversion factor	High precision, open to standardization and automation, compatibility of results obtained in different laboratories, effective in combination with isotope technique to label microbial cells in situ
Kinetic	Rates of specific metabolic reactions estimated in incubation experiments with amended soil samples	Found as parameters of mathematical model describing microbial growth on added substrate. Precise and objective procedure	Underestimates the resting forms of natural microbial populations	All technical advantage of biochemical methods (see above), exact calibration, differentiates microbial biomass into functional groups, assessment of the physiological state of microorganisms in situ

choice of method depends on the targets of a particular study, and there can be no absolute preference for one unique methodological tool. It is sufficient to mention that even plating and MPN techniques (which have been subjected to most severe criticism last decades) remain to be valuable and indispensable in some specific fields of research.

In the last decade, growing interest was paid to nontraditional approaches based on biochemical determinations (class 3, Table 4.4). New generations of these methods gave strong impetus to the development of quantitative microbial ecology. However, these methods have at least two drawbacks: (a) they use doubtful conversion factors from measured chemical index (ATP, DNA, or chitin content, fumigation flush, etc) to real biomass, and (b) they generally neglect diversity of soil microbial community. The most popular method today remains direct UV microscopy with new "functional" staining methods, analysis of phospholipids profile and various techniques based on extraction and sequencing of the total community DNA: wide range of techniques starting from specific oligonucleotide probes (FISH) to metagenome analysis.

3.4. Estimating Microbial Growth Rates In Situ

The majority of biologists who are unfamiliar with microbial ecology naively believe that bacteria multiply very fast. This is a gross misconception. First, we should acknowledge that some bacteria do grow very fast, but there are plenty of slow growing *K*-selected microorganisms with multiplication speeds essentially lower than, say, rats or herring. Second, even opportunistic bacteria displaying explosive growth rate with generation time down to 15–20 min on specially designed laboratory media fail to grow fast in situ, the main restrictive factor being amount and quality of nutrients. In this section, we briefly survey the available literature on techniques used to measure the actual growth rate of microbial populations in situ. This issue is especially important for the development of environmental biotechnologies for a very simple reason: microbial growth rate in situ is an integral parameter related to the actual activity of microbial populations in their technological performance. Additionally, the actual growth rate of indigenous and released to natural environment populations must be known to predict their fate after termination of biotechnological processes.

3.4.1. Microscopy In Situ

Direct microscopic observations of this type are normally done only in aquatic habitats with the use of a submerged-slide technique. At regular intervals, glasses with microbes attached are removed from water for microscopy and afterward are returned back. Instead of standard glass slides, microcapillaries may be used (31). An alternative approach, which has only been used by the most courageous ecologists, is to immerse a microscope directly into the pond and to carry out a diurnal observation of individual cells attached to glass surface (32). Obviously, this technique requires the discrimination between true growth of attached bacteria and their immigration from surrounding waters. The cell settlement or detachment could be accounted for by a microscopic count of UV-sterilized control slides. The generation time of aquatic bacteria was found to vary from 2 to 30 h.

3.4.2. Methods Based on the Analysis of the Cell-Division Cycle

In eukaryotes, the cell cycle consists of four phases: mitosis, G_1, S, and G_2. Mitosis can be recognized morphologically. In many cell types, the time of mitosis (t_m) represents a constant fraction of the total cell-division cycle. If t_m is known, then the generation time, g, can be found from the relationship $t_m/g = 1.44\,R$, where R is the fraction of cells in mitosis. This method was used initially to measure the growth rate of the protozoa *Entodinium* in the rumen. The division frequency at night was higher than during the day, and the average generation time was about 15 h.

Hagström et al. (33) suggested that the growth rate of Gram-negative bacteria could be estimated from the frequency of occurrence of dividing cells. The division event (formation of septa and subsequent separation of the two daughter cells) is known to occupy a more or less constant time within the bacterial cell cycle. So, the higher the growth rate, the higher the probability of finding a cell at this stage. The calibration of the method was achieved using a mixed chemostat culture of marine bacteria. In the coastal region of the Baltic Sea, the frequency of dividing cells was below 5%, and the estimated mean generation time varied seasonally from 10 to 100 h.

3.4.3. Genetic Methods

Meynell (34) devised an elegant method to measure microbial growth rate using bacteria with a nonreplicating genetic marker. At each cell division, the fraction of the population which contains the label is halved. Once the dynamics of total and labeled populations are determined, the doubling time can then be calculated from the rate of marker dilution. Meynell studied the growth of pathogenic enterobacteria in the gut and blood circulation system of laboratory animals. The genetic markers were various superinfecting mutants of phages, which enter the bacterial cells, but do not replicate. It was observed that after intravenous inoculation into a mouse, virulent *Salmonella typhimurium* cells became lodged in the spleen. Their viable count doubled every 24 h, whereas the true doubling time as determined from the rate of marker dilution was 8 to 10 h. The same strain grew 20 times faster on nutrient broth ($g = 0.5$ h).

3.4.4. Techniques Stemming from Chemostat Theory

Many natural habitats are open systems, with a continuous supply of nutrients and the simultaneous elimination of cells. Under such conditions, the growth rate μ is eventually adjusted to the elimination rate, D (similar to the dilution rate in the chemostat). Now, the value of D is often easier to measure than μ. For example, in the case of bacteria growing in an animal's intestines, D is measured by feeding the animal food tagged with some inert label (lignin, silica-gel, dyes, etc). The time of 50% reduction in the output label concentration is expressed as $t_{0.5} = \ln 2/D$. The steady-state (or quasi steady-state) cell concentration, is measured in the gut of sacrificed animals. Using this method, enterobacteria in laboratory rodents (mice, rats, hamsters) were shown to yield between one and six generations per day.

Another ingenious technique was developed by Brock for measuring the growth rate of thermophilic algae in hot-spring drainways (35). The technique involved measuring the algal wash-out rate after growth was prevented by darkening the system. The spring was sheltered

from ambient light by a screen and the exponential decrease in cell concentration in the effluent stream was monitored. The value of D, which is equal numerically to μ under normal day-night cycles, was found to be 0.4/day ($g = 40$ h).

In static aquatic environments such as lakes and ponds, the main factor responsible for microbial cell elimination is no longer wash-out, but their predation by protozoa and probably other small animals. The growth of cells and their grazing rate are about to be balanced. If under in situ experiments, predation is completely suppressed by passing the water sample through filters retaining large protozoan cells, then the μ value may be measured from the recorded increase in the bacterial population (7). However, this approach should be used with care. Suppose we are to measure the value of μ in a chemostat culture from the x dynamics after stopping the flow. It is obvious that by halting the pump operation, we terminate not only cell washout but also the substrate input with fresh medium. Therefore, the use of this method is restricted to only nonlimited microbial growth. These conditions are fulfilled in the chemostat only at $s \gg K_s$ (i.e., at high s_0 and subcritical D), so that reliable application of the method is limited to natural eutrophic habitats.

3.4.5. Isotope Techniques

The high sensitivity of radio-isotope techniques allows for the measurement of the rates of consumption of labeled substrates added to water at nearly background concentration. The main problems include: (a) how to estimate the ratio between added labeled and natural nonlabeled compounds, and (b) how to derive the rate of microbial growth from the measured rate of label consumption. Several examples are provided below.

Dark $^{14}CO_2$ fixation rate as a measure of total heterotrophic bacterial production was originally suggested by Romanenko (36). Heterotrophic CO_2 fixation is an anaplerotic metabolic reaction, serving to regenerate those metabolic intermediates which are "lost" from the TCA cycle for the synthesis of macromolecules. Hence, the measured fixation rate is expected to be tightly coupled to the overall cell growth, through metabolic control. However, the experimentally observed ratio of carbon fixed from CO_2 to total carbon assimilated has been found to vary in a wide range, from 0.01 to 0.12. In view of this fact, it was suggested (37) that measurements of CO_2 fixation should be accompanied by a determination of the activity of PEP-carboxylase, the principal anaplerotic enzyme. This would allow for more rigorous conclusions about the stoichiometry involved.

The primary productivity of phytoplankton is determined by the measurement of the rate of $^{14}CO_2$ photoassimilation. In recent modifications of the technique, it was suggested that the label incorporation be determined in the fraction of chlorophyll *a* rather than in whole particulate matter. This allows the estimation of phytoplankton biomass and avoids possible underestimations caused by label transfer from algae to bacteria and zooplankton via excretion and grazing respectively (38).

Nowadays, the most promising technique for the evaluation of secondary productivity (microbial growth rate) in waters is considered to be the measurement of the uptake of labeled precursors of nucleic acids biosynthesis, thymidine, uridine and adenine (39). The use of isotopes with high specific activity guarantees minimal alterations of in situ growth conditions. At the same time, the amount of added nucleoside should be large enough to suppress their

synthesis de novo from endogenous cell compounds. The main deficiency of this routine is the ambiguity of conversion factors from a nucleoside uptake rate to a microbial growth rate.

3.4.6. Assessment of Productivity from Fluctuation Frequency of Microbial Biomass

The first systematic studies of bacterial production in soil were undertaken by Aristovskaya (40). The work involved daily measurements of the number and size of bacterial cells by direct microscopy of soil smears. Bacterial production was evaluated from the shape of the dynamic curve $x(t)$. This curve was always characterized by a seesaw pattern. Every 3–8 days increases in x were observed followed by declines down to background level. Fluctuations did not depend immediately on environmental factors and occurred even under stable hydrothermal conditions. This type of fluctuating dynamics was first observed as early as the beginning of the century and was explained by two mechanisms: (a) by a predator-prey interaction of soil bacteria with microfauna (mainly with amoebae), which usually gives rise to oscillations in the population densities of both prey and predator (41); and (b) by the accumulation in soil of self-inhibitory metabolic products (H_2, ethylene oxide, a hypothetical compound "periodine," etc.), which are susceptible to spontaneous autoxidation, decomposition or dispersion (40).

For calculating productivity, Aristovskaya assumed that bacterial growth is periodically interrupted by toxin accumulation while grazing of microbes was executed continuously. From this, the overall production of "seesaw" bacterial growth was calculated as the following sum: apparent x increase (measured during intervals where $dx/dt > 0$)+bacterial biomass elimination (estimated as x decreases at time intervals when $dx/dt < 0$). This calculation algorithm may underestimate as well as overestimate the true bacterial productivity. The generation time was found to vary in the seasonal dynamics from 7 to 100 h, with a seasonal bacterial production of 1–6 tons of dry weight per hectare. When compared with natural waters, the microbial growth rate in soils was roughly the same, whereas the seasonal productivity was higher by an order of magnitude. For example, the net bacterial production over one season in the Rybinsk water reservoir was as low as 200 kg/ha, while in podzolic soil of the same bio-climatic zone it was 1,500 kg/ha.

3.4.7. Estimation of Productivity from C-Balance

A simple relationship exists between the respiration rate of aerobic chemoorganotrophs v_{resp}, their biomass (x) and specific growth rate (μ):

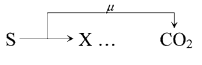
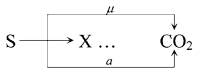
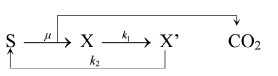
$$v_{resp} = Y_{p/x}\mu x \tag{13}$$

Although the biomass yield, $Y_{p/x}$, depends on numerous factors, it can be measured as the net average value in calibration experiments for the entire microbial community of a particular soil. The main advantage offered by this method is the possibility for continuous and exact recording of in situ metabolic rates by CO_2 analysis. Of course, one must be able to distinguish microbial and plant roots activity to the overall soil respiration, but this is basically feasible. A rough estimation based on soil respiration data (42), revealed lower productivity of soil microorganisms as compared with previous calculations, but systematically this approach has not been implemented.

Table 4.5a
Estimation of soil microbial growth from mass balance of entire ecosystem

Ecosystem, ref	Microbial biomass, kg/ha	Warm period, day/year	Input of plant litter, kg/ha/year	Mean generation time (day) according to equation			Microbial production, t/ha/year
				Eq. (14)	Eq. (15)	Eq. (16)	
Subarctic bog (43)	310	100	1,560	34.4	$\mu < 0$	20.7	1.0
Mixed forest (44)	148.4	150	12,000	3.2	3.6	1.9	8.1
Coniferous forest (45)	192	150	4,920	10.1	15.6	6.1	3.3
Soil under wheat (46)	400	100	7,080	9.8	14.8	5.9	6.8
Soil under continuous wheat (28)	1,140	100	2,400	82.3	$\mu < 0$	49.4	1.6
Virgin steppe (47)	800	190	23,900	11.0	17.8	6.6	16.0

Table 4.5b
Mathematical expressions used for the calculation of microbial productivity in soils

Model	Graph	Equation of mass balance	Production term	
Simple chemostat-type model		$\dot{s} = F - \mu x / Y$ $\dot{x} = \mu x - kx$	$\mu x = YF$	(14)
Account of maintenance		$\dot{s} = F - \mu x / Y^{max} - ax / Y^{max}$ $\dot{x} = \mu x - kx$	$\mu x = Y^{max} F - ax$	(15)
Account of microbial biomass reutilization		$\dot{s} = F - \mu x / Y + k_2 x'$ $\dot{x} = \mu x - k_1 x$ $\dot{x}' = k_1 x - k_2 x'$	$\mu x = \frac{FY}{1 - Y}$	(16)

Symbols: S organic substrate, X viable biomass, X' necromass or microbial detritus, F input of OM, μ is specific growth rates of microorganisms, Y and Y^{max} are yield coefficients, k_1, k_2 and a are specific death or decay rates.

The second type of mass balance evaluations of microbial production in soil is more common and involves the estimation of C input to soil from plant litter and root deposition. These values are supposed to be equal to C-substrate consumption by the heterotrophic microbial community.

Tables 4.5a and 4.5b present the available experimental data on C-budget of several terrestrial ecosystems, which we used to calculate the rates of microbial growth by different methods. Equations (14) and (16) give, respectively, an upper and lower boundary of the microbial growth rate. Calculated generation times vary within a range of 5 to 50 days, corresponding to 3–25 generations per season. (The only exception is soil 5, where microbial

biomass is definitely overestimated; as a result, average generation time increased here up to 3 months). The seasonal production of microbes is of the order of tons dry weight per hectare and exhibits a steady increase parallel to the primary production of an ecosystem from 1 ton/ha for tundra to 16 tons/ha for chernozem under virgin steppe. When compared with the "seesaw" method, the mass balance calculation yields smaller microbial growth rates, but the difference is small when more realistic models [like Eq. (16)] are used. Thus, in four out of six soils, the generation time is less than one week, which corresponds to the oscillation period found in most observations. Calculations with the use of incongruous kinetic models [like Eq. (15)] yield longer generation times, the difference being up to several orders of magnitude. Sometimes, such models "predict" that microbial communities in particular soils are not sufficiently supplied by energy even to maintain their viability. Ironically, it was this type of incongruous kinetic model which shaped the dominating concepts on the physiological state of soil microorganisms.

4. DIVERSITY OF MICROBIAL HABITATS IN NATURE

Traditionally, the *biosphere* (ecosphere) is divided into atmo-, hydro-, and litho-ecospheres to describe the portions of the global expanse inhabited by living things in air, water, and soil environments, respectively. Here, we will survey the major types of microbial habitats to underpin the origin of their diversity and importance for various biotechnological applications.

4.1. Terms and General Principles (How to Classify Habitats)

Each habitat has a set of physical, chemical, and biological parameters that determine the microbial populations that may thrive there. As a result of natural selection forces, characteristic communities develop within each habitat. In some cases, particularly in extreme habitats such as salt lakes and thermal springs, the indigenous microbial populations exhibit adaptations to their physical and chemical surroundings that permit their survival. In other habitats, intense competition dictates which populations survive and become the autochthonous members of the communities living there.

A *habitat* is the physical location where an organism is found. The term *ecological niche* includes also "the profession" of a respective population, i.e., what organisms do there. The niche is the functional role of an organism within an ecosystem.

Some microorganisms are *autochthonous* or indigenous within a given habitat. They occupy the available environmental niches and are able to escape predation and compete successfully with the other members of the microbial community. Other so-called *allochthonous* microorganisms are grown elsewhere and transported into a given habitat to be there a transient member of community. They do not occupy the functional niches and typically they are weak competitors (although temporary could be abundant).

Although the definitions of autochthonous and allochthonous microorganisms are mutually exclusive, it is often difficult to determine whether a microorganism found in a particular ecosystem is indeed autochthonous or allochthonous. The truly autochthonous organisms can temporarily slow down growth and activity in situ, while allochthonous microorganisms that

have arrived into a new habitat may be able to survive, grow, and carry out active metabolism and perhaps to become autochthonous microorganisms.

For higher organisms, such as animals that range over wide territories, the habitat may be on the scale of a landscape. By contrast, the habitat for microorganisms often occurs on a microscale. Hence, for microorganisms, one must consider not only the overall characteristics of the general habitat but also the fine features of the microhabitats in which the microorganisms live.

Most natural habitats (soil, subsoil, sediments, and wetlands) are heterogeneous on a microscale. They form mosaics of relatively independent microsites, each of which can be drastically dissimilar in the amount and nature of growth substrates, aeration level, texture and moisture, etc. However, there are several very general features which are the most essential for determining the fate and functioning of microorganisms occupying respective macrohabitat or microsite:

1. *The way of delivery of nutrient substrate(s) to habitat.* The quality and quantity of microbial substrates vary widely. If we consider only the limiting and preferential/available substrates, then the most essential factor controlling microbial growth in situ is the dynamic pattern of substrate input, which can be a continuous (*cont*) and a discontinuous (*Dis*), single-term delivery of respective chemical species to particular loci.
2. *The elimination of growing cells.* Bacteria or fungi growing in situ can be retained within a microhabitat (*Ret*) or removed/eliminated (*Rem*). Examples of elimination include: cell washout, predation (consumption of microbial cells by protozoa, nematodes, microarthropods) or lysis by parasites, active migration due to taxis (motile bacteria) and tropism (vectorized apical growth of hyphal organisms). With low or zero elimination and continuous supply of nutrients, biofilms or microbial mats are formed, which are visible multilayer cell accumulation on various inert solid surfaces. In soil and waters, such accumulated immobile cells perform an important function of *geochemical barrier* to key elements used as nutrient substrates: the element's concentration in water or air drops by several orders of magnitude after passing through the microbial layer.
3. *The spatial organization of habitat.* Habitats can be homogeneous (*Hom*) and heterogeneous (*Het*) or spatially organized. In the first type of system, we have an even or at least random distribution of cells, substrate, and metabolic products across the space; in the second type of habitat, we can see regular spatial gradients. Typically, most aquatic habitats (ponds, lakes, sea) are rather homogeneous and well mixed at micro- to mesoscale (from 10^{-6} to 1 m) although there is distinct vertical stratification at the higher scale (see below). Solid habitats (soils, subsoils, sediments) are generally much more heterogeneous and mosaic; however, there are many situations when the soil environment can be safely considered homogeneous, e.g., strong homogenization is done by soil tillage and by fossorial animals. Homogeneous soil microenvironments are also formed when the growth substrates are mobile, i.e., gases and volatiles.
4. *Extreme and favorable habitats.* Favorable physico-chemical conditions are vaguely defined as those which are close to a "physiological optimum" of the majority of organisms: pH $\sim$ 7, mild hydrothermal conditions, absence of toxic compounds and any other stressful factors (moderate salinity, pressure, radiation level, etc). Under these favorable conditions, competition is very strong and plays the most essential role in community dynamics. Extremely unfavorable ecotopic conditions imply that one or more environmental factors are outside of the tolerance limits for most of known organisms: too cold or too hot, strongly acidic or alkaline, dried, irradiated, intoxicated, etc. Biological competition between *extremophiles* occupying a given habitat is minimal; the main selection factor is resistance to the key unfavorable factor.

Table 4.6
Matrix of growth patterns in situ and ex situ for favorable habitats (after (7))

Spatial organization	Substrate input			
	Continuous		**Dis**continuous	
	Cell **Rem**oved	Cell **Ret**ained	Cell **Rem**oved	Cell **Ret**ained
Homogeneous	*Con-Rem-Hom*	*Con-Ret-Hom*	*Dis-Rem-Hom*	*Dis-Ret-Hom*
Heterogeneous	*Con-Rem-Het*	*Con-Ret-Het*	Forbidden combination	Forbidden combination

The first three independent characteristics of ecotopic conditions produce $2^3 = 8$ potentially possible combinations (Table 4.6), some of them logically disallowed, e.g., any heterogeneous habitats are not compatible with the single-term delivery of substrates because even in the simplest case, molecules of substrates are *continuously* delivered to microbial cells across the concentration gradient.

Having reviewed the general principles, we now proceed to a short survey of the major types of microbial habitats in the biosphere. The main focus is an assessment of the quality of respective ecosystem as microbial habitat, discussion of the degree of understanding of the key mechanism controlling growth, elimination, competition and functioning of microbial inhabitants. Such information seems to be the most essential for the development of biotechnological and bioengineering applications based on an understanding of the functional mechanisms rather than on an empirical trial-and-error approach.

4.2. Atmosphere

The atmosphere consists of 79% nitrogen, nearly 21% oxygen, 0.038% carbon dioxide, and trace amounts of some other gases.

4.2.1. Atmosphere as Extreme Habitat

The atmosphere is saturated with water vapor to varying degrees, and it may contain water droplets, ice crystals, and dust particles. The atmosphere is divided into regions (Fig. 4.13), the troposphere interfacing with both the hydrosphere and the lithosphere. Above the troposphere is the stratosphere, and above this lies the ionosphere.

For the most part, the chemical and physical parameters of the atmosphere do not allow microbial growth and even survival: low temperatures (from -43°C to -83°C), lack of substrates, low moisture content and intensive UV radiation. Therefore, we should classify the atmosphere as the largest of the Earth's *extreme* microbial environments. The supply of substrates which could potentially support heterotrophic, methanotrophic or autotrophic growth (respectively volatile organic compounds like VFA, alcohols, aldehydes, methane and CO_2) is continuous via turbulent and diffusive flux, but the ambient concentration is so low (ppb range) that it could be kinetically manageable only if air is pumped through a microbial cell layer retained on some immobile support (types *Con-Ret-Hom* or *Con-Ret-Het,* in the Table 4.6). However, the retention of cells in air is fully excluded and formally

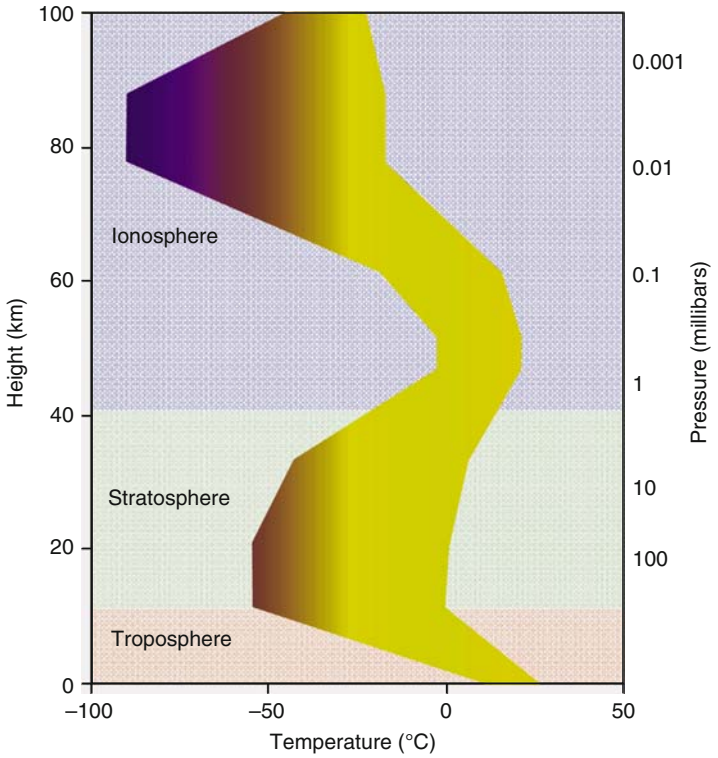


Fig. 4.13. Atmosphere as microbial habitat. Divisions of the atmosphere showing temperature and pressure gradients. The two lines indicate seasonal shifts in temperature. After (5), with permission from Pearson Education Publisher.

this type of habitats belongs to the category *Con-Rem-Hom*. Exposure to UV is probably the most powerful elimination factor; as the atmosphere thins at increased heights and offers less shielding from UV radiation, it causes lethal mutations and the death of microorganisms.

The stratosphere contains a layer of high ozone concentration, which acts to absorb UV light, protecting the Earth's surface from excessive UV radiation (48). There is a justified concern today that certain human activities, such as the flying of supersonic military and commercial jets, excessive use of fluorocarbons, and increased use of fertilizers (which results in increased release of N_2O from microbial denitrification), will decrease concentrations of ozone in the stratosphere, thus allowing increased amounts of UV light to reach the Earth's surface. The seasonal development of an Antarctic ozone hole is a clear symptom of the lessening atmospheric concentration of ozone.

4.2.2. Organisms

The stratosphere represents a barrier to the transport of living microorganisms to or from the troposphere and is characterized by a slow mixing of gases. Organisms in the stratosphere are thus transported slowly and are exposed for prolonged periods to the prevailing concentrations of ozone and high UV light intensities. Only microorganisms shielded from these conditions in the stratosphere – as perhaps within a spacecraft – could survive passage out of the Earth's atmosphere. For all practical purposes, the atmoecosphere does not extend above the troposphere (5).

Even though the atmosphere is a hostile environment for microorganisms, there are substantial numbers of microorganisms in the lower troposphere, where, because of thermal gradients, there is a rapid mixing of air (6). Some microorganisms have evolved specialized adaptations that favor their survival in and dispersal by the atmosphere. Several viral, bacterial, and fungal diseases are spread through the atmosphere; outbreaks of disease from such microorganisms often follow prevailing winds.

Temporary locations in the troposphere may provide habitats for microorganisms. Clouds possess concentrations of water that permit growth of microorganisms. Light intensities and carbon dioxide concentrations in cloud layers are sufficient to support growth of photoautotrophic microorganisms, and condensation nuclei may supply some mineral nutrients. In industrial areas, there may even be sufficient concentrations of organic chemicals in the atmosphere to permit growth of some heterotrophs. Nevertheless, such "life in the sky" is only a fascinating possibility; conclusive proof is lacking, and the practical importance of such life appears to be negligible (5).

Although many microorganisms that grow in the hydrosphere or lithosphere can become airborne, there are no known autochthonous atmospheric microorganisms. During dispersal, aquatic and soil microorganisms may enter and pass through the atmosphere before reaching other favorable aquatic or terrestrial ecosystems.

4.2.3. Significance for Environmental Engineering

The most important bioengineering task today is the development of monitoring of the atmosphere for potential biohazardous organisms, first of all pathogenic bacteria, fungi and viruses. Airborne pathogens are especially dangerous because of their direct invasion into the respiratory tract; on the other hand, bacterial aerosols could be easily detected (with higher speed and better sensitivity and precision) as compared with bacterial populations in soils and waters. The most promising procedure for scanning bacterial aerosols seems to be laser-based IR spectroscopy. A new and highly intriguing direction of environmental biotechnology is to regulate the physical state of the atmosphere by introducing bacterial aerosols of ice-nucleation bacteria which affect snowfall, freeze-resistance of plants, cloud formation, etc.

4.3. Aquatic Ecosystems

The hydrosphere is divided into freshwater (lakes, ponds, springs, swamps, streams and rivers) and marine habitats (seas, oceans and estuaries). All these habitats are interconnected to each other and terrestrial systems (Fig. 4.14). The world's oceans occupies 71% of the Earth's

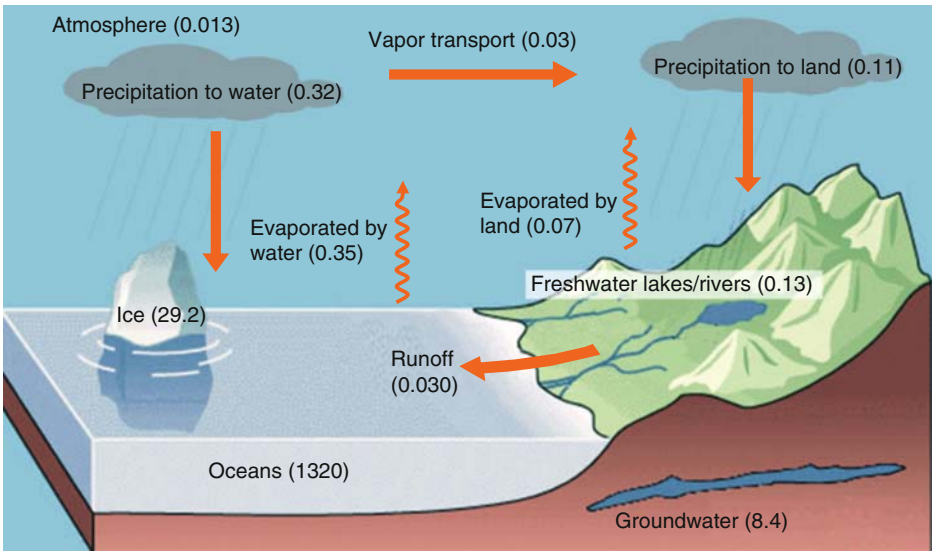


Fig. 4.14. The present-day surface hydrologic cycle. The numbers in parentheses refer to volumes of water in millions of cubic kilometers, and the fluxes adjacent to the arrows are in millions of cubic kilometers of water per year. After (49).

surface. Its huge water masses have an important buffering effect on the global climate, serving as the ultimate reservoir and receptacle of the global water and energy cycle. About 50% of the incident solar energy is consumed in the evaporation of water. Water vapor eventually precipitates as rain or snow, releasing the stored energy. The precipitation returns water to ocean directly or after passing over/through land as runoff. Therefore, the ocean is the ultimate basin for all water-soluble minerals and soluble recalcitrant organic matter derived from the terrestrial environment.

Freshwater habitats are classified based on their physical and chemical properties. Those with standing water (lakes, ponds) are called *lentic habitats*; those with running water are *lotic habitats* (rivers, streams, and brooks).

4.3.1. Lakes

Lakes are divided into several zones based on the penetration of light. In the upper *euphotic zone,* light is available to support photosynthesis. The deeper *profundal zone* is practically dark and does not support photosynthesis; two zones separated at a so-called *compensation depth* where photosynthesis is equal to respiration (usually here the photon flux is $\sim$ 1% of the full sunlight intensity). The *littoral zone* is the region of a lake where light penetrates to the bottom (Fig. 4.15). The *limnetic zone* refers to open waters inhabited by plankton. The bottom of the lake, or *benthos,* is the interface between water (hydrosphere) and solid sediments (part of lithosphere). Particulate nutrients (dead cells and cell aggregates of phototrophic organisms) are deposited by gravitational forces and concentrate on the surface of the benthic sediments.

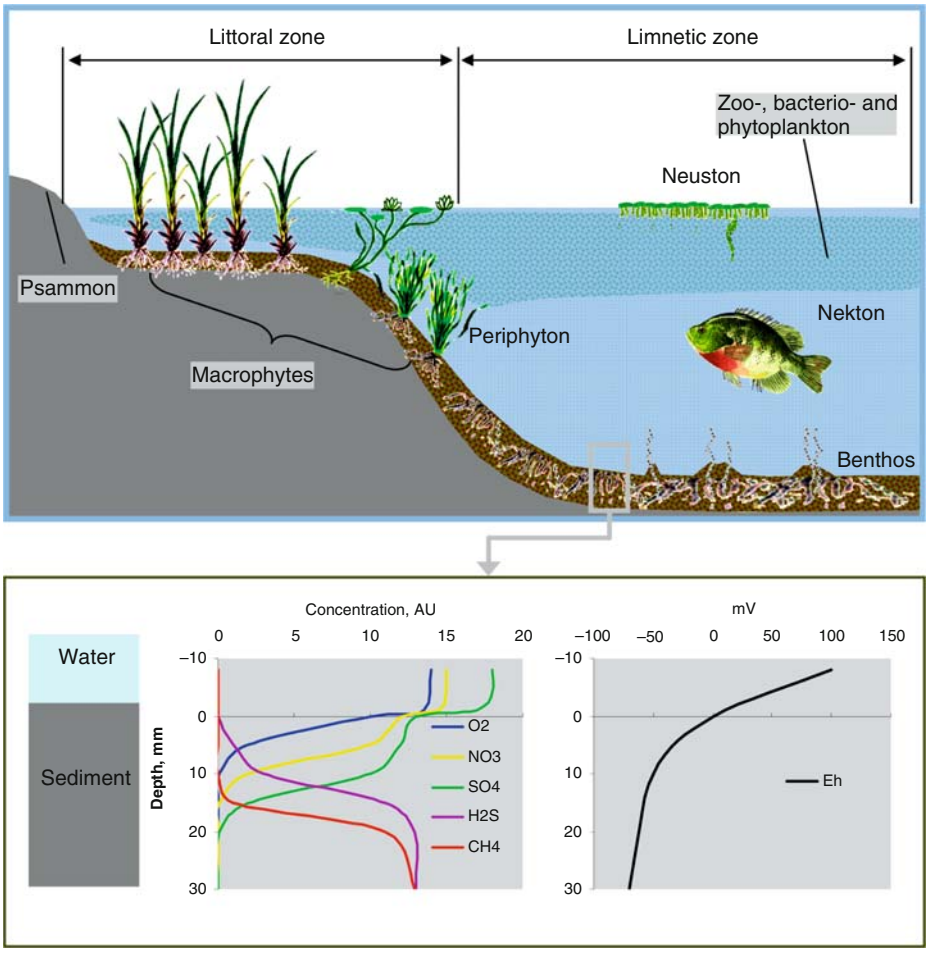


Fig. 4.15. The vertical profile of the typical lake. Insert shows vertical gradient of hydrochemical characteristics; note the sequential order of switching from one predominant electron acceptor to another with sediment depth.

The oxygen diffusion from the water to underlying sediment is rather low, thus only first several mm of sediment are aerated. Deeper layers accommodate anaerobic microorganisms which sequentially use alternative electron acceptors in the order of decrease of respective redox potential: $O_2 > NO_3^- > SO_4^{2-} > H_2O$. Generally, stratification of silt material may be found at the scale of micrometers into aerobic layers, denitrifiction, Fe-reduction, sulfate reduction and methanogenesis.

In addition, the lower portion of the water column in most freshwater lakes becomes seasonally anoxic. The mechanism is explained and illustrated by Fig. 4.16. The starting point is the fact that the maximal density of water corresponds to the temperature $+4^{\circ}C$ and both warming and cooling decrease water density. In the spring, as the sun warms the water, a

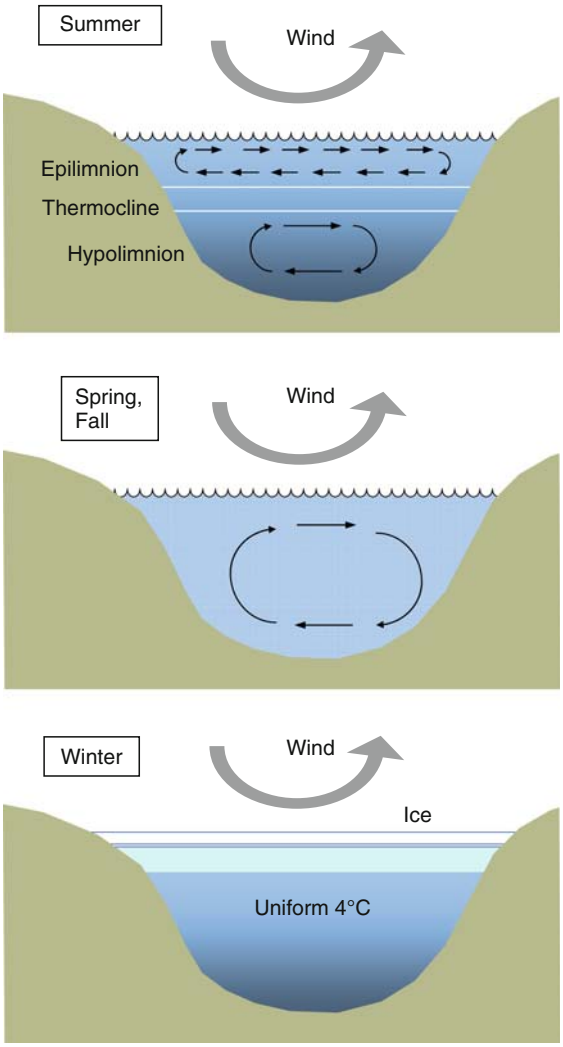


Fig. 4.16. Annual circulation patterns in a dimictic lake. The typical dimictic lake undergoes stratification in the summer and complete overturn in the autumn and spring. During winter, surface ice prevents further mixing by the wind. Small differences in density and temperature exist, with cooler water (0°C) staying near the surface and warmer, more dense water (4°C) extending to the bottom.

warm surface layer called the *epilimnion,* is formed. This warm, lightweight water ceases to mix with the lower, colder and denser layer (*hypolimnion*). The boundary between these layers is the *metalimnion* or *thermocline*, a zone of rapid temperature change. With the onset of autumn, the epilimnion cools and the water becomes denser, sinking and mixing with the hypolimnion. The work required to mix the two layers is provided by wind, and the lake

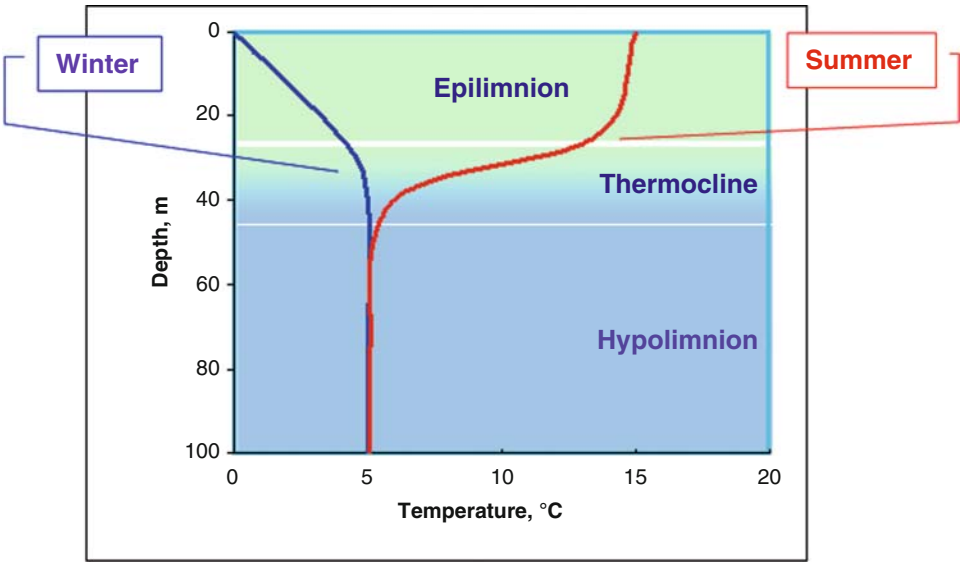


Fig. 4.17. Temperature-driven stratification of lake in summer and winter.

circulates, or overturns, completely. Circulation continues until the surface ice protects the lake from further wind action. The lake overturns again in spring after surface ice melts, and by summer it is stratified once again (Fig. 4.17).

Thermal stratification has a strong impact on the nutrient status of habitats. The epilimnion is not only warm, but also oxygen rich; the vigorous growth of phototrophic organisms tends to deplete the mineral nutrients. The cold and dark hypolimnion does not support high biological activity, the phototrophic organisms being suppressed more than the heterotrophic ones, therefore oxygen is partly depleted while mineral nutrients tend to be relatively abundant. In the fall, the thermocline breaks down, resulting in complete mixing of the lake.

In deep, freshwater lakes, the primary producers (plants) are found either at the shallow edges of the lake (emergent, submerged, or floating macrophytes) or free-floating within its upper layers (microscopic algae, cyanobacteria, and photosynthetic bacteria of the plankton community) (Fig. 4.15). Plants are found only in the photic zone. Animals and decomposers are found in both the photic and aphotic zones.

Other major biological components include:

- *Plankton*, which contains tiny floating plants (*phytoplankton*) and animals (*zooplankton*) as well as microbes (bacterioplankton);
- *Benthos* (bottom-dwelling organisms);
- *Nekton* (free-swimming forms in the water column);
- *Periphyton* (microscopic biota on submerged objects);
- *Psammon* (biota buried in sediments); and
- *Neuston* (biota associated with surface film).

The population density of microorganisms is significantly higher in so-called *eutrophic lakes* (high primary productivity and nutrient content, partial diurnal anoxia, usually shallow with large epilimnion and small or zero hypolimnion) as compared with *oligotrophic* lakes (low primary productivity and low nutrient content, high oxygen content, usually deep and clean with large hypolimnion). A more detailed classification in quantitative terms (Tables 4.1 and 4.2) gives several distinct categories of habitats:

- *Limnetic zone – a* homogeneous habitat with a continuous supply of organic substrates for heterotrophic microorganisms (release of exometabolic products by photosynthetic organisms, products of their cell lysis) and elimination of bacteria via protozoan grazing; photosynthetic microorganisms probably are limited by inorganic compounds whose availability significantly increases by inter-season mixing, formally it corresponds to a discontinuous supply of limiting substrate.
- *Littoral zone* should be characterized as a more eutrophic habitat with a continuous supply of mineral nutrients from terrestrial source; probably, most heterotrophic bacteria are directly associated with macrophytes (see below rhizosphere and phyllopshere sections).
- *Sediments* are split into at least two different categories. The first is the aerobic interface of sediment with water, the kinetic analog of the top soil layer with aerobic conditions and a continuous supply of C-substrates as deposition of particulate necromass of plankton; elimination due to grazing or washing should be very low. The second type of habitat is deeper and preferentially anaerobic layers of accumulated silt material, the major C-substrates are products of depolymerization and fermentation of necromass; these products are delivered slowly and continuously across the concentration gradient.
- Digestive tract of aquatic animals (see below special section).

4.3.2. Rivers

Contrary to lakes, rivers are characterized by flowing waters (Fig. 4.18). They have zones of rapid water movement and pools with reduced currents. The first type of habitats occurs at shallow parts of river, while pools are associated with deep water column and intensive accumulation of silt similar to lakes sediments. Rivers do not form a high degree of thermal and chemical stratification due to the continuous mixing of water. The zones of rapid water movement contain the sessile (firmly attached to the rocky stony bottom of river) forms of

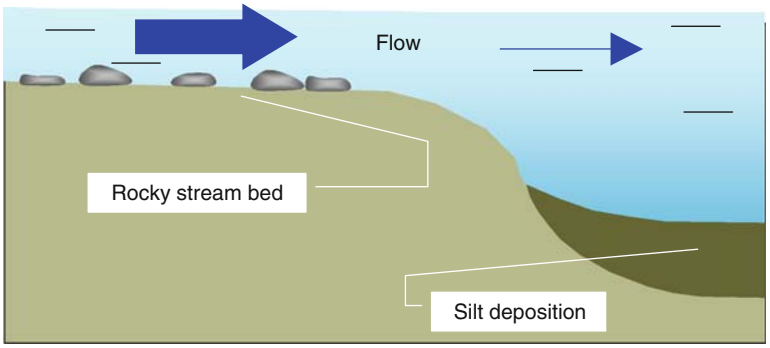


Fig. 4.18. Water flow in river provides different types of microbial habitats at shallow and deep parts.

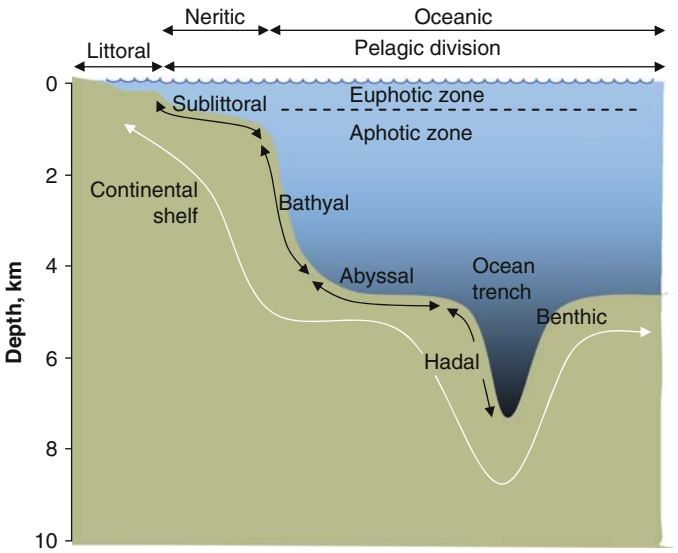


Fig. 4.19. Major vertical zones of an ocean profile.

life, such as macrophytes rooted to river bed and unicellular organisms forming biofilms. This attachment prevents the elimination of growing microorganisms by running water and provide a continuous supply of nutrients.

4.3.3. Marine Ecosystems

The availability of *light* is crucial for differentiating marine environment (49). The greater the depth of the water, the less light can penetrate until below a certain depth there is no light whatsoever. This area of inky darkness, which occupies the great bulk of the ocean, is called the *aphotic zone* (Fig. 4.19). The illuminated region above it is called the *photic zone*, within which are distinguished the euphotic (receives enough light for *photosynthesis* to occur) and *disphotic zones* (illuminated so poorly that rates of respiration exceed those of photosynthesis). Marine organisms are particularly abundant in the photic zone; however, many organisms inhabit the aphotic zone and migrate vertically to the photic zone every night.

Marine environments consist of water, or *pelagic, environment* and a bottom, or *benthic, environment* (Fig. 4.19). Within the pelagic environment, the waters are divided into the *neritic* province above the continental shelf, and the open oceanic waters. The neritic province is a much more eutrophic environment resulting from dissolved materials in riverine runoff. The pelagic water body is divided into several zones (epipelagic, mesopelagic, bathypelagic, and abyssalpelagic) according to depth. The intertidal, or *littoral, zone* ranges from the high-tide mark to the shallow, offshore waters. The sublittoral is the environment beyond the low-tide mark and is often used to refer to continental shelf (150–300 m). Sediments of the continental shelf that influence marine organisms generally originate from the land, particularly in the form of riverine runoff, and include clay, silt, and sand. Beyond the continental shelf is the

bathyal zone, which occurs at depths of 150 to 4,000 m and includes the descending continental slope and rise. The *abyssal zone* (between 4,000 and 6,000 m) represents a substantial portion of the oceans. The deepest region of the oceans (greater than 6,000 m) is the hadal zone of the deep-sea trenches. Sediments of the deep sea primarily originate from a rain of dead marine organisms and their wastes.

Summarizing the oceanographic data (Fig. 4.19), we can conclude that the trophic status of marine ecosystems depends on both the vertical and horizontal positions of a particular site. The supply of mineral substrates is smallest in a pelagic environment and grows while approaching coastal line (runoff). A significant increase in the nutrient level encourages circulation of oceanic waters and upwelling (Fig. 4.20). Probably, the majority of heterotrophic/saprotrophic marine organisms are limited by a supply of available organic substrates derived mainly from soluble exometabolites and the dead bodies of primary producers. The latter (photosynthetic bacteria such as *Prochlorococcus* and numerous algae) are limited mainly by mineral nutrients, among which are nitrogen, phosphorus and especially iron (9, 50). In most cases, a supply of substrates should be considered continuous with seasonal and diurnal fluctuations dependent on fluctuation of temperature and photone flux.

Marine protozoa have been shown to be important grazers of both prokaryotic secondary and microbial primary production. Enigmatic marine viruses, which proved extremely abundant in the sea, appear to be an important source of prokaryotic mortality, perhaps forming a smaller "viral" loop within the microbial loop.

Interactions between microorganisms in marine sediments differ from those in planktonic communities. Sediment assemblages are much more densely populated ($\sim 10^9$ cells/g vs. $\sim 10^6$ cells/mL in the water column), more diverse, with a rather slow turnover rate. Actually, the sediment community should be considered as being in mid-way between aquatic and terrestrial habitats (soils and subsoils). Sediment prokaryotes are probably limited by the

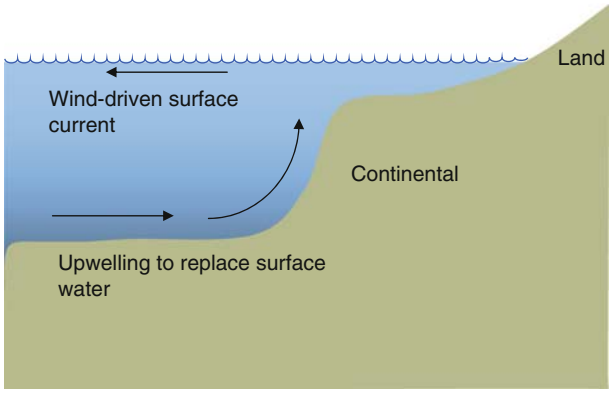


Fig. 4.20. Upwelling of deep ocean waters along continental slope to replace surface waters driven offshore by wind.

availability of electron donors and electron acceptors, which creates the fine vertical zonation described earlier.

4.3.4. Significance for Environmental Engineering

Most aquatic habitats are essentially less resistant to pollution than their terrestrial counterparts. In contrast to soil, which has a large active surface area and tremendous absorbtion capacity, aquatic habitats have a limited "buffering" capacity to resist pollutants. On the other hand, rivers, lakes and marine ecosystems are more homogenous and transparent, making them easier to monitor and control the course of remediation. Probably, the introduction of "beneficial" microbial cultures to waters is more efficient and feasible as compared to heterogeneous natural habitats. The composition and functions of the aquatic microbial community is better understood because it is less complicated (500–1,000 16 rRNA phylotypes as compared with 10^4–10^6 species in typical soils). Therefore, aquatic habitats are more probable candidates for development of the so called *ecosystem-based management* of these natural environments, probably first of all for controlled fishery (51). These include the fields of food processing and chemical production, medicine and bioactive materials production, cleaning of the oceans and of the air, and others.

4.4. Terrestrial Ecosystems

Within the terrestrial habitats, we will focus on arable and virgin soils, subsurface and continental wetlands as the most important natural objects.

4.4.1. Soil

Soil is typically known as the boundary upper layer of lithosphere supporting plant growth. There are several layers or horizons in the vertical soil profile which are morphologically distinguishable and associated with different quality of soil as microbial and plants habitats (Fig. 4.21). Apart from continuous soil horizons, there is a mosaic of rhizosphere soil which consists of soil particles firmly adhered to the plant roots and considerably affected by root exudates (low molecular weight compounds, mainly organic acids) as well as by *root sloughing*, the release into soil of polymeric polysaccharides and proteins from the surface of growing roots.

Soil differentiation into specific habitats is driven mainly by plants (roots development, supply of available organic matter, leaching of minerals with aggressive plant and microbial metabolites) and is dramatically different as compared with lakes and oceans: instead of continuous and regular vertical gradients of the key environmental parameters (temperature, light intensity, oxygen and nutrients), we can envisage various *microgradients* which form a mosaic of *microhabitats* or *microloci*. Figure 4.21 depicts several types of such habitats. The most spacious and the poorest/oligotrophic habitat is the *dispersion zone* (*Con-Ret-Hom*), the subsoil and the patches of bare top soil devoid for some reasons plant roots or fresh litter. Numerous microbial populations inhabiting these habitats grow very slowly on volatile or readily soluble compounds which continuously diffuse from other soil loci, where monomeric concentration is high due to intensive decomposition (plant litter) or excretory activity of plants (rhizosphere). The lack of elimination (no motility in majority of soil bacteria and no

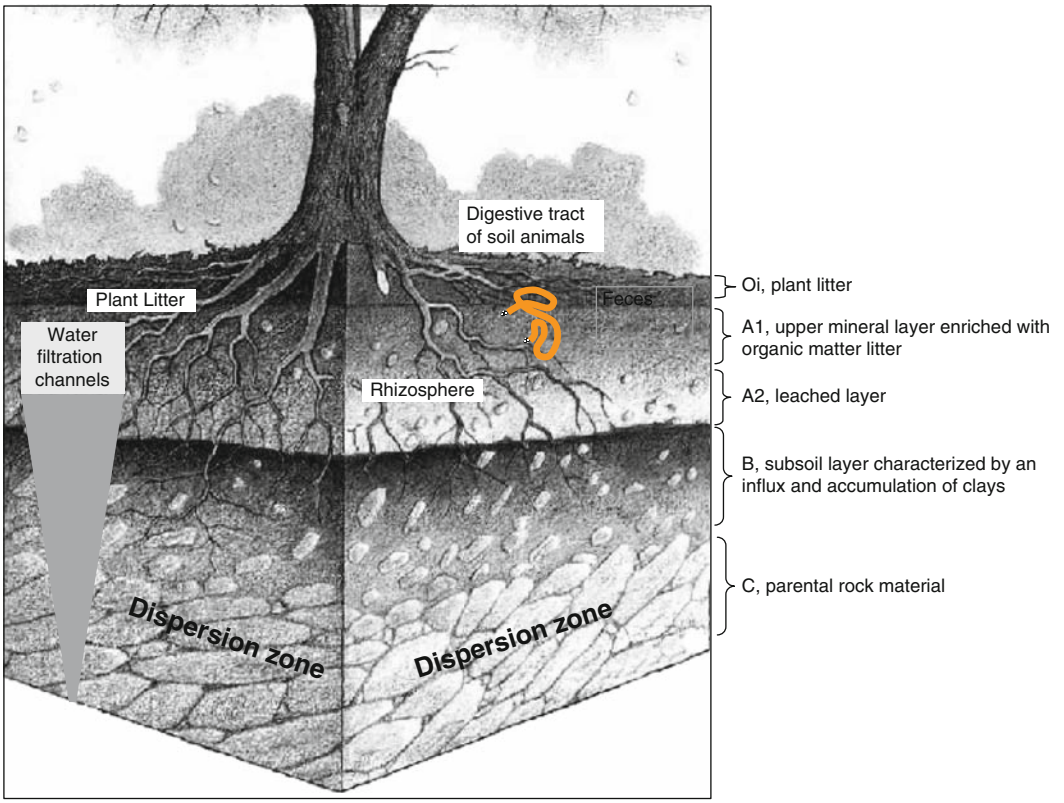


Fig. 4.21. Soil profile and major microbial habitats (see text and Table 4.6 for explanation). After (18) with permission of Elsevier.

predation due to low prey density) combined with slow but uninterrupted continuous growth results eventually in significant build-up of half-dormant cell mass.

The highest and continuous microbial activity is localized in the soil *litter layer, rhizosphere* and *digestive tract* of soil animals (habitats of the types *Con-Rem-Hom* and *Con-Rem-Het*. The C-substrates for microbial populations are the monomeric labile compounds (sugars and organic acids) derived from plants either as root exudation in the rhizosphere or released by extracellular hydrolytic enzymes from the lignocellulose and other polymeric material in plant litter. The litter layer on the soil surface is formed from the fall of aboveground plant remnants, while belowground plant senescence (root litter and root sloughing) provide microbial C-substrates in the rhizosphere. Root exudation is closely related to plant photosynthesis and displays diurnal dynamics, while hydrolytic release is monotonous. Spatially, all these habitats are rather heterogeneous; however, the random distribution of microloci combined with macroscopic sampling size allows us to use homogeneous kinetic models. Clear vertical special gradients are formed in the litter layer: from uncolonized fresh plant debris on the

top to highly decomposed sublayer at the interface with mineral soil. On a microscale, the spatial heterogeneity of microbial colonies (mainly fungi) is manifested in its differentiation into growing extension zone and nongrowing reproductive compartments (Fig. 4.22).

Within the rhizosphere, there are also several spatial gradients of different scales: (a) the vertical gradient of the root phytomass which reflects the spatial pattern of belowground allocation of photosynthate, (b) the horizontal gradient between distant trees or tussocks, and (c) the microscale gradient around root hair with a maximal concentration of microbial cells, microscopic grazers and substrates on the root surface (rhizoplane) and exponential decline outward (sometimes bacterial density declines in the vicinity of the plant surface due to excessive grazing or excretion of antibiotic compounds by plants).

The soil millipedes, isopods, some earthworms and other *primary decomposers* inhabit the litter layer and feed on plant debris. The ingested lignocellulose material is mechanically

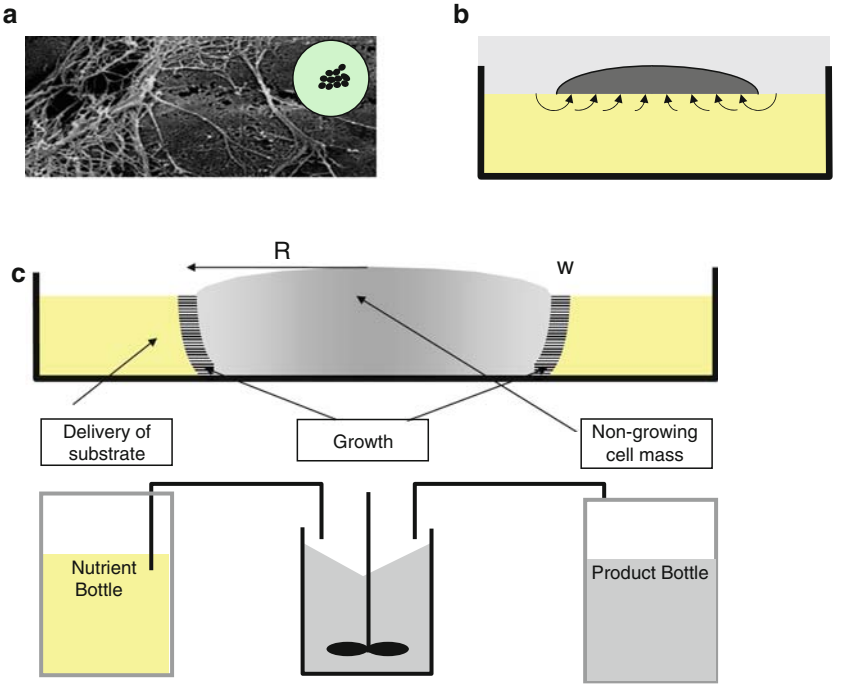


Fig. 4.22. The colony growth: (**a**) in the soil; (**b**) bacterial growth on agar plate; (**c**) fungal growth in nutrient agar. After (18) with permission of Elsevier. Note that bacteria grow only on the agar surface and the colony expansion is controlled by diffusion of substrates from outside of the colony. The fungi and actinomycetes are able to penetrate into the depth of the agar layer, so their mycelium expansion is not dependent on nutrient diffusion within agar layer. The fungal colony follows a chemostat-type growth pattern, being (**a**) continuous, (**b**) steady state, and (**c**) limited by substrate availability. The role of the fermentation vessel of the conventional chemostat is played by the peripheral zone of the colony; the product bottle is analogous to the central part of the colony, while the pump is substituted by the chemotropic movement of hyphae tips along the substrate concentration gradient.

disrupted by the decomposers' mandibles, moistened with saliva and then passed to mid- and hindgut. It is important that the digestive tract of various soil invertebrates harbor not only specific symbionts, but also the normal free-living microorganisms occurring in soil or plant litter, e.g., *Pseudomonas, Flavobacterium, Vibrio, Enterobacter, Streptomyces*. Acceleration of their growth in the hindgut is due to favorable conditions such as neutral pH, optimal moisture, elevated concentrations of nutrient and growth factors (amino acids, peptides, vitamins) as well as the continuous input of fresh substrate and concomitant removal of digestion products (glucose) to prevent the negative feedback (catabolic repression) on cellulase synthesis. Because of peristaltic motion, the content of the gut is mixed and homogeneous. The secondary decomposers (i.e., the earthworms *Allolobophora chlorotica*) feed on amorphous humus containing bacteria and fungal mycelium; they eliminate some microbial species and greatly stimulate the growth of others.

Discontinuous explosive microbial growth occurs within hot spots initiated by a sudden increase in the available organic substrate/nutrient in the soil (*Dis-Rem-Hom – Dis-Ret-Hom*) coming from feces and carcasses of animals, rain washing of organic compounds from the plant foliage, drying-rewetting or freezing-thawing cycles, application of manure, soil fumigation, etc. Growth is usually accompanied by elimination in the form of grazing, myco- and bacteriolytic activity, as well as by the active migration of microbial cells.

4.4.2. Deep Subsurface

In the past decade, it was found that terrestrial microbial life was not limited to the dark-colored humus-containing upper soil layers: both plating and direct microscopy revealed up to 10^6–10^8/cells per cc of bacteria, yeasts and fungi in the subsoils going down to a thousand meters. The deepest samples that yielded bacteria were 3,900–4,200 m deep and contained thermophilic fermentative bacteria. Much of this research was supported in the USA and Europe to explore the consequences of the subsurface disposal of hazardous nuclear and chemical wastes (52). Obviously, microbial activity in subsurface geological formations could influence the fate and mobility of waste materials. The findings were surprising and had significance much beyond subsurface waste disposal.

At least in undisturbed formations, the age of geological layers increases with their depth. Some geological layers are water-permeable and constitute aquifers; others are water-impermeable. Aquifers separated from the surface by one or more water-impermeable layers are called "confined" aquifers (Fig. 4.23).

The obvious and still unresolved question is what food and energy resources do these subsurface bacteria survive on. Most of the bacteria appear to be heterotrophic and anaerobic species (methanogens, sulfate reducers, fermenting yeasts and bacteria). Photosynthetic production is impossible, and the leaching of undegraded but soluble organic matter to deep soil layers is very limited. There could be bacterial primary production based on chemosynthesis: oxidation of ammonium, sulfur and especially molecular hydrogen. The deposited organic carbon in sedimentary rock may become available at a slow rate to support heterotrophic activity, and mobile (gaseous and dissolved) organics may enter aquifers from fossil gas, oil, or lignite deposits. The population density of the deep subsurface microbes should be

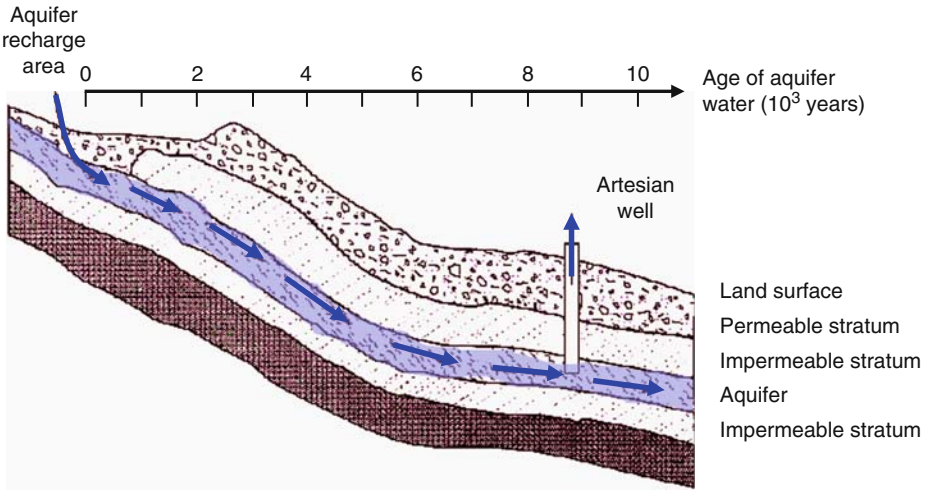


Fig. 4.23. Cross section of a geological formation with water-permeable and water-impermeable strata and a confined aquifer. Deep subsurface bacteria may be sampled in the water of artesian wells. The distance of the wells from the aquifer recharge area correlates with the time the water spends in the aquifer. Bacteria are present and sometimes abundant (10^6/mL) in aquifer waters from more than 1,000 m deep; these bacteria have spent several thousand years in the aquifer. After (5) with permission from Pearson Education Publisher.

essentially lower than that which supports intensive grazing by Protozoa (just imagine how efficient the feeding of wolves would be on mosquitoes!).

4.4.3. Wetlands

The term wetland implies at least two environmental qualities: water saturation and anoxia (lack of oxygen). Some wetlands are nonpeat-forming, such as intermittently flooded marshes, but most of them belong to category peatland or mires, ecosystems where the long-term production of organic material exceeds the rate of decomposition, leading to peat accumulation. Mires are usually classified as: a) bogs, which are fed by rainwater (ombrotrophic) and are therefore poor in dissolved nutrients; or b) fens, which are fed by ground water (minerotrophic) and therefore richer in mineral solutes (N, K, P, Mg...) from terrestrial sources (53, 54). In this section, we deal primarily with Sphagnum bogs, which also contain some other plants (sedges, ericads, and dwarf trees), but have in common such qualities as acidity (pH 3–5), and oligotrophy (low content of mineral compounds).

Sphagnum bogs are the predominant type of peatlands covering about 3% of the total land surface. Of the total mire area, 90% lies in the subarctic, boreal and temperate zones of the northern hemisphere, while the remaining 10% is found in the tropics. Geographic regions with an especially high density of peatlands include Alaska and Eastern Canada in North America as well as West Siberia and Northern Europe in Eurasia. Here, mires can cover as much as 10–30% of the land surface. The two largest continuous peatlands are those of the Hudson Bay lowland, Canada, covering $320,000\,\mathrm{km}^2$, and the Western Siberian Lowland,

covering 540, 000 km^2. Peatlands are estimated to contain about 450 Pg of carbon (55), which represents approximately 30% of all terrestrial carbon in biomass.

As a microbial habitat, the *Sphagnum* peat has several specific qualities: (a) an extremely low content of mineral nutrients delivered mainly through rain, (b) toxicity of Sphagnum metabolites, (c) low pH, (d) weak buffering capacity of soil solution, (e) predominantly low temperature, (f) anoxia, and (g) stagnation. There is a lack of consensus among wetland ecologists as to why microbial decomposition in mires is more restricted than plant growth. Three explanations are usually given: (a) the simultaneous action of all restrictive factors together (54), (b) "intrinsic" inability of microbes to degrade phenolic compounds under anaerobic conditions (56), and c) the severe limitation of microbial activity by mineral nutrients (7).

According to the last view, the peatland quality of having an extremely low nutrient content is the most essential, while low temperature and anoxia could not be restrictive thanks to the wide distribution of psychrophilic and anaerobic organisms. Other peatland qualities (b–d) are in fact not primary: they are derived from quality a (low nutrient content). Exudation of acidic exometabolites by Sphagnum results from C-overflow under limitation by N, P, and K and ample supply of CO_2 from air. The excreted organic acids remain mobile and aggressive because of the lack of free bases. These free organic acids are toxic at pH 3–5 near their pK's as a result of passive diffusion of uncharged molecules into the cell interior with subsequent ionization (pH $\sim$ 7 in the cell's interior) and discharge of transmembrane proton gradient. Finally, peatland quality g (stagnation) exacerbates self-poisoning due to extremely slow physical removal, leaching or volatilization of accumulated acids.

Contrary to the most familiar types of oligotrophic habitats (lakes, subsoils, sediments) which are C-limited and mineral-sufficient (57), the *Sphagnum* bog is mineral-limited and C-sufficient (practically unlimited supply of CO_2 from atmosphere to plants, and continuous flux of rhizodeposition from plants to soil microorganisms). We can hypothesize that indigenous microbes have evolved special metabolic mechanisms to live in an unbuffered, low-mineral, C-sufficient and toxic environment. That could be the main obstacle for microbial isolation because the conventional microbiological technique is a complete antithesis: C-limited buffered media with excess of mineral salts, agitation or exposure to fresh anaerobic mixture with removal of metabolic products.

4.4.4. Significance for Environmental Engineering

Terrestrial habitats are the major type of environment involved in agricultural production and remediation, the most essential branches of biotechnology and bioengineering. Resistance to pollution is very high due to these habitats' ability to absorb molecules and ions of pollutants by soil clays and humic polymers (all of them having acidogenic functional groups). On the other hand, accumulation of toxic compounds in the soil can be so severe that short-term remediation could be problematic. It makes the problem of early diagnosis of soil contamination especially important for environmental engineering to prevent irreversible chemical damage.

The main obstacle in many biotechnological developments remains the enormous complexity of the soil microbial community, the bulk of which is represented by unknown unculturable organisms with unknown metabolic features. Most are slowly growing microbes,

and restoration of the natural community after significant environmental perturbations can take years.

Subsoils are extremely important in relation to several industrial problems, such as prevention and prediction of subsurface contamination with heavy metals including radionuclides; pesticides and other inorganic and organic pollutants; as well as the development of bioremediation techniques aimed at cleaning and restoration of polluted subsurface environment. Other areas of environmental bioengineering as related to subsurface habitats include biometallurgy (use of microorganisms for leaching, separation and transformation of metals in ore deposits), enhancement of oil recovery (activation of microorganisms within the deep oil-carrying subsurface which allows to build-up the pressure and liquefies the oil forcing it up from the partly exhausted oilfields), deposit stabilization by microbial polysaccharides, etc. In all known examples, the positive effects are achieved either by deliberate stimulation of the indigenous microbial populations (adding growth substrates, aeration, amelioration) or by direct release of the specially prepared microbial biomass preliminary grown in fermentor.

Finally, the wetlands are now considered a major component of the global C-budget essential for controlling global warming, flooding and desertification. The reason is that peat accumulated in wetland's area concentrates significant resources of organic carbon and greenhouse gases (CO_2, CH_4, NO_x) affecting the Earth's thermal balance. Accelerated decomposition of the peat leads to CO_2 accumulation in the atmosphere, instability of the climate system expressed as frequent flooding, hurricanes, uneven distribution of water across terrestrial space (desertification of some areas combined with flooding in others).

Besides natural and arable soils and wetlands, the terrain is covered by numerous, man-made industrial objects that are becoming an essential part of the Earth's system. The concept of *industrial metabolism* was developed as a functional analog of the cellular metabolic network (58): the use of materials and energy by industry and the way these materials flow through industrial systems and are transformed and then dissipated as wastes. It is possible to trace the mass and energy flows and identify inefficient processes that result in accumulation of industrial waste and pollution. Further development of this concept combined with the attractive idea of making industrial systems emulate more efficient and sustainable natural systems, eventually led to the birth of a new branch of ecology called *industrial ecology* (59, 60). In an ideal industrial ecosystem, the waste produced by one company would be used as resources by another. No waste would leave the industrial system or negatively impact natural systems.

Industrial ecology relies on a *systems approach* which provides a holistic view of environmental problems, including the links between industrial activities and environmental processes, making them easier to model, identify and solve. A goal of industrial ecology is to change the linear nature of our industrial system "raw materials → products → wastes," to a cyclical system where the wastes are reused as energy or raw materials for another product or process.

NOMENCLATURE

s = (limiting) substrate concentration in external environment, mg/g soil, mL water
x = cell biomass concentration, mg/g soil, mL water
p = product concentration, mg/g soil, mL water
s_0, x_0, p_0 = the initial values (at time $t = 0$) of respectively s, x and p
N = cell population density or cell number, 10^6/g soil, mL water
C_i = intracellular content of the ith cell component (g/g cell mass)
Δx, Δs = changes in x and s respectively for a finite time interval Δt
dx, ds = respectively changes of x and s for infinitesimally small time interval dt
Y = stoichiometric parameter, the yield of cell mass per unit of consumed substrate, g cell mass per g substrate
$Y_{x/s}$, $Y_{p/x}$, $Y_{p/s}$. . . = yield of cell mass per unit of taken up substrate, yield of product per unit of cell mass produced, yield of product per unit of substrate consumed respectively, g/g
μ = specific growth rate, the gross cell growth rate (dx/dt) per unit of cell concentration x, h^{-1}
μ_m = maximal specific growth rate attained under ideal conditions $s >> K_s$, h^{-1}
K_s = saturation constant, parameter of Monod equation equal to such limiting substrate concentration which supports growth rate $\mu = 0.5\,\mu_m$, mg/g soil or mg/L
q = specific rate of substrate consumption, the gross uptake rate per unit of cell concentration x, g substrate/g cell mass per h
m = maintenance coefficient, the $q-$value at $\mu = 0$, g substrate/g cell mass per h
Y^m or Y^{max} = maximal biomass yield under idealized conditions $m = 0$, g cell mass per g of consumed substrate
Y^{min} = the minimal yield observed in the real microbial culture under progressive slowing down of growth rate ($Y \rightarrow$ when $\mu \rightarrow 0$)
σ_s = the intracellular content of deficient element S or *cell quota,* g element per g of cell mass
a = specific death rate (mortality rate), the gross rate of cell decline per unit of the current cell concentration x, h^{-1}
μ_{app} = apparent specific growth rates which is difference between true growth μ and death rate a, $\mu_{app} = \mu - a$, h^{-1}
r = the birth or reproduction rate of population, $r \equiv \mu_{app}$, h^{-1}
K = carrying capacity of ecosystem, the maximal population density supported by available resources, number per g soil or L of water of m_2 of surface area
P- and U-components = the terms of Synthetic Chemostat Model designating nonconstitutive (changeable) cell constituents, P-constituents are needed for intensive cell growth, while U-components provide cell survival under growth restrictive conditions, g component per g cell mass
r^* = the master variable of SCM which is generalized measure of the relative amount of P-components (star is introduced to avoid confusion with r parameter of logistic equation), dimensionless

Glossary

Acidophiles microorganisms that show a preference for growth at low pH, e.g., bacteria that grow only at very low pH values, ca. 2.0.

Actinomycetes members of an order of bacteria in which species are characterized by the formation of branching and/or true filaments.

Adhesins substances involved in the attachment or adherence of microorganisms to solid surfaces; factors that increase adsorption.

Adhesion factors substances involved in the attachment of microorganisms to solid surfaces; factors that increase adsorption.

Aerobes microorganisms whose growth requires the presence of air or free oxygen.

Aerobic having molecular oxygen present; growing in the presence of air.

Aerosol a fine suspension of particles or liquid droplets sprayed into the air.

Algae a heterogeneous group of eucaryotic, photosynthetic organisms, unicellular or multicellular, but lacking true tissue differentiation.

Allochthonous an organism or substance foreign to a given ecosystem.

Amensalism an interactive association between two populations that is detrimental to one and does not adversely affect the other.

Anaerobes organisms that grow in the absence of air or oxygen; organisms that do not use molecular oxygen in respiration.

Anaerobic the absence of oxygen; able to live or grow in the absence of free oxygen.

Anoxic absence of oxygen; anaerobic.

Antagonism the inhibition, injury, or killing of one species of microorganism by another; an interpopulation relationship in which one population has a deleterious (negative) effect on another.

Aquifer a geological formation containing water, such as subsurface water bodies that supply the water for wells and springs; a permeable layer of rock or soil that holds and transmits water.

Archaea (archaebacteria) prokaryotes with cell walls that lack murein, having ether bonds in their membrane phospholipids; analysis of rRNA indicates that the Archaea represent a primary biological domain distinct from both Bacteria and Eucarya.

Autecology branch of ecology that examines individual organisms in relation to their environment, emphasizing the "self-properties" of an organism's physiological attributes.

Autochthonous microorganisms and/or substances indigenous to a given ecosystem; the true inhabitants of an ecosystem; referring to trie common microbiota of the body or soil microorganisms that fend to remain constant despite fluctuations in the quantity of fermentable organic matter.

Autotrophs organisms whose growth and reproduction are independent of external sources of organic compounds, the required cellular carbon being supplied by the reduction of CO_2 and the needed cellular energy being supplied by the conversion of light energy to ATP or the oxidation of inorganic compounds to provide the free energy for the formation of ATP.

Bacteria members of a group of diverse and ubiquitous procaryotic, single-celled organisms; organisms with procaryotic cells, i.e., cells lacking a nucleus.

Bactericidal any physical or chemical agent able to kill some types of bacteria.

Bacteriophage a virus whose host is a bacterium; a virus that replicates within bacterial cells.

Bacteriostatic an agent that inhibits the growth and reproduction of some types of bacteria but need not kill the bacteria.

Barophiles organisms that grow best or grow only under conditions of high pressure, e.g., in the ocean's depths.

Barotolerant organisms that can grow under conditions of high pressure but do not exhibit a preference for growth under such conditions.

Benthos the bottom region of aquatic habitats; collective term for the organisms living at the bottom of oceans and lakes.

Biocide an agent that kills microorganisms.

Biodegradable a substance that can be broken down into smaller molecules by microorganisms.

Biodegradation the process of chemical breakdown of a substance to smaller molecules caused by microorganisms or their enzymes.

Biodeterioration the chemical or physical alteration of a product that decreases the usefulness of that product for its intended purpose.

Biofilm a microbial community occurring on a surface as a microlayer.

Biogenic element an element that is incorporated into the biomass of living organisms.

Biogeochemical cycling the biologically mediated transformations of elements that result in their global cycling, including transfer between the atmosphere, hydrosphere, and lithosphere.

Biological control the deliberate use of one species of organism to control or eliminate populations of other organisms; used in the control of pest populations.

Biomagnification an increase in the concentration of a chemical substance, such as a pesticide, as the substance is passed to higher members of a food chain.

Biomass the dry weight, volume, or other quantitative estimation of organisms; the total mass of living organisms in an ecosystem.

Bioremediation the use of biological agents to reclaim soils and waters polluted by substances hazardous to human health and/or the environment; it is an extension of biological treatment processes that have traditionally been used to treat wastes in which microorganisms typically are used to biodegrade environmental pollutants.

Biosphere the part of Earth in which life can exist; all living things together with their environment.

Carbon cycle the biogeochemical cycling of carbon through oxidized and reduced forms, primarily between organic compounds and inorganic carbon dioxide.

Carrying capacity the largest population that a habitat can support.

Chemoautotrophs microorganisms that obtain energy from the oxidation of inorganic compounds and carbon from inorganic carbon dioxide; organisms that obtain energy through chemical oxidation and use inorganic compounds as electron donors; also known as chemolithotrophs.

Chemocline a boundary layer in an aquatic habitat formed by a difference in chemical composition, such as a halocline formed in the oceans by differing salt concentrations.

Chemolithotrophs microorganisms that obtain energy through chemical oxidation and use inorganic compounds as electron donors and cellular carbon through the reduction of carbon dioxide; also known as chemoautotrophs.

Chemoorganotrophs organisms that obtain energy from the oxidation of organic compounds and cellular carbon from preformed organic compounds.

Chemostat an apparatus used for continuous-flow culture to maintain bacterial cultures in a selected phase of growth, based on maintaining a continuous supply of a solution containing a nutrient in limiting quantities that controls the growth rate of the culture.

Chemotaxis a locomotive response in which the stimulus is a chemical concentration gradient; movement of microorganisms toward or away from a chemical stimulus.

Chitin a polysaccharide composed of repeating A'-acetyl-glucosamine residues that is abundant in arthropod exoskeletons and fungal cell walls.

Circadian rhythms daily cyclical changes that occur in an organism even when it is isolated from the natural daily fluctuations of the environment.

Climax community the organisms present at the end-point of an ecological succession series.

Colonization the establishment of a site of microbial reproduction on a material, animal, or person without necessarily resulting in tissue invasion or damage.

Colony the macroscopically visible growth of microorganisms on a solid culture medium.

Colony-forming units (CPUs) number of microbes that can replicate to form colonies, as determined by the number of colonies that develop.

Colony hybridization hybridization that is combined with conventional plating procedures in which bacterial colonies or phage plaques are transferred directly onto hybridization filters; the colonies or phage containing plaques are then lysed by alkaline or enzymatic treatment, after which hybridization is conducted.

Cometabolism the gratuitous metabolic transformation of a substance by a microorganism growing on another substrate; the cometabolized substance is not incorporated into an organism's biomass, and the organism does not derive energy from the transformation of that substance.

Commensalism an interactive association between two populations of different species living together in which one population benefits from the association, and the other is not affected.

Community highest biological unit in an ecological hierarchy composed of interacting populations.

Competition an interactive association between two species, both of which need some limited environmental factor for growth and thus grow at suboptimal rates because they must share the growth-limiting resource.

Competitive exclusion principle the statement that competitive interactions tend to bring about the ecological separation of closely related populations and preclude two populations from occupying the same ecological niche.

Competitive inhibition the inhibition of enzyme activity caused by the competition of an inhibitor with a substrate for the active (catalytic) site on the enzyme; impairment of the function of an enzyme due to its reaction with a substance chemically related to its normal substrate.

Composting the decomposition of organic matter in a heap by microorganisms; a method of solid waste disposal.

Consortium an interactive association between microorganisms that generally results in combined metabolic activities.

Copiotrophic populations organisms adapted to live in habitats with plentiful supply of nutrients (syn: **eutrophic populations**, antonym: **oligotrophic populations**).

Coprophagous capable of growth on fecal matter; feeding on dung or excrement.

Crenarchaeota kingdom of archaea consisting of extreme thermophiles.

Cross-feeding the phenomenon that occurs when two organisms mutually complement each other in terms of nutritional factors or catabolic enzymes related to substrate utilizations; also termed syntrophism.

Culture to encourage the growth of particular microorganisms under controlled conditions; the growth of particular types of microorganisms on or within a medium as a result of inoculation and incubation.

Cyanobacteria procaryotic, photosynthetic organisms containing chlorophyll a, capable of producing oxygen by splitting water; formerly known as blue-green algae.

Cyst a dormant form assumed by some microorganisms during specific stages in their life cycles, or assumed as a response to particular environmental conditions in which the organism becomes enclosed in a thin- or thick-walled membranous structure, the function of which is either protective or reproductive.

Decomposers organisms, often bacteria or fungi, in a community that convert dead organic matter into inorganic nutrients.

Desiccation removal of water; drying.

Detrital food chain a food chain based on the biomass of decomposers rather than on that of primary producers.

Detritivore an organism that feeds on detritus; an organism that feeds on organic wastes and dead organisms.

Detritus waste matter and biomass produced from decom-positional processes.

Direct counting procedures methods for the enumeration of bacteria and other microbes that do not require the growth of cells in culture but rather rely upon direct observation or other detection methods by which the undivided microbial cells can be counted.

Direct viability count a direct microscopic assay that determines whether or not microorganisms are metabolically active, i.e., viable.

Dispersion zone an oligotrophic (nutrient-poor) soil or subsoil space which receives C-substrates mainly as diffusive flux of volatiles (hydrocarbons, alcohols, aldehydes, VFA, etc)

Diversity the heterogeneity of a system; the variety of different types of organisms occurring together in a biological community.

Dormant an organism or a spore that exhibits minimal physical and chemical change over an extended period of time but remains alive.

Ecological niche the functional role of an organism within an ecosystem; the combined description of the physical habitat, functional role, and interactions of the microorganisms occurring at a given location.

Ecological succession a sequence in which one ecosystem is replaced by another within a habitat until an ecosystem that is best adapted is established.

Ecology the study of the interrelationships between organisms and their environments.

Ecosystem a functional self-supporting system that includes the organisms in a natural community and their environment.

Ectomycorrhiza a stable, mutually beneficial (symbiotic) association between a fungus and the root of a plant where the fungal hyphae occur outside the root and between the cortical cells of the root.

Endomycorrhiza mycorrhizal association in which there is fungal penetration of plant root cells.

Endophytic a photosynthetic organism living within another organism.

Endospores thick-walled spores formed within a parent cell; in bacteria, heat-resistant spores.

Endosymbiotic a symbiotic (mutually dependent) association in which one organism penetrates and lives within the cells or tissues of another organism.

Endosymbiotic evolution theory that bacteria living as endosymbionts within eucaryotic cells gradually evolved into organelle structures.

Enrichment culture any form of culture in a liquid medium that results in an increase in a given type of organism while minimizing the growth of any other organism present.

Epilimnion the warm layer of an aquatic environment above the thermocline.

Epiphytes organisms growing on the surface of a photo-synthetic organism, e.g., bacteria growing on the surface of an algal cell.

Epizootic an epidemic outbreak of infectious disease among animals other than humans.

Estuary a water passage where the ocean tide meets a river current; an arm of the sea at the lower end of a river.

Eubacteria procaryotes other than archaebacteria.

Eucaryotes cellular organisms having a membrane-bound nucleus within which the genome of the cell is stored as chromosomes composed of DNA; eucaryotic organisms include algae, fungi, protozoa, plants, and animals.

Euphotic the top layer of water, through which sufficient light penetrates to support the growth of photosynthetic organisms.

Eurythermal microorganisms that grow over a wide range of temperatures.

Eutrophic containing high nutrient concentrations, such as a eutrophic lake with a high phosphate concentration that will support excessive algal blooms.

Eutrophication the enrichment of natural waters with inorganic materials, especially nitrogen and phosphorus compounds, that support the excessive growth of photosynthetic organisms.

Evolution the directional process of change of organisms by which descendants become distinct in form and/or function from their ancestors.

Extreme environments environments characterized by extremes in growth conditions, including temperature, salinity, pH, and water availability, among others.

Extreme thermophiles organisms having an optimum growth temperature above 80˚ C.

Fastidious an organism with stringent physiological requirements for growth and survival; an organism difficult to isolate or culture on ordinary media because of its need for special nutritional factors.

Floc a mass of microorganisms cemented together in a slime produced by certain bacteria, usually found in waste treatment plants.

Flocculate to aggregate or clump together individual, tiny particles into small clumps or clusters.

Food web an interrelationship among organisms in which energy is transferred from one organism to another; each organism consumes the preceding one and in turn is eaten by the next higher member in the sequence.

Fungi a group of diverse, unicellular and multicellular eucaryotic organisms, lacking chlorophyll, often filamentous and spore-producing.

Fungicides agents that kill fungi.

Fungistasis the active prevention or hindrance of fungal growth by a chemical or physical agent.

Grazers organisms that prey upon primary producers; protozoan predators that consume bacteria indiscriminately; filter-feeding zooplankton.

Greenhouse effect rise in the concentration of atmospheric CO_2 and a resulting warming of global temperatures.

Gross primary production total amount of organic mat ter produced in an ecosystem.

Growth rate increase in the number of microorganisms per unit of time.

Guild populations within a community which use the same resources.

Habitat a location where living organisms occur.

Halophiles organisms requiring NaCl for growth; extreme halophiles grow in concentrated brines.

Heterotrophs organisms requiring organic compounds for growth and reproduction; the organic compounds serve as sources of carbon and energy.

Hot springs thermal springs with a temperature greater than 37°C.

Humic acids high-molecular-weight irregular organic polymers with acidic character; the portion of soil organic matter soluble in alkali but not in acid.

Humus the organic portion of the soil remaining after microbial decomposition.

Hyperthermophiles organisms having an optimum growth temperature above 80°C; some grow best at 110°C.

Hypolimnion the deeper, colder layer of an aquatic environment; the water layer below the thermocline.

In situ in the natural location or environment.

Ex situ outside the natural environment, under artificial laboratory conditions (∼ in vitro).

In vitro in glass; a process or reaction carried out in a culture dish or test tube.

In vivo within a living organism.

Indigenous native to a particular habitat.

Lichens a large group of composite organisms consisting of a fungus in symbiotic association with an alga or a cyanobacterium.

Lignin a class of complex polymers in the woody material of higher plants, second in abundance only to cellulose.

Limnetic zone in lakes, the portion of the water column excluding the littoral zone where primary productivity exceeds respiration.

Lithosphere the solid part of Earth.

Lithotrophs microorganisms that live in and obtain energy from the oxidation of inorganic matter; chemo-autotrophs.

Littoral situated or growing on or near the shore; the region between the high and low tide marks.

Mesophiles organisms whose optimum growth is in the temperature range of 20–45°C.

Methanogens methane-producing procaryotes; a group of archaea capable of reducing carbon dioxide or low-molecular-weight fatty acids to produce methane.

Methylation the process of substituting a methyl group for a hydrogen atom.

Mineralization the microbial breakdown of organic materials into inorganic materials brought about mainly by microorganisms.

Mixotrophs organisms capable of utilizing both autotrophic and heterotrophic metabolic processes, e.g., the concomitant use of organic compounds as sources of carbon and light as a source of energy.

Most probable number (MPN) a method for determination of viable organisms using statistical analyses and successive dilution of the sample to reach a point of extinction.

Mutualism a stable condition in which two organisms of different species live in close physical association, each organism deriving some benefit from the association; symbiosis.

Mycelia the interwoven mass of discrete fungal hyphae.

Mycobiont the fungal partner in a lichen.

Mycorrhiza a stable, symbiotic association between a fungus and the root of a plant; the term also refers to the root-fungus structure itself.

Net primary production amount of organic carbon in the form of biomass and soluble metabolites available for heterotrophic consumers in terrestrial and aquatic habitats.

Neuston the layer of organisms growing at the interface between air and water.

Neutralism the relationship between two different microbial populations characterized by the lack of any recognizable interaction.

Niche the functional role of an organism within an ecosystem; the combined description of the physical habitat, functional role, and interactions of the microorganisms occurring at a given location.

Nitrogen fixation the reduction of gaseous nitrogen to ammonia, carried out by certain procaryotes.

Nitrogenase the enzyme that catalyzes biological nitrogen fixation.

Numerical taxonomy a system that uses overall degrees of similarity and large numbers of characteristics to determine the taxonomic position of an organism; allows organisms of unknown affiliation to be identified as members of established taxa.

Obligate aerobes organisms that grow only under aerobic conditions, i.e., in the presence of air or oxygen.

Obligate anaerobes organisms that cannot use molecular oxygen; organisms that grow only under anaerobic conditions, i.e., in the absence of air or oxygen; organisms that cannot carry out respiratory metabolism.

Obligate intracellular parasites organisms that can live and reproduce only within the cells of other organisms, such as viruses, all of which must find suitable host cells for their replication.

Obligate thermophiles organisms restricted to growth at high temperatures.

Oligotrophic lakes and other bodies of water that are poor in those nutrients that support the growth of aerobic, photo-synthetic organisms; microorganisms that grow at very low nutrient concentrations.

Osmophiles organisms that grow best or only in or on media of relatively high osmotic pressure.

Osmotic pressure the force resulting from differences in solute concentrations on opposite sides of a semipermeable membrane.

Osmotolerant organisms that can withstand high osmotic pressures and grow in solutions of high solute concentrations.

Parasites organisms that live on or in the tissues of another living organism, the host, from which they derive their nutrients.

Parasitism an interactive relationship between two organisms or populations in which one is harmed and the other benefits; generally, the population that benefits, the parasite, is smaller than the population that is harmed.

Pathogens organisms capable of causing disease in animals, plants, or microorganisms.

Pelagic zone the portion of the marine environment beyond the edge of the continental shelf, comprising the entire water column but excluding the sea floor.

Pest a population that is an annoyance for economic, health, or aesthetic reasons.

Pesticides substances destructive to pests, especially insects.

Photoautotrophs organisms whose source of energy is light and whose source of carbon is carbon dioxide; characteristic of plants, algae, and some procaryotes.

Photoheterotrophs organisms that obtain energy from light but require exogenous organic compounds for growth.

Photosynthesis the process in which radiant (light) energy is absorbed by specialized pigments of a cell and is subsequently converted to chemical energy; the ATP formed in the light reactions is used to drive the fixation of carbon dioxide, with the production of organic matter.

Phototaxis the ability of bacteria to detect and respond to differences in light intensity, moving toward or away from light.

Phototrophs organisms whose sole or principal primary source of energy is light; organisms capable of photophosphorylation.

Phycobiont the algal partner of a lichen.

Phytoplankton passively floating or weakly motile photosynthetic aquatic organisms, primarily cyanobacteria and algae.

Phytoplankton food chain a food chain in aquatic habitats based on the consumption of primary producers.

Plankton collectively, all microorganisms and invertebrates that passively drift in lakes and oceans.

Plasmid an independent self-replicating DNA molecule, which compared to a bacterial chromosome carries relatively few genes which are not essential for survival under nonselective growth conditions.

Plate counting method of estimating numbers of microorganisms by diluting samples, culturing on solid media, and counting the colonies that develop to estimate the number of viable microorganisms in the sample.

Predation a mode of life in which food is primarily obtained by killing and consuming animals; an interaction between organisms in which one benefits and one is harmed, based on the ingestion of the smaller organism, the prey, by the larger organism, the predator.

Predators organisms that practice predation.

Prey an animal taken by a predator for food.

Primary producers organisms capable of converting carbon dioxide to organic carbon, including photoautotrophs and chemoautotrophs.

Profundal zone in lakes, the portion of the water column where respiration exceeds primary productivity.

Proto-cooperation synergism; a nonobligatory rela-tion-ship between two microbial populations in which both populations benefit.

Protonmotive force potential chemical energy in a gradient of protons and electrical energy across the membrane.

Protozoa diverse eucaryotic, typically unicellular, non-photosynthetic microorganisms generally lacking a rigid cell wall.

Psychrophile an organism that has an optimum growth temperature below 20°C.

Psychrotroph (or psychroactive microbe) a mesophile that can grow at low temperatures.

Pure culture a culture that contains cells of one kind; the progeny of a single cell.

Recalcitrant a chemical that is totally resistant to microbial attack.

Rhizosphere an ecological niche that comprises the surfaces of plant roots and the region of the surrounding soil in which the microbial populations are affected by the presence of the roots.

Rhizosphere effect evidence of the direct influence of plant roots on bacteria, demonstrated by the fact that microbial populations usually are higher within the rhizosphere (the region directly influenced by plant roots) than in root-free soil.

Self-purification inherent capability of natural waters to cleanse themselves of pollutants based on biogeochemical cycling activities and interpopulation relationships of indigenous microbial populations.

Seston all material, both organic and inorganic, suspended in a waterway; all the fine particulate matter which drifts passively in lakes, seas and other bodies of water, including living organisms.

Soil horizon a layer of soil distinguished from layers above and below by characteristic physical and chemical properties.

Solfatara hot, sulfur-rich environment; a volcanic area or vent which yields sulfur vapors, steam, and the like.

Solid waste refuse; waste material composed of both inert materials – glass, plastic, and metal – and decomposable organic wastes, including paper and kitchen scraps.

Stenothermophiles microorganisms that grow only at temperatures near their optimal growth temperature.

Stenotolerant highly specialized and therefore having a narrow tolerance for a specific growth factor.

Succession the replacement of populations by other populations better adapted to fill the ecological niche.

Symbiosis an obligatory interactive association between members of two populations, producing a stable condition in which the two organisms live together in close physical proximity to their mutual advantage.

Synecology the study of the ecological interrelationships among communities of organisms.

Synergism in antibiotic action, when two or more antibiotics are acting together, the production of inhibitory effects on a given organism that are greater than the additive effects of those antibiotics acting independently; an interactive but nonobligatory association between two populations in which each population benefits.

Syntrophism the phenomenon that occurs when two organisms mutually complement each other in terms of nutritional factors or catabolic enzymes related to substrate utilization; also termed cross-feeding.

Thermal stratification division of temperate lakes into an epilimnion, thermocline, and hypolimnion, subject to seasonal change; zonation of lakes based on temperature where warm and cold water masses do not mix.

Thermal vents hot areas located at depths of 800–1,000 m on the sea floor, where spreading allows seawater to percolate deeply into the crust and react with hot core materials; life around the vents is supported energetically by the chemoautotrophic oxidation of reduced sulfur.

Thermocline zone of water characterized by a rapid decrease in temperature, with little mixing of water across it.

Thermophiles organisms having an optimum growth temperature above 40°C.

Tolerance range the range of a parameter, such as temperature, over which microorganisms survive.

Transposons translocatable genetic elements; genetic elements that move from one locus to another by non-homologous recombination, allowing them to move around a genome.

Trickling filter system a simple, film-flow aerobic sewage treatment system; the sewage is distributed over a porous bed coated with bacterial growth that mineralizes the dissolved organic nutrients.

Trophic level the position of an organism or population within a food web: primary producer, grazer, predator, etc.

Trophic structure the collection of steps in the transfer of energy stored in organic compounds from one to another.

Trophozoite a vegetative or feeding stage in the life cycle of certain protozoa.

Turbidostat a system in which an optical sensing device measures the turbidity of the culture in a growth vessel and generates an electrical signal that regulates the flow of fresh medium into the vessel and the release of spent medium and cells.

Ultraviolet light (UV) short wavelength electromagnetic radiation in the range 100–400 nm.

Vectors organisms that act as carriers of pathogens and are involved in the spread of disease from one individual to another.

Vesicular-arbuscular mycorrhiza a common type of mycorrhiza characterized by the formation of vesicles and arbuscules.

Viable nonculturable microorganism microorganisms that do not grow in viable culture methods, but which are still metabolically active and capable of causing infections in animals and plants.

Viable plate count method for the enumeration of bacteria whereby serial dilutions of a suspension of bacteria are plated onto a suitable solid growth medium, the plates are incubated, and the number of colony-forming units is counted.

Virus a noncellular entity that consists minimally of protein and nucleic acid and that can replicate only after entry into specific types of living cells; it has no intrinsic metabolism, and its replication is dependent on the direction of cellular metabolism by the viral genome; within the host cell, viral components are synthesized separately and are assembled intracellularly to form mature, infectious viruses.

Volatile organic compounds (VOC) vaporizes into the atmosphere.

Water activity (a_w) a measure of the amount of reactive water available, equivalent to the relative humidity; the percentage of water saturation of the atmosphere.

Xenobiotic a synthetic product not formed by natural biosynthetic processes; a foreign substance or poison.

Xerotolerant able to withstand dry ness; an organism capable of growth at low water activity.

Yeasts a category of fungi defined in terms of morphological and physiological criteria; typically, unicellular, saprophytic organisms that characteristically ferment a range of carbohydrates and in which asexual reproduction occurs by budding.

Zymogenous term used to describe opportunistic soil microorganisms that grow rapidly on exogenous substrates.

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5
Microbial Metabolism: Importance for Environmental Biotechnology

Aharon Oren

CONTENTS

Abstract Microorganisms are the main agents responsible for biogeochemical transformations of carbon, nitrogen, sulfur, iron, and other elements. The prokaryotic world (domains *Archaea* and *Bacteria*) presents us with a far larger variety of metabolic types than are found among the eukaryotes (fungi, higher plants, protozoa and animals). The range of substrates used by prokaryotes as carbon sources for growth (assimilatory metabolism) is far greater than in the eukaryotic world. In addition, many groups of prokaryotes perform types of energy generation (dissimilatory reactions) that are altogether unknown among the eukaryotes. This chapter provides a general overview of the metabolism of microorganisms, with special emphasis on the prokaryotic world. Processes such as oxygenic and anoxygenic

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photosynthesis, aerobic and anaerobic respiration, and chemolithotrophic metabolism are discussed. Finally, it is shown how these processes together enable the functioning of the biogeochemical cycles of the elements on Earth.

Key Words Prokaryotes • metabolic diversity • energy generation • assimilatory metabolism • oxygenic photosynthesis • anoxygenic photosynthesis • respiration • anaerobic respiration • fermentation • methanogenesis • chemoautotrophs • biogeochemical cycles.

1. INTRODUCTION: THE METABOLIC DIVERSITY OF PROKARYOTIC AND EUKARYOTIC MICROORGANISMS

Microorganisms – prokaryotic as well as eukaryotic – are the main agents responsible for biogeochemical transformations of carbon, nitrogen, sulfur, iron, and other elements. Therefore, they are of utmost importance to the environmental engineer: in processes such as bioremediation, water purification, and many other applications of environmental biotechnology the microbes do the work. A thorough understanding of the ways in which microorganisms function is therefore essential in all aspects of environmental engineering.

The prokaryotic world (domains *Archaea* [Archaebacteria] and *Bacteria* [Eubacteria]) presents us with a far larger variety of metabolic types than are found among the eukaryotes (fungi, higher plants, protozoa, and animals) (1–6). First of all, the range of substrates used by prokaryotes as carbon sources for growth (assimilatory metabolism) is far greater than in the eukaryotic world. Secondly, many groups of prokaryotes perform types of energy generation (dissimilatory reactions) that are altogether unknown among the eukaryotes. Such dissimilatory processes are of great interest in environmental biotechnology as the amounts of substrates transformed in the course of the energy-generating process are generally much larger than the amounts of substrates necessary for assimilatory purposes, i.e., cell growth and multiplication. Processes, such as denitrification (dissimilatory reduction of nitrate to dinitrogen and other gaseous nitrogen compounds), nitrification (oxidation of ammonium ions to nitrate with nitrite as intermediate), dissimilatory reduction of sulfate to badly smelling and corrosive hydrogen sulfide, the formation of acidic wastewater loaded with yellow–brown iron hydroxides in the neighborhood of coal mines and other mining operations, and formation of the greenhouse gas methane in the course of anaerobic degradation of organic matter are just a few examples of such microbial processes that the environmental biotechnologist has to understand before he/she can try to prevent such processes from occurring, or stimulate desirable processes. Another reason why microorganisms are so important in the environment, both natural and manipulated by the environmental engineer, is their rapid growth and their high metabolic rates. As a result, the processes they perform are often extremely rapid. We will also encounter many cases in which a small community of microorganisms has a huge impact on the properties of the ecosystem – terrestrial as well as aquatic. An understanding of these processes and of the role of the different types of microorganisms that mediate them is therefore essential for the environmental engineer.

The following sections provide a general overview of the metabolism of microorganisms, with special emphasis on the prokaryotic world. They aim at an understanding of why the different processes occur, and of what use they are to the organisms that perform them.

2. DISSIMILATORY METABOLISM OF MICROORGANISMS: THERMODYNAMIC AND MECHANISTIC PRINCIPLES

2.1. General Overview of the Metabolic Properties of Microorganisms: A Thermodynamic Approach

If we want to gain insight into the ways in which microorganisms function and the nature of the transformations they perform in nature, we need to understand both the assimilatory and the dissimilatory processes they perform. Each cell has to obtain building blocks to produce new cellular components necessary for growth and multiplication. These building blocks need to contain all the elements of which the cell is built: carbon, hydrogen, oxygen, nitrogen, sulfur, phosphorus, iron, and many others that are required in small quantities ("trace elements"). Some cells can build all their complex molecules, such as proteins, nucleic acids, cell walls, etc. from simple inorganic components such as carbon dioxide as carbon source, nitrate or ammonium ions as nitrogen source, and sulfate and phosphate as sources of sulfur and phosphorus, respectively. Such cells that do not require any organic carbon compounds for growth are designated autotrophic (Fig. 5.1). Green plants and eukaryotic algae are such autotrophs, but many more types of autotrophic microorganisms are known, such as phototrophic purple and green sulfur bacteria that use sulfide rather than water as electron

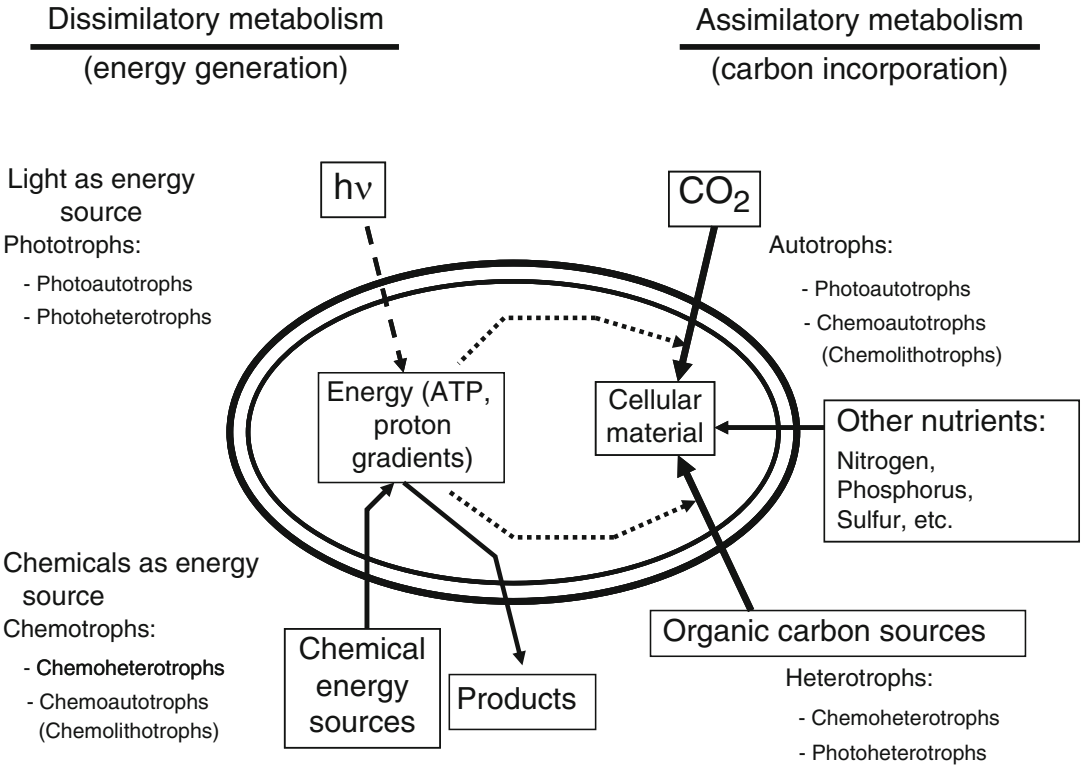

Fig. 5.1. Types of microorganisms, classified according to their energy and carbon sources.

donor to fix CO_2, aerobic chemoautotrophic (chemolithotrophic) bacteria that obtain their energy from the oxidation of inorganic compounds such as ammonium or nitrite ions or sulfide (see Sect. 6.1), and anaerobic prokaryotes such as those methanogenic *Archaea* that obtain their energy from the reduction of CO_2 to methane using hydrogen as electron donor and also use CO_2 as their sole carbon source for growth. Other microorganisms – many bacteria, the fungi, the protozoa, and also all higher animals – are heterotrophs that require organic carbon compounds, and often organic sources of nitrogen and sulfur as well, as building blocks for the production of more cell material.

Biosynthesis of proteins, nucleic acids, and other cellular macromolecules from simple precursors is an energetically expensive process. An autotrophic organism that has to produce all its chemical components from CO_2 and other simple, generally oxidized components will need far more energy to produce the same amount of cell material than a heterotrophic organism that takes up sugars, amino acids, etc. from the medium and needs only to assemble these components into proteins, nucleic acids, and cell wall polysaccharides. The amount of new biomass that can be formed, thus, depends both on the availability of building blocks – organic and/or inorganic – and on the generation of sufficient amounts of energy by the cells to enable the biosynthesis and assembly processes to take place.

A basic understanding of the principles of chemical thermodynamics is required to obtain insight into the metabolism of microorganisms and into the transformations these microorganisms perform in nature. For our purpose, the most relevant parameter of the different reactions involved in the energy metabolism of cells is the change in free energy (Gibbs free energy, ΔG) that accompanies any reaction. This free energy change represents the amount of energy that can be used to perform useful work, this in contrast to energy released as heat that cannot be further used by the organism. When the change in free energy is positive, i.e., the amount of free energy in the reaction products exceeds the amount of free energy in the reagents, the reaction is called endergonic ($\Delta G > 0$); when the total free energy in the products is lower than in the reagents, the reaction is exergonic ($\Delta G < 0$). Such reactions can be used by the cell to generate energy, and they form the core of the dissimilatory metabolism of the cell. Reactions associated with the assimilatory metabolism of the cell are typically endergonic, and they are driven by the energy obtained in the dissimilatory processes.

The change in free energy of any reaction or process determines whether it can (at least theoretically) be used for energy generation. The amount of free energy released in the course of the dissimilatory process performed is therefore the most important bioenergetic parameter for any living organism (7, 8).

The amount of free energy released or required in the course of chemical reactions, expressed in kilojoules (kJ), can be calculated according to the equation:

$$\Delta G^{o} = \Sigma \Delta G_{f}^{o}(\text{products}) - \Sigma \Delta G_{f}^{o}(\text{reagents}) \quad (1)$$

where

ΔG^{o} = the free energy change associated with the reaction, when molar amounts of the reagents and products are converted according to the reaction stoichiometry. The sign ^{o} refers to standard conditions, i.e., concentrations of all compounds involved

in the reaction being 1 M or 1 atmosphere in the case of gases, and at a temperature of 25°C.

ΔG_f = the free energy (in kJ/mol) required for synthesis of the reagents or the reaction products from the elements of which they are composed.

Based on the known ΔG_f^o values of common substrates and metabolic products such as those found in Table 5.1, the free energy yield or demand of different reactions performed by microorganisms can easily be calculated.

For example, the free energy change associated with the alcoholic fermentation of yeast:

$$C_6H_{12}O_6 \text{ (glucose)} \rightarrow 2CH_3CH_2OH \text{ (ethanol)} + 2CO_2 \text{ (carbon dioxide)} \quad (2)$$

under standard conditions can be calculated according to:

$$-917.2 - (2 \times -181.8) + (2 \times -386)\text{kJ} = -218.4\,\text{kJ per mol glucose fermented} \quad (3)$$

Many reactions, which occur during cellular metabolism involve the participation of protons (H^+). For such reactions, calculation of the ΔG values under standard conditions would imply an H^+ concentration of 1 mol/l, i.e., a pH of zero. Such conditions are of little relevance to the metabolism of the cell, in which reactions proceed under near-neutral to slightly alkaline conditions. It is therefore customary to calculate all ΔG^o values at neutral pH (H^+ concentration of 10^{-7} M). The free energy change is then indicated as $\Delta G^{o\prime}$. The $\Delta G'_f$ for protons is 39.8 kJ/mol (see Table 5.1).

Another important parameter to be taken into account when estimating the energy yield or energy requirement of metabolic reactions is the actual concentrations of the reactants and the reaction products. The $\Delta G^{o\prime}$ value defined above refers to standard conditions of

Table 5.1
Free energy of formation (ΔG_f^o) for some substances relevant to microbial metabolism. For more extensive tables see (1) and (8)

CO_2 (aqueous)	−386.0	N_2	0
HCO_3^-	−586.9	NO	+86.6
CH_4	−50.8	NO_2^-	−37.2
CO	−137.2	NO_3^-	−111.3
Formate $^-$	−351.0	NH_4^+	−79.4
Acetate $^-$	−369.4	N_2O	+104.2
Propionate $^-$	−361.1		
Butyrate $^-$	−352.6		
Ethanol	−181.8	S^o	0
Lactate $^-$	−517.8	SO_4^{2-}	−744.6
Succinate $^{-2}$	−690.2	H_2S (aqueous)	−27.9
Glucose	−917.2	HS^-	+12.1
H_2O	−237.2		
H^+	−39.8 (pH 7)	Fe^{2+}	−78.9
OH^-	−198.8 (pH 7)	Fe^{3+}	−4.6
O_2	0		

concentrations and temperature. The true ΔG value of the reaction is determined both by the $\Delta G^{o\prime}$ and by the concentrations at which the reagents and the end products of the reaction are present, according to:

$$\Delta G' = \Delta G^{0'} + RT \ln \frac{[C]^c \cdot [D]^d}{[A]^a \cdot [B]^b} \tag{4}$$

where $\Delta G'$ is the free energy change associated with the reaction (in kJ/mol), $\Delta G^{o'}$ is the free energy change associated with the reaction under standard conditions (in kJ/mol), R is the gas constant (8.29 J/mol.K), T is the temperature in K,

for a reaction between reagents A and B to yield products C and D with stoichiometries of a, b, c, and d, respectively.

Equation (4) implies that the true free energy change of a reaction may change from exergonic to endergonic and vice versa according to the concentrations of the reactants and the products. This is also predicted from Le Chatelier's principle ("If some stress is brought to bear upon a system in equilibrium, a change occurs, such that the equilibrium is displaced in a direction which tends to undo the effect of the stress"). As discussed in Sect. 5.7, such effects may have a profound impact on the progress of degradation of organic compounds and other metabolic pathways, especially under anaerobic conditions.

To mediate between energy-yielding (exergonic, dissimilatory) reactions and energy-consuming (endergonic, assimilatory) reactions, all cells use adenosine triphosphate (ATP), a compound that contains two energy-rich anhydride bonds linking the phosphate groups. Synthesis of ATP from ADP (adenosine diphosphate) and inorganic phosphate requires a large amount of energy:

$$\text{ADP} + \text{phosphate} \rightarrow \text{ATP} + \text{H}_2\text{O} \quad (\Delta G^{o'} = +33\,\text{kJ mol}) \tag{5}$$

$$(\Delta G' = \text{approximately} + 44\,\text{kJ/mol})$$

In practice, the amount of free energy needed to drive formation of ATP is approximately 70 kJ/mol (8, 9). This value takes into account both the true concentrations of ATP, ADP and phosphate typically found within the cell and the inevitable amount of energy lost as heat during nonequilibrium situations. Hydrolysis of ATP in the cell is coupled with energy-requiring reactions, thus driving thermodynamically unfavorable reactions.

ATP can be formed in biological systems in either or both of two ways:

1. ATP can be synthesized by "substrate-level phosphorylation," in which the formation of ATP is directly coupled to a strongly exergonic reaction in which an intermediate that carries a "high-energy" phosphate group, (i.e., a phosphate group whose hydrolytic cleavage is associated with a highly negative $\Delta G^{o\prime}$), transfers the phosphate group to ADP. Examples are the formation of ATP and 3-phosphoglycerate from 1,3-bisphosphoglycerate and the formation of ATP from phosphoenolpyruvate to yield pyruvate in the glycolytic Embden–Meyerhof pathway (Fig. 5.2, upper part). An analogous reaction is the formation of GTP (guanosine triphosphate) from GDP (guanosine diphosphate) and inorganic phosphate coupled to the formation of succinate from the high-energy compound succinyl-CoA in the tricarboxylic acid cycle (Krebs cycle) (Fig. 5.2, lower part).

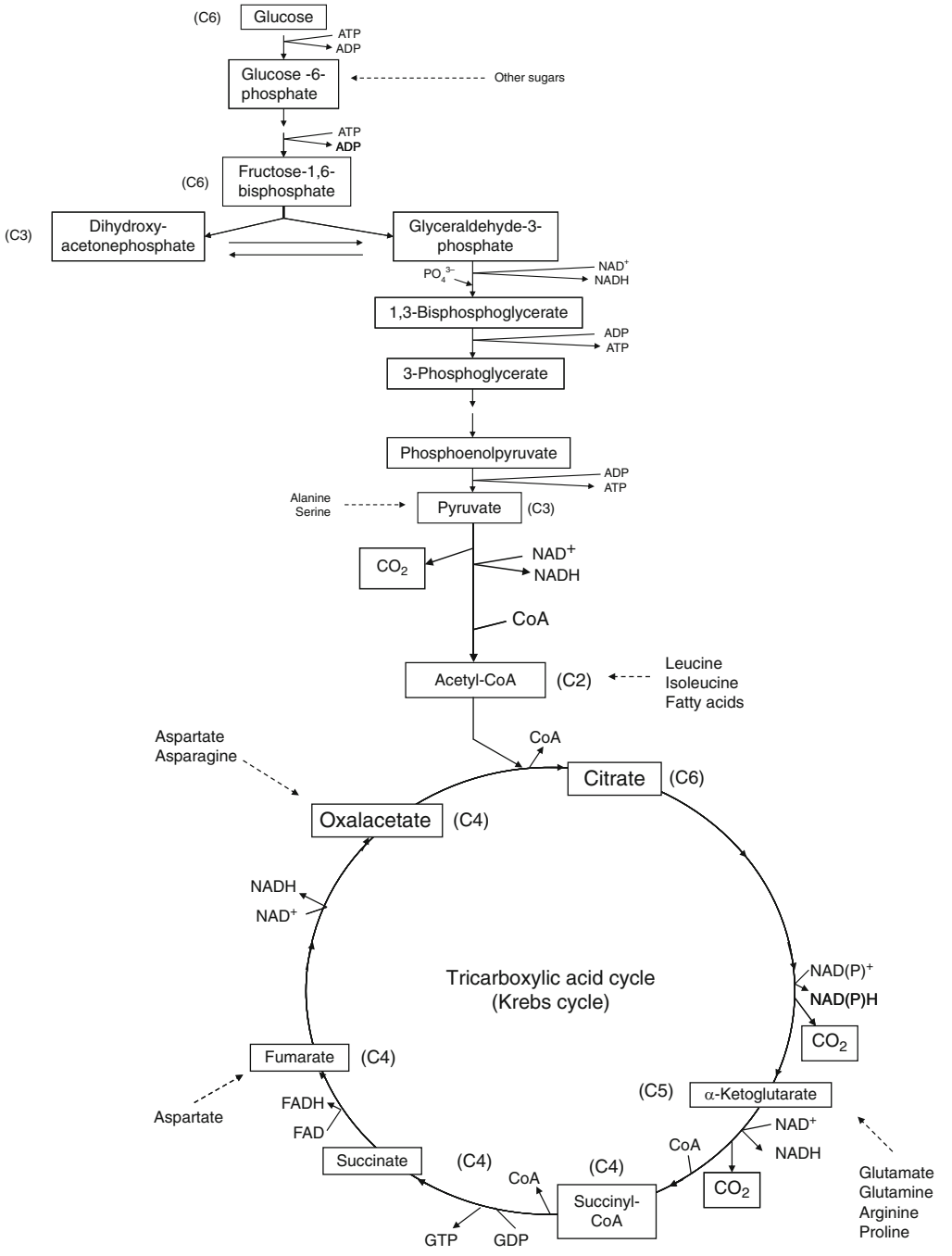

Fig. 5.2. Glycolysis (the Embden–Meyerhof pathway) and the tricarboxylic acid cycle as the backbone of dissimilatory metabolism in heterotrophic bacteria.

Table 5.2
The most important high-energy compounds involved in substrate-level phosphorylation and their $\Delta G^{o\prime}$ of hydrolysis (1, 8)

High-energy compound	$\Delta G^{o\prime}$ of hydrolysis (kJ/mol)
1,3-Bisphosphoglycerate	−51.9
Phosphoenolpyruvate	−51.6
Acetyl-CoA	−35.7
Succinyl-CoA	−35.1
Acetyl phosphate	−44.8
Carbamyl phosphate	−39.3
Adenosine-5′-phosphosulfate	−88.0

The number of high-energy compounds that can be used for ATP production by substrate-level phosphorylation is limited (1, 5, 8, 10). The most important of these compounds are listed in Table 5.2.

2. Energy is also available to living systems in the form of gradients of protons (H^+) across biological membranes. In many dissimilatory (energy-yielding) processes in prokaryotes, energy is conserved in the form of proton gradients generated by transport of protons from the cytoplasmic side of the membrane to the extracellular environment. Such electrochemical gradients of protons involve both a pH difference (alkaline inside, acidic outside) and a membrane potential (negative inside, positive outside). In eukaryotes, similar processes take place across the membranes of mitochondria and chloroplasts. Controlled entry of protons through the enzyme ATP synthase ("ATPase") located within the membrane (which otherwise is highly impermeable to protons) is coupled with the synthesis of ATP from ADP and inorganic phosphate. The generation of a proton electrochemical gradient at the expense of ATP can occur as well. ATP and proton electrochemical gradients across membranes may therefore be considered as fully interconvertible forms of energy, to be used to drive energy-requiring processes within the cell, including biosynthesis of new cellular components enabling growth.

Many reactions performed by the cell, both in dissimilatory and assimilatory metabolism, are electron transfer processes in which electrons flow from an electron donor to an electron acceptor. Aerobic respiration in which organic substrates are oxidized, coupled to the transfer of the electrons to molecular oxygen with the formation of water is just one example. The tendency of different compounds to gain electrons by reduction or to donate electrons and become oxidized can be expressed in terms of the standard reduction potential of the redox couples. Figure 5.3 presents the standard redox potentials of the most important compounds that become oxidized and/or reduced in the course of cellular metabolism. The more negative the standard reduction potential, the stronger the tendency of the reduced form to donate electrons to an oxidized compound with a higher reduction potential. The amount of energy to be invested or to be gained during such redox reactions is directly proportional to the difference in the standard reduction potential of the reductant and the oxidant involved according to:

$$\Delta G^{o\prime} = -n\,F\,\Delta E'_{o} \tag{6}$$

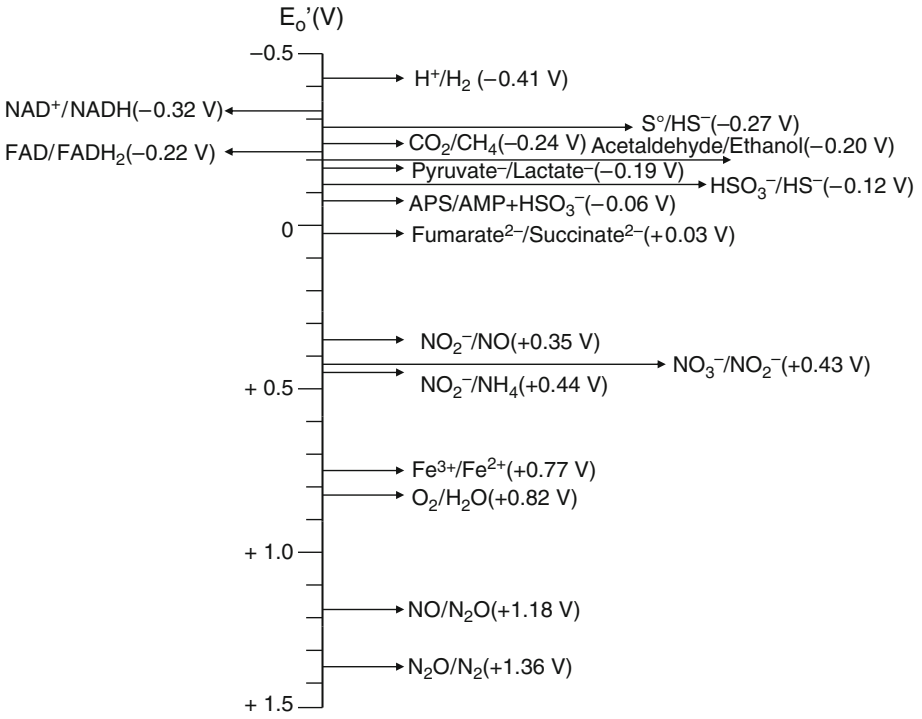

Fig. 5.3. Standard reduction potentials of selected redox couples relevant to microbial metabolism.

where $\Delta G^{o\prime}$ = the free energy change associated with the reaction under standard conditions (in kJ/mol), n = the number of electrons transferred in the reaction, F = the Faraday constant (96.5 kJ/V), and $\Delta E_o{}'$ = the difference in standard reduction potential of the redox couples participating in the reaction.

Flow of electrons from a reductant with a low reduction potential to an oxidant with a high reduction potential gives rise to an exergonic reaction; "uphill" flow of electrons to form a stronger reductant from a weak reductant is an endergonic process that can only proceed with the expenditure of energy. From the data presented in Fig. 5.3, it can for example be concluded that oxidation of ammonium ions to nitrite with transfer of the electrons to molecular oxygen is an energy-yielding process (the process that *Nitrosomonas* and other nitrifying bacteria use to gain energy for growth), while the same bacteria have to invest energy in order to use electrons from ammonium ions to reduce $NADP^+$ to NADPH, which is required by the cell to serve as the electron donor for autotrophic fixation of CO_2 in the Calvin cycle, the major carbon assimilation pathway used by nitrifying bacteria (see also Sect. 6.1).

2.2. *Modes of Energy Generation of Prokaryotic and Eukaryotic Microorganisms*

Microorganisms display a tremendous diversity in the modes of energy generation that can support their growth. In this respect, their abilities greatly exceed those of the eukaryotic microorganisms as well as the macroorganisms.

The metabolic diversity of the prokaryotes, especially as far as their dissimilatory processes are concerned, will form the topic of most of the following sections in this chapter. An in-depth insight in to the diversity in dissimilatory metabolism is essential when we need to understand the conversions performed by microorganisms in the natural environment. Here again, the nitrifying bacteria provide an excellent example. Nitrification (see also Sects. 2.1 and 6.1) consists of two steps, the first being the aerobic oxidation of ammonium ions (NH_4^+) to nitrite (NO_2^-) by organisms such as *Nitrosomonas* (a six-electron transfer in which N^{3-} is oxidized to N^{3+}), followed by oxidation of the nitrite to nitrate (NO_3^-, N^{5+}) by organisms such as *Nitrobacter*, again using molecular oxygen as electron acceptor. Due to the fact that the electron donors (ammonium and nitrite, respectively) are relatively weak reductants (see Fig. 5.3), the amount of energy gained per mol of substrate oxidized is relatively small, as can be calculated from Eqs. (1) and (6):

$$NH_4^+ + 1.5O_2 \rightarrow NO_2 + 2H^+ + H_2O \quad \Delta G^{o'} = -274.6\,\text{kJ} \tag{7}$$

$$NO_2^- + 0.5O_2 \rightarrow NO_3^- \quad \Delta G^{o'} = -74.1\,\text{kJ} \tag{8}$$

Moreover, for the autotrophic fixation of CO_2, both groups of nitrifying bacteria need NADPH as electron donor, a strong reductant that can only be formed at the expenditure of much energy from the weak reductants available, i.e., ammonium and nitrite respectively. The result is that to fix one molecule of CO_2, *Nitrosomonas* has to oxidize in the order of 30–40 ammonium ions, while *Nitrobacter* needs around 100 ions of nitrite to provide both the energy and the electrons necessary for the fixation of one CO_2 molecule. If we further assume that the ratio of carbon to nitrogen in cell material is 6.6:1, *Nitrosomonas* will need to assimilate (based on the general empirical formula $C_{106}H_{263}O_{110}N_{16}P$ for [phytoplankton] cell material, known as the Redfield ratios) 4.5 mmol of ammonium per gram of dry cell material, while as much as 0.9–1.2 mol ammonium are needed in the dissimilatory reaction used to provide the energy and the electrons for the autotrophic carbon fixation. A similar calculation for *Nitrobacter* shows that the ratio between the amount of inorganic nitrogen (as nitrite) converted in the dissimilatory reaction and the amount of inorganic nitrogen assimilated into proteins and other nitrogen-containing cellular components is more than 600. Such calculations clearly show that the dissimilatory processes that the bacteria perform have the most profound impact on the environment, and that small numbers of bacteria and accordingly small amounts of biomass may influence the chemical composition of the environment in the most dramatic way. This, together with their generally rapid growth and their accordingly short generation times makes the prokaryotic microorganisms (*Bacteria* and certain *Archaea*, notably the methanogenic species) responsible for the greatest part of the biogeochemical transformations of matter, quantitatively spoken, and the often very rapid turnover rates in the biogeochemical cycles.

A general overview of the different modes of life, as based on the diversity of dissimilatory and assimilatory pathways, was presented in Fig. 5.1. According to the energy source used, we can divide the organisms living on Earth into phototrophs – organisms that use photons of light in the visible and sometimes the near-infrared range as their source of energy, and chemotrophs – organisms that use chemical energy to produce ATP. The phototrophic organisms can further be divided into photoautotrophs, i.e., organisms that use CO_2 as their carbon source with light providing the energy for autotrophic carbon fixation, and photoheterotrophs, which derive their energy from light, but obtain their cellular carbon from organic compounds rather than from carbon dioxide (11).

Most phototrophic microorganisms use chlorophyll, a tetrapyrrole derivative with a central bound magnesium atom, as the central molecule responsible for the photochemical processes. Excitation of the chlorophyll in the reaction center liberates an electron at a low reduction potential. This electron can return to the reaction center through a chain of electron carriers, including quinones and cytochromes ("cyclic electron flow"), or reduce an electron acceptor such as NAD^+ or $NADP^+$. An external electron donor is then required in order to replenish the missing electron in the reaction center.

Among the prokaryotic photoautotrophs, there is considerable diversity with respect to the electron donors used. Eukaryotic phototrophs (green plants, macro- and microalgae) invariably use water as electron donor, and they excrete molecular oxygen as a waste product. Therefore, this process is called oxygenic ("oxygen-forming") photosynthesis. In the eukaryotic phototrophs, the photosynthetic machinery is localized in intracellular organelles, named chloroplasts. The same kind of metabolism is also used by one group of photoautotrophic prokaryotes, the cyanobacteria. A wide range of alternative electron donors are available for photosynthetic CO_2 fixation by other prokaryotes. These include reduced sulfur compounds (sulfide, elemental sulfur, thiosulfate), molecular hydrogen, and others. As no oxygen is evolved in these cases, the process is termed anoxygenic photosynthesis. In both types of phototrophic life, energy is conserved as a proton electrochemical gradient, which may serve for the generation of ATP.

Figure 5.4 presents a schematic overview of the metabolism of oxygenic photoautotrophs (a), anoxygenic photoautotrophs (b), and anoxygenic photoheterotrophs (c). The metabolism of the different types of phototrophs, the habitats in which they are found, and the impact they have on their environment, are discussed in further depth in Sects. 4.1 and 4.2.

The chemoorganotrophic microorganisms use organic compounds, both as energy source and as a source of carbon to be taken up and incorporated into cell material. There are many different ways in which energy can be derived from conversion of organic compounds. One mode of metabolism is aerobic respiration, i.e., oxidation of the organic carbon while using molecular oxygen as terminal electron acceptor. In most cases, this oxidation proceeds all the way to form CO_2 and H_2O as only products. The amount of free energy released is large, as may be expected based on the high standard reduction potential of the couple O_2/H_2O (Fig. 5.3). The principles of aerobic respiration are summarized in Fig. 5.5a. Aerobic respiration is by far the most common way of energy generation in the animal world. It is also used by plants to obtain energy during the night when light is not available as energy source, thus gaining energy by aerobic oxidation of storage polymers (starch and others) that had

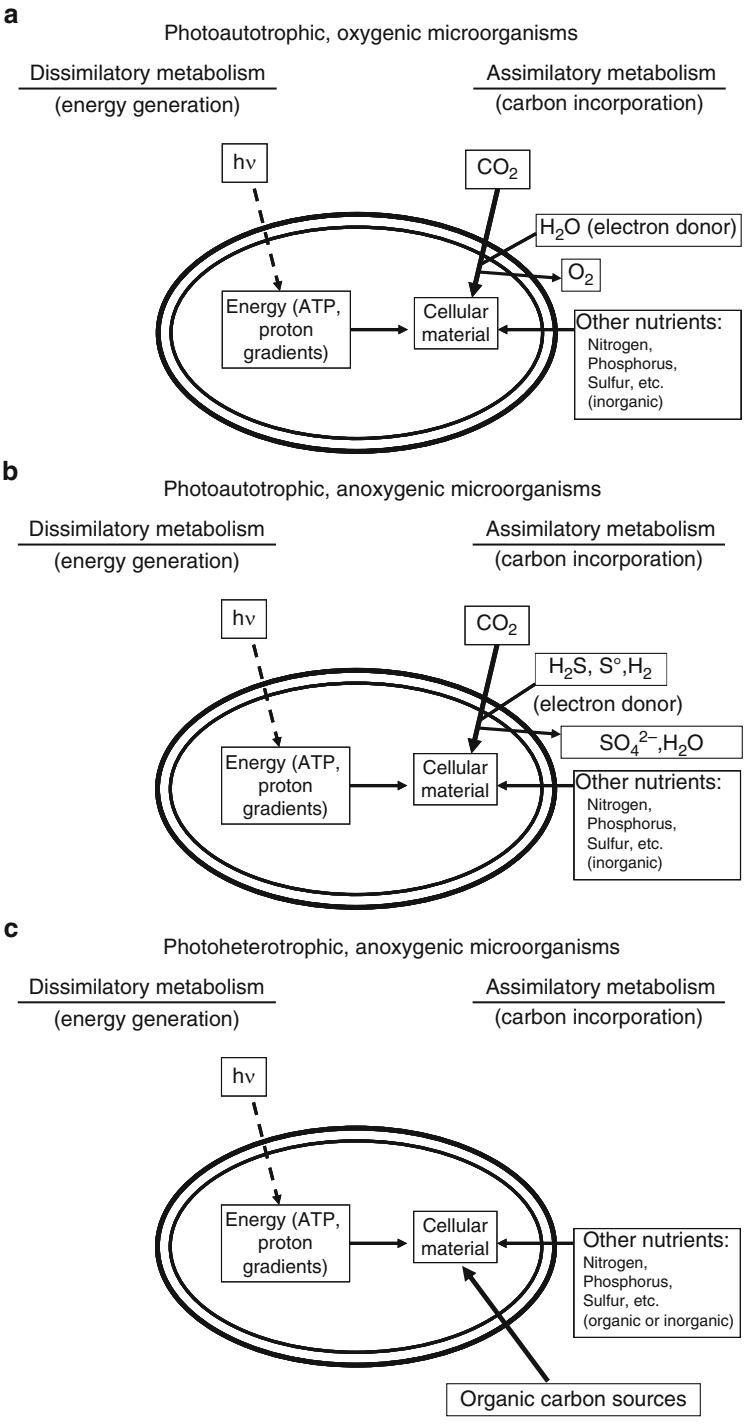

Fig. 5.4. The principles of oxygenic, anoxygenic photoautotrophic, and anoxygenic photoheterotrophic life.

been accumulated in the course of the photoautotrophic metabolism during daytime. Fungi use aerobic respiration as their major type of metabolism. However, the ability to proliferate anaerobically by fermentation is also widespread in the fungi, and especially in the yeasts. Also, many bacteria obtain their energy from the aerobic oxidation of an often tremendously wide variety of organic substrates. Among the prokaryotes we find many obligate aerobes, while others use aerobic respiration only when oxygen is available. In the absence of oxygen they shift to alternative modes of energy generation, such as anaerobic respiration (see below), fermentation, and even photoheterotrophic growth, in some cases.

Aerobic breakdown of complex organic compounds generally proceeds through the reactions of the central cellular metabolic pathways such as the glycolytic Embden–Meyerhof pathway and the tricarboxylic acid cycle (Krebs cycle) (Fig. 5.2). Large polymeric biodegradable compounds (polysaccharides, proteins, lipids) are first split outside the cell into monomers by extracellular depolymerizing enzymes. The resulting small molecules are taken up by the cells and converted in one or more enzymatic steps to intermediates of these nearly universal central metabolic pathways, enabling complete oxidation to CO_2. It should be stated here that not all aerobic chemoorganotrophic microorganisms use the Embden–Meyerhof pathway for sugar degradation. Alternative pathways exist, such as the Entner–Doudoroff pathway in which 6-phosphogluconate and 2-keto-3-deoxy-6-phosphogluconate are key intermediates, or the oxidative pentosephosphate cycle, in which sugar conversions by the enzymes transaldolase and transketolase play a great role.

In some organisms, aerobic respiration does not lead to complete oxidation of the organic substrates to CO_2 but rather to other, more reduced products. Well-known examples are the acetic acid bacteria *Acetobacter* and *Gluconobacter*, which oxidize ethanol while using oxygen as electron acceptor. The acetic acid formed is excreted into the medium. *Acetobacter* can oxidize the acetic acid to CO_2 under suitable conditions, while *Gluconobacter* cannot further metabolize the acetic acid formed.

The electrons released during the oxidation of organic material to CO_2 are transferred to molecular oxygen through a chain of cytochromes and other electron carriers located in the cytoplasmic membrane in prokaryotes, leading to the formation of proton electrochemical gradients that can be converted to ATP. In eukaryotes, this respiratory electron transport is localized in the inner membranes of the mitochondria, specialized intracellular organelles that are used for energy generation.

When oxygen is not available as electron acceptor, respiration may still be possible when other potential electron acceptors are present in the medium. Such processes of "anaerobic respiration" are known to occur in the prokaryotic world only. Depending on the organism, electron acceptors that can be used for the purpose are oxidized nitrogen compounds (nitrate, nitrite), sulfur compounds such as sulfate and elemental sulfur, trivalent iron, tetravalent manganese, and others. Such alternative electron acceptors are generally more reduced than molecular oxygen, and the amount of energy gained by respiratory electron transport from NADH generated during oxidation of the organic electron donor to the acceptor molecule is therefore less than what can be obtained during aerobic respiration (see also Fig. 5.2 and Eq. (6)). Fig. 5.5b explains the principles of anaerobic respiration, principles that are explained in further depth in Sects. 5.2, 5.4, and 5.5. Also in organisms performing anaerobic respiration,

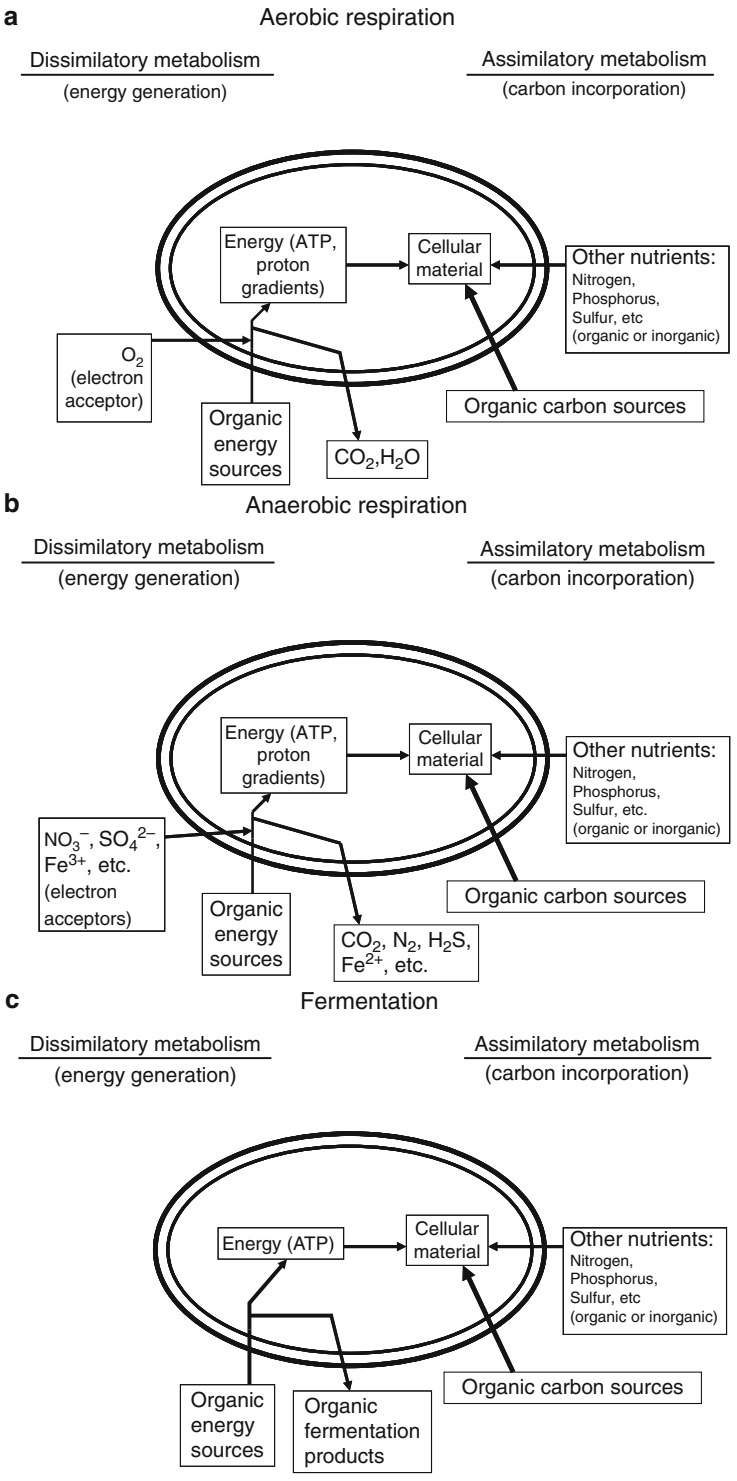

Fig. 5.5. The principles of respiratory and fermentative life.

incomplete oxidation processes may occur, such as shown by the case of the sulfate-reducing bacteria of the genus *Desulfovibrio*, which oxidize lactic acid to acetic acid $+CO_2$ while using sulfate as the electron acceptor.

When no potential electron acceptors are available, energy may still be gained by fermentation. In fermentative processes, organic substrates are degraded to other, generally smaller, organic products. Energy generation proceeds via substrate-level phosphorylation only, and is based on ATP formation from those high-energy intermediates of the metabolic pathways presented in Table 5.2. No respiratory electron transport from NADH to an acceptor occurs in the cell membrane during fermentation that would enable the generation of a transmembrane proton gradient. A schematic representation of the principle of fermentative life is given in Fig. 5.5c (12).

Fermentation is seldom found in the eukaryotes. Higher animals, including man, have a limited potential of energy generation by fermentation; muscles that do not receive a sufficient supply of oxygen, e.g., as a result of excessively high body activity, can still form two molecules of ATP per molecule of glucose taken up from the blood by fermenting it to two molecules of lactic acid ("homolactic fermentation"), in a reaction that is similar to that performed by lactic acid bacteria (*Lactobacillus*, *Streptococcus*) that produce lactic acid from sugars during the fabrication of cheese, yogurt, sauerkraut, etc., and during the formation of silage (see Sect. 5.3). One well-known group of fermentative eukaryotes is that of the yeasts (which generally can live by aerobic respiration as well when oxygen is supplied). Yeasts involved in the production of bread, wine, beer, and similar products ferment sugars to ethanol and CO_2, with the formation of two molecules of ATP per hexose molecule. Both the homolactic fermentation and the alcohol fermentation proceed via the reactions of the glycolytic pathway (see also Fig. 5.2). The intermediate pyruvate is in the first case reduced by NADH to lactic acid, and in the second it is decarboxylated to acetaldehyde which is then reduced to ethanol.

The third mode of energy generation in nature is chemoautotrophy or chemolithotrophy (13, 14). Chemoautotrophs are organisms that use inorganic compounds as energy sources for the generation of ATP as well as electron donors for assimilatory metabolism – the autotrophic fixation of CO_2. Chemoautotrophs, thus, depend on inorganic compounds only for growth and are independent of light energy. No chemoautotrophs are known among the eukaryotes, but a wide diversity of chemoautotrophic types is found in both the *Bacteria* and the *Archaea*. A variety of electron donors are used by different chemoautotrophs. Thus, aerobic chemoautotrophs can be found that use ammonium or nitrite as energy sources, producing nitrite and nitrate, respectively (the nitrifying bacteria, see also Sect. 6.1), and there are colorless sulfur bacteria that use sulfide, elemental sulfur, and other reduced sulfur compounds as energy source and electron donor, producing sulfate in the process (see Sect. 6.2). Other types make a living by oxidizing molecular hydrogen to water (Sect. 6.4), divalent iron to trivalent iron, or divalent manganese to tetravalent manganese (Sect. 6.3). The amount of energy to be gained by the transfer of electrons from the respective electron donors to molecular oxygen, the electron acceptor in all these cases, depends on the difference in standard reduction potential of the electron donating reactions involved and the reduction potential of the O_2/H_2O couple (+0.82 V), in accordance with Eq. (6) (see also Fig. 5.3). As explained above for the nitrifying

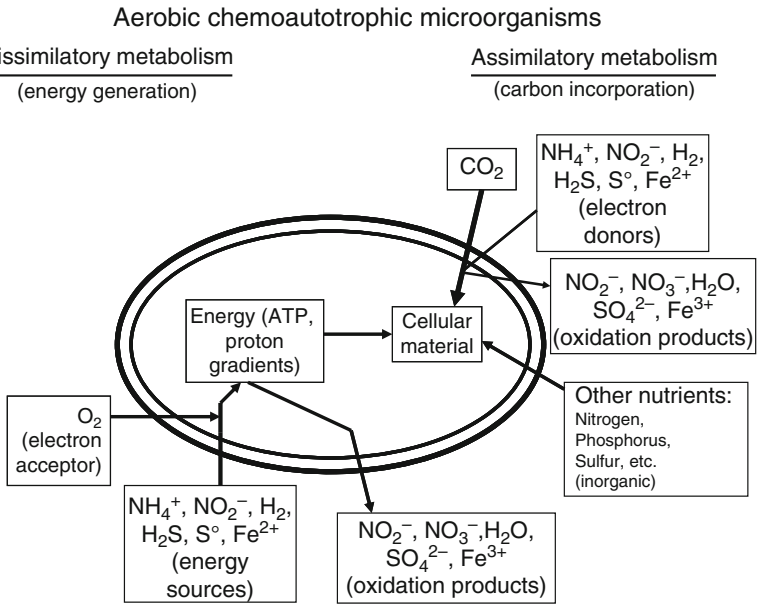

Fig. 5.6. The principles of aerobic chemoautotrophic (chemolithotrophic) life.

bacteria, chemoautotrophic prokaryotes that use relatively oxidized electron donors will have to invest considerable amounts of energy to produce reducing equivalents (as NADPH) for the autotrophic fixation of CO_2, and large amounts of substrate have therefore to be oxidized for the production of only a small amount of new cell material. Figure 5.6 provides an overview of the principles behind the metabolism of aerobic chemoautotrophs.

Chemoautotrophic life is possible under anaerobic conditions as well. As long as the electron transport occurs "downhill" from an electron donor with a redox potential lower than that of the electron acceptor (see Fig. 5.3), energy can be gained. We thus know bacteria that couple the oxidation of sulfide or elemental sulfur to sulfate with the reduction of nitrate (e.g., *Thiobacillus denitrificans*, an organism that can also grow aerobically using oxygen as electron acceptor). Many thermophilic and hyperthermophilic *Archaea* grow autotrophically by coupling the oxidation of molecular hydrogen with the reduction of elemental sulfur. Oxidation of hydrogen can also be coupled with the reduction of sulfate to sulfide in some sulfate-reducing bacteria.

Hydrogen can be used as electron donor for the dissimilatory reduction of CO_2 under anaerobic conditions in two types of metabolism. One yields methane as the product, the other acetic acid. All known methanogens are *Archaea*, and most are able to obtain their energy from the oxidation of hydrogen with CO_2 as electron acceptor. Such methanogens also use CO_2 as assimilatory carbon source, and they can therefore be termed chemoautotrophs. A schematic representation of the reactions performed by the hydrogen-oxidizing methanogens can be found in Fig. 5.7a. It should be noted that the details of the energy-yielding reactions involved differ greatly from those of the other chemoautotrophs. In the aerobic nitrifying and sulfur-oxidizing prokaryotes, energy is gained by the generation of a transmembrane proton

gradient during transport of electrons through a respiratory chain consisting of cytochromes, quinones, and other components, from the inorganic electron donor to molecular oxygen (or to nitrate in special cases, as stated above). The proton gradient is then used for the production of ATP. Also in the case of the methanogens, a proton gradient is generated, but here the formation of the proton gradient (and in some cases a sodium ion gradient as well) is directly coupled to some of the reactions that occur during the stepwise reduction of CO_2 to CH_4. Such hydrogen-oxidizing methanogenic *Archaea* even do not possess cytochromes. It should be stated here that not all methanogens are autotrophs. Some types, for example *Methanosarcina* and *Methanosaeta*, use acetate both as energy source and as their main carbon source (Fig. 5.7b), and accordingly they should be classified as heterotrophs. Such methanogens do possess cytochromes in their membranes, and their mode of energy generation thus differs quite significantly from that of the autotrophic methanogens.

A second type of metabolism that exploits the reduction of CO_2 with hydrogen as electron donor to generate energy exists in homoacetogenic bacteria. These organisms produce no methane but acetic acid, formed from two molecules of CO_2. More in-depth information about the function of methanogenic and the homoacetogenic prokaryotes in nature is given in Sect. 5.6.

A highly intriguing novel mode of autotrophic growth under anaerobic conditions was discovered a few years ago, when it was shown that ammonium ions can be oxidized with nitrite serving as the electron acceptor with the formation of molecular nitrogen as the product (15). In fact, the possible existence of such a process had already been predicted by Engelbert Broda in 1977 on purely thermodynamic grounds (16), as it was calculated that such a reaction is exergonic:

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O \quad \Delta G^{o\prime} = -357.8\,\text{kJ} \tag{9}$$

The process, now called the "anammox reaction" (*an*aerobic *amm*onium *ox*idation), was first found to occur in a laboratory-scale bioreactor for wastewater treatment, but it is now becoming clear that it commonly occurs in nature in anaerobic environments in which both nitrite (or nitrate) and ammonium ions are available. The organisms responsible for the anaerobic oxidation of ammonium appear to be representatives of the *Planctomyces* group (*Bacteria*). They use CO_2 as carbon source, which is reduced using nitrite as electron donor with the production of nitrate. The discovery of the anammox process proves once more that the prokaryotic world can use (nearly) any reaction that is thermodynamically feasible for energy generation, and that the appropriate enzymatic mechanisms can be developed to exploit such reactions. In the case of the anammox bacteria, some of these reactions are very unusual indeed; intermediates in the process of ammonium oxidation are exotic compounds as nitric oxide (NO) and even hydrazine (N_2H_2), a compound better known as a rocket propulsion fuel than as an intermediate in biochemical pathways. It is yet to be seen whether two other autotrophic processes calculated as thermodynamically feasible by Broda are also realized in the microbial world. One is the anaerobic oxidation of ammonium ions with nitrate as electron acceptor:

$$5NH_4^+ + 3NO_3^- \rightarrow 4N_2 + 9H_2O + 2H^+ \quad \Delta G^{o\prime} = -1483.5\,\text{kJ} \tag{10}$$

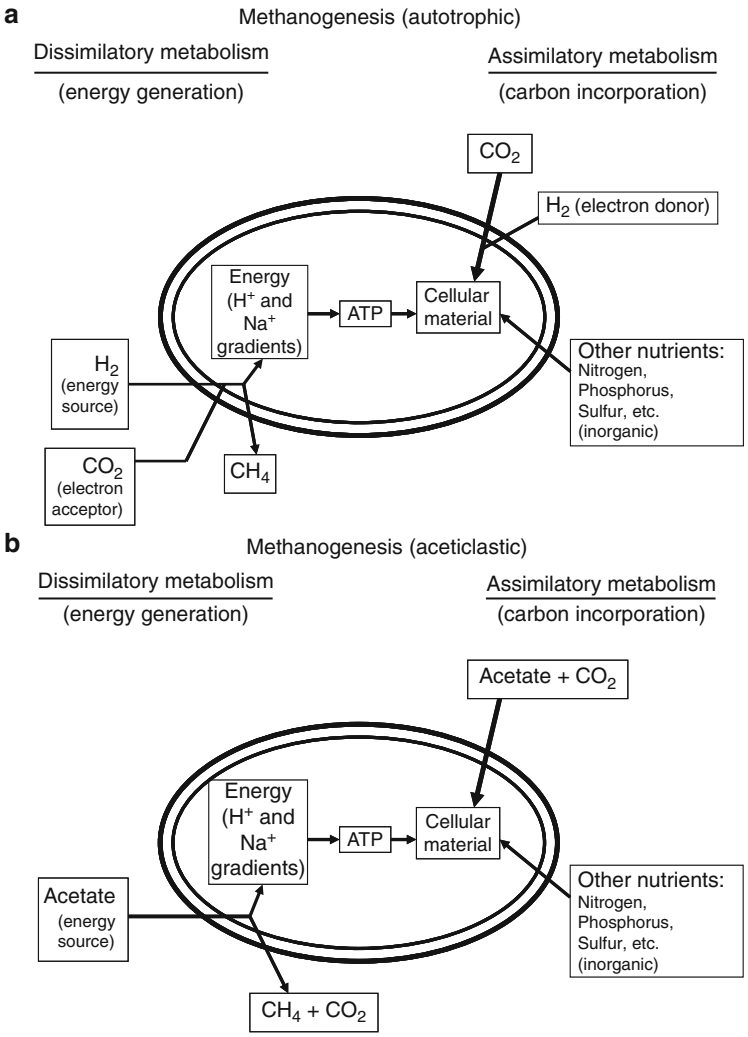

Fig. 5.7. The principles of the metabolism of methanogenic bacteria.

As far as known, this reaction is only realized in two steps, with "conventional" nitrate-reducing bacteria reducing the nitrate to nitrite, and the anammox organisms then performing the remaining part of the reaction as given in Eq. (9). The second process envisaged by Broda (16) is the use of ammonium ions as electron donor in anoxygenic photosynthesis and photoautotrophic growth, analogous to the use of reduced sulfur compounds by the purple and green sulfur bacteria (see also Sect. 4.2). No organisms have been discovered thus far that perform such a type of metabolism.

3. ASSIMILATORY METABOLISM OF MICROORGANISMS

Each cell that grows and multiplies needs a sufficient supply of building blocks for the synthesis of all components found in the cell. Molecules such as amino acids, sugars, and nucleotides must be available, and these have to be polymerized to form biological macromolecules at the expense of energy. In addition to carbon, many other elements are needed to fulfill the nutritional demands of the cell. They include nitrogen, sulfur, phosphorus, and a large number of other elements that are required in small amounts, such as iron, manganese, nickel, cobalt, and other metals that are needed in trace concentrations.

The prokaryotes display not only a large diversity in their dissimilatory metabolism, exploiting many different ways of generating energy, but they are also extremely resourceful in finding different modes of obtaining and exploiting different sources of nutrients to supply their assimilatory demands and to synthesize all those compounds needed for the proper functioning of the cell. Like in the dissimilatory metabolism, the prokaryotes are considerably more diverse than the eukaryotic micro- and macroorganisms when it comes to the variety of building blocks that can be used and the ways these are incorporated by the cells.

This section provides a brief overview of the ways prokaryotic microorganisms fulfill their assimilatory demands for the different elements of which the living cell is composed.

3.1. Carbon Assimilation

As explained in Sect. 2.1, we can divide the microorganisms into two groups with respect to the nature of their assimilatory carbon source; autotrophs that use CO_2 and heterotrophs that depend on organic carbon for growth.

Among the heterotrophic microorganisms, there is a wide diversity in the demands for organic carbon compounds. Some bacteria have very limited biosynthetic abilities and can only grow when supplied with a complex mixture of amino acids, nucleotides, and vitamins. The lactic acid bacteria (*Lactobacillus* and relatives) are well-known such highly fastidious organisms. For many other heterotrophs, a single compound such as glucose is sufficient to provide the cellular carbon. An organism such as *Escherichia coli* can grow aerobically on a simple medium that contains glucose as carbon source with inorganic nitrogen being supplied as ammonium or nitrate ions, and other essential elements, such as sulfur and phosphorus, being present as inorganic salts. Certain bacteria can use a tremendous variety of different carbon compounds for growth. Some representatives of the genus *Pseudomonas* can use no less than 70–80 different organic compounds to provide both the cellular carbon and the energy for biosynthesis. The list of such single carbon sources for growth includes carbohydrates, fatty acids, dicarboxylic acids, amino acids, alcohols, and more exotic substrates such as aromatic compounds (benzoate, phenol), including many that are toxic and that are degraded by a few microorganisms only. Between the extreme cases of the lactic acid bacteria with their extremely limited biosynthetic potential and *Pseudomonas* species that can grow on nearly any bioavailable carbon source are many intermediate cases of microorganisms that require the presence of a number of different organic compounds (e.g., amino acids, vitamins) for growth as their biosynthetic machinery is unable to synthesize these from simpler components.

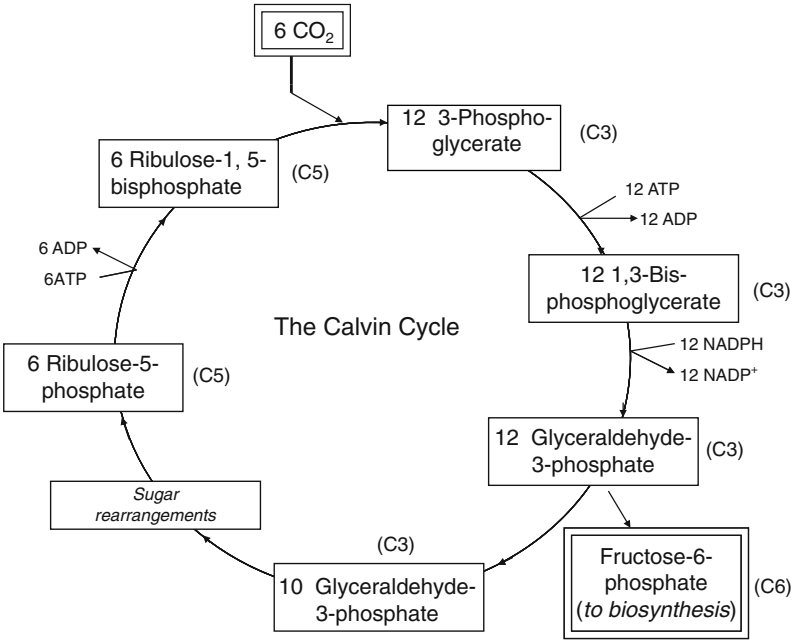

Fig. 5.8. Autotrophic CO_2 fixation by the Calvin cycle.

As shown in the earlier sections, the autotrophic way of life is widespread in the prokaryotic world. We know oxygenic phototrophs (the cyanobacteria) (see Sect. 4.1), anoxygenic phototrophic bacteria (see also Sect. 4.2), aerobic chemoautotrophs such as the nitrifying and the colorless sulfur bacteria (see Sects. 6.1 and 6.2), and anaerobic chemoautotrophs such as many methanogenic and homoacetogenic bacteria (see Sects. 5.6 and 6.4). These groups differ in the nature of their energy sources and the electron donors used for the reduction of CO_2.

Considerable diversity exists in the biochemical pathways enabling autotrophic CO_2 fixation. Green plants use the Calvin cycle, in which ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) is the key enzyme responsible for the conversion of inorganic to organic carbon (Fig. 5.8). The same pathway is used to drive autotrophic growth in eukaryotic algae, in cyanobacteria, in purple sulfur bacteria, and in aerobic chemoautotrophic bacteria such as the nitrifiers (*Nitrosomonas*, *Nitrobacter* and relatives) and the colorless sulfur bacteria (*Thiobacillus*, *Beggiatoa*, and related organisms). However, the Calvin cycle is by no means the only way carbon dioxide can be fixed by autotrophic organisms. For example, the green sulfur bacteria (*Chlorobium*, *Prosthecochloris*) fix CO_2 by reversing the reactions of the tricarboxylic acid cycle Krebs cycle, see also the lower part of Fig. 5.2). This, combined with the reductive carboxylation of acetyl-CoA to pyruvate, enables the biosynthesis of sugars and other cellular components. Another strategy for autotrophic carbon fixation, found both in certain representatives of the *Bacteria*, e.g., a number of autotrophic sulfate-reducing bacteria and homoacetogens, as well as in many of the methanogens, starts with the reduction of carbon

dioxide to carbon monoxide, which is then coupled with methyl groups (bound to coenzymes such as coenzyme B_{12}) to form acetyl-CoA.

A limited number of prokaryotes can grow on methane as sole carbon and energy source. Strictly spoken such methanotrophs cannot be considered autotrophs their substrate, CH_4, is an organic compound. However, the use of a one-carbon compound, such as methane as their carbon source, presents the cells with similar problems as autotrophic growth on CO_2. Two different pathways lead to the goal. One (the "ribulose monophosphate cycle") uses formaldehyde, formed by partial oxidation of the methane, as precursor of cellular carbon, with ribulose 5′-monophosphate serving as the acceptor of the one-carbon units. The second (the "serine pathway") uses two reactions in which one-carbon compounds enter the metabolism, one being the formation of serine from glycine and formaldehyde (bound to the coenzyme tetrahydrofolate), the second binding CO_2 to phosphoenolpyruvate to form oxalacetate. A full discussion of the details of these unique pathways is outside the scope of the present review; more details can be found in textbooks (1, 4, 5) and in specialized review articles.

3.2. Nitrogen Assimilation

After carbon, nitrogen is the most important nutrient to be incorporated by any cell to produce proteins, nucleic acids, and other compounds. Many heterotrophic microorganisms (and all heterotrophic macroorganisms as well) are unable to obtain their cell nitrogen from inorganic sources, and they have to take up organic forms of nitrogen such as amino acids that had been produced by the autotrophs in their ecosystem. Extracellular proteases are often excreted by such heterotrophs to degrade proteins to amino acids that can be taken up.

Inorganic nitrogen sources that can be used by many groups of prokaryotes, both autotrophic and heterotrophic, include nitrate (NO_3^-), ammonium ions (NH_4^+), and molecular nitrogen (N_2). Nitrate and ammonium are also suitable nitrogen sources for higher plants and eukaryotic algae. However, the ability of using dinitrogen as assimilatory nitrogen source is restricted to the prokaryotic world. The nitrogen in proteins and other cellular components mainly exists in the form of $–NH_2$ groups, i.e., in the same oxidation state as ammonium (N^{3-}). The incorporation of ammonium ions into amino acids is therefore relatively simple, and ammonium is generally available both under aerobic and under anaerobic conditions. One way of ammonium incorporation is by reductive amination of α-ketoglutarate, an intermediate of the tricarboxylic acid cycle, (see Fig. 5.2) to form the amino acid L-glutamate. This reaction is mediated by the enzyme glutamate dehydrogenase. The disadvantage of this pathway is the low affinity of the enzyme for ammonium ions, making the reaction little effective when ammonium concentrations in the medium are low, as they often are. A quantitatively much more important pathway starts with the amidation of glutamate to form glutamine by glutamine synthase, a reaction that proceeds under expenditure of ATP by an enzyme that has a high affinity for ammonium ions. The amino group is subsequently transferred to α-ketoglutarate (α-oxoglutarate) to form glutamate in a reaction mediated by an enzyme known as GOGAT (glutamine–oxoglutarate aminotransferase). The overall result of both pathways is that glutamate is formed from α-ketoglutarate and ammonium, in the second case at the cost of ATP, allowing nitrogen incorporation also at low ammonium concentrations. From

glutamate, the newly formed amino group can be transferred to other carbon compounds to form all other amino acids, purine and pyrimidine bases of DNA and RNA, and other organic nitrogen compounds present in the cell.

Nitrate is another widely used source of nitrogen for assimilation. It is generally available in aerobic environments only, as anaerobically it is preferentially used as an electron acceptor for anaerobic respiration (denitrification, see Sect. 5.2). To serve as assimilatory nitrogen source, nitrate first has to be reduced to ammonium, whereafter it is incorporated into amino acids by the reactions outlined in the previous paragraph. When both ammonium and nitrate-nitrogen are available in the environment, most microorganisms that can use both preferentially take up the ammonium and repress the assimilatory reduction of nitrate at the expense of reducing power and energy.

The ability to use molecular nitrogen (dinitrogen; N_2), the most abundant form of nitrogen in the biosphere, as a source of cellular nitrogen is limited to the prokaryote world, and only a limited number of prokaryotes possess nitrogenase, the enzyme complex needed to fix nitrogen. Most nitrogen-fixing microorganisms are found in the domain *Bacteria*. Many photosynthetic prokaryotes have nitrogenase, both oxygenic species (many, but not all cyanobacteria) and anoxygenic phototrophs. Among the free-living chemoheterotrophs that can use dinitrogen as N-source are aerobes (*Azotobacter*, *Beijerinckia*, and others) and anaerobes (some members of the genus *Clostridium*, many sulfate-reducing bacteria). The finding of nitrogenase activity in some methanogens proves that nitrogen fixation is also possible in representatives of the *Archaea*. There are many known cases of symbiotic associations between nitrogen-fixing prokaryotes and higher organisms. The best-known such association is that of the aerobic bacterium *Rhizobium* that lives in the root nodules of leguminous plants and supplies the plant with nitrogen in exchange for organic nutrients. Other examples are the association of *Azospirillum* with the roots of many plants, and the symbiosis of filamentous cyanobacteria related with the genus *Anabaena* within the leaves of the water fern *Azolla*.

The nitrogenase complex reduces dinitrogen to ammonium ions, which are then available for assimilation into amino acids as outlined above. From the point of view of bioenergetics, nitrogen fixation is a very expensive process. The triple bond between the two atoms in the nitrogen molecule is very stable, and the activation energy needed to break this bond is accordingly high. It has been estimated that between 6 and 15 molecules of ATP are required to provide the energy for the fixation of one molecule of nitrogen. It can therefore be expected that when other forms of nitrogen are available (ammonium, nitrate), nitrogen-fixing microorganisms shut down the synthesis and the activity of nitrogenase, and use energetically more favorable nitrogen sources instead.

Nitrogenase is a complex enzyme that contains both iron and molybdenum. A common property of all bacterial nitrogenases, both from aerobic and from anaerobic microorganisms, is their high sensitivity to molecular oxygen. This is not a problem for the many anaerobic prokaryotes that fix nitrogen, but microorganisms that lead an aerobic life and at the same time need to fix nitrogen will need special arrangements to protect the nitrogen-fixing machinery against damage by oxygen. Many (but not all) filamentous nitrogen-fixing cyanobacteria produce differentiated cells called heterocysts. These cells lack activity of photosystem II, so that no photosynthetic oxygen evolution takes place intracellularly, but they do contain

photosystem I, enabling the production of ATP at the expense of light. Moreover, the cell wall of the heterocyst is particularly thick, restricting entry of oxygen from outside. Some other nitrogen-fixing cyanobacteria protect their nitrogenase by separating the processes of photosynthesis and nitrogen assimilation in time, evolving oxygen during daytime and fixing nitrogen during the night driven by energy released during the oxidation of storage polymers (starch and others) that had been accumulated during the light periods. *Azotobacter*, a free-living heterotrophic bacterium common in the soil, and responsible for a significant flux of nitrogen from the atmosphere into the soil, has among other adaptations an extremely high rate of respiration, efficiently removing oxygen before it can damage the sensitive nitrogenase complex. Finally, complex mechanisms exist in the symbiotic association of *Rhizobium* in the root nodules of leguminous plants to regulate the oxygen in the surroundings of these aerobic bacteria to levels optimal for both survival and nitrogen fixation, mechanisms to which both the plant and the bacterium contribute. A hemoglobin-like protein is present in the root nodules, which binds oxygen and enables its controlled release when needed.

An interesting storage polymer named cyanophycin can be accumulated by many cyanobacteria. Cyanophycin is a polymer in which two amino acids, arginine (which contains four nitrogen atoms) and aspartate (containing one nitrogen atom) alternate. Cyanophycin is formed when suitable nitrogen sources are abundantly available, and it can be degraded later when nitrogen is in short supply. Another possible function of cyanophycin is energy storage; conversion of arginine to the amino acid ornithine releases carbamyl phosphate, a high-energy compound (see Table 5.2) that can be split to ammonium ions and CO_2 with the formation of ATP.

3.3. Phosphorus Assimilation

Phosphorus is almost exclusively found in nature in its most oxidized state (P^{5+}), either as inorganic phosphate ($PO_4{}^3-$) or as organically bound phosphate. Uptake of phosphate occurs in the inorganic form, and organic phosphorus compounds present in the environment can be used by microorganisms as assimilatory source of phosphorus only, following the release of the phosphate groups catalyzed by extracellular phosphatases.

Many microorganisms, including heterotrophic bacteria and cyanobacteria, can accumulate phosphorus intracellularly as storage granules ("volutin granules") in the form of inorganic polyphosphate when phosphorus is abundantly present in their medium. Such polyphosphates can later serve as source of phosphorus.

3.4. Sulfur Assimilation

Sulfur is found in the cell mainly in two amino acids: cysteine and methionine. The sulfur is here present in its reduced form. Reduced sulfur is present in the cell in other forms as well, in organic molecules such as coenzyme A, in iron–sulfur centers that make part of the respiratory electron transport chain, etc.

Many heterotrophic bacteria (as well as most heterotrophic higher organisms, including animals) can obtain sulfur only in the form of amino acids. Autotrophs (higher plants, photosynthetic and chemosynthetic bacteria, as well as many heterotrophic bacteria) use inorganic

sources of sulfur. The direct precursor of the organic sulfur in the sulfur-containing amino acids is H_2S. In anaerobic environments, sulfide is generally abundantly found, derived both from degradation of proteins and from dissimilatory reduction of sulfate during anaerobic respiration by sulfate-reducing bacteria (sees Sect. 5.5). Therefore, sulfide is generally directly available for incorporation into amino acids. In the aerobic world, sulfate (SO_4^{2-}) is the only abundant form of available sulfur. Assimilatory use of sulfate to be incorporated into amino acids requires its prior reduction to sulfide. Assimilatory reduction of sulfate is an energy requiring process, in which the sulfate is coupled to ATP with the formation of adenosine-5′-phosphosulfate (APS) at the expense of two high-energy bonds of ATP, following by the phosphorylation of APS to PAPS (adenosine-3′-phosphate-5′-phosphosulfate). PAPS is then reduced to form sulfite (SO_3^{2-}), which is subsequently further reduced to sulfide in a single enzymatic step, followed by its incorporation into sulfur-containing amino acids.

3.5. Iron Assimilation

Iron is needed by almost all cells as components of cytochromes, ferredoxin (a low potential mediator of redox reactions in photosynthesis, nitrogen fixation, and other cellular functions), and other enzymes. The lactic acid bacteria, a group of fastidious bacteria that lack respiration and have very limited biosynthetic potential, are probably the only organisms that do not require any iron for growth.

Iron can be taken up from the medium by different mechanisms. These often involve special carrier molecules (“siderophores”) that act as chelators and thus keep trivalent iron in solution, preventing its precipitation as iron oxides or other poorly soluble compounds.

A special case of an exceptionally high requirement for iron to be assimilated by the cells is that of the magnetotactic bacteria. Certain groups of bacteria, generally microaerophilic types that thrive best at low oxygen concentrations, synthesize small magnetic particles consisting of either magnetite (Fe_3O_4) or greigite (Fe_3S_4). These are accumulated in the cytoplasm, and are arranged in one or more well-ordered rows. These rows of “magnetosomes” enable the cells to orient themselves in the Earth magnetic field and to direct their swimming movement along the downward oriented magnetic field lines to reach optimal oxygen concentrations near the surface of the sediment.

4. THE PHOTOTROPHIC WAY OF LIFE

The previous pages have provided a general introduction, describing the principles of the dissimilatory and the assimilatory metabolism of different groups of microorganisms. We have outlined the tremendous metabolic diversity that is found especially in the prokaryote world (*Bacteria* and *Archaea*). In the sections below a more in-depth picture will be given of the different types of microorganisms found in nature, of the ways they exploit the resources present in their environment, and of the modes they act together to enable the functioning of the major biogeochemical cycles in nature: the cycles of carbon, nitrogen, and sulfur. First the phototrophic way of life will be discussed.

4.1. Oxygenic Photosynthesis

We owe the presence of molecular oxygen in the atmosphere to the process of oxygenic photosynthesis (photosynthesis that uses water as electron donor with the evolution of oxygen, see Sect. 2.2). This type of photosynthesis is found not only in eukaryotic microalgae (as well as in macroalgae and in higher plants), but also in the cyanobacteria, a branch of the *Bacteria*.

Nearly, all oxygenic phototrophs all use chlorophyll *a* as the major pigment in the photosynthetic reaction centers, generally accompanied by other forms of chlorophyll such as chlorophyll *b* in the green algae. In most representatives of the cyanobacteria, we find chlorophyll *a* as the only chlorophyll. The prochlorophytes, which phylogenetically are affiliated with the cyanobacteria, are exceptional among the prokaryotes as they contain chlorophyll *b* derivatives as well. An ecologically highly important member of this group is the small unicellular phototroph *Prochlorococcus*, one of the most abundant photosynthetic organisms in the ocean (17). Accessory pigments are generally present to aid in the harvesting of light. These include carotenoid pigments and the phycobiliproteins – the blue phycocyanin and the red phycoerythrin of the cyanobacteria, the latter also being found in the red algae. The range of wavelengths used by the oxygenic phototrophs is from 400 nm (blue light) to 700 nm (red light), spanning whole range of the visible light. We find oxygenic phototrophic microorganisms anywhere in nature where light and water are available and aerobic conditions prevail. The extent of development of oxygenic phototrophic planktonic organisms in water bodies generally depends mainly on the availability of essential inorganic nutrients, such as nitrogen (see Sect. 3.2), phosphorus (Sect. 3.3), and in certain cases such in the central parts of the Pacific Ocean, iron (see Sect. 3.5).

4.2. Anoxygenic Photosynthesis

The world of phototrophs is far much diverse than appears from the abundance of oxygenic phototrophs only. Use of light energy mediated by chlorophyll derivatives (bacteriochlorophylls) not coupled to the evolution of oxygen is possible in many ways, as shown by a variety of groups of prokaryotes, all belonging to the domain *Bacteria*, but phylogenetically affiliated with very different phyla within the *Bacteria* (11, 18) With very few exceptions these anoxygenic phototrophs develop under anaerobic conditions only. Due to the high standard reduction potential of the couple H_2O/O_2 (+0.82 V, see Fig. 5.3), the reduction of $NADP^+$ needed for autotrophic CO_2 fixation with electrons from water during oxygenic photosynthesis is an energetically expensive process. Two photons are required per electron transferred to $NADP^+$, and oxygenic photosynthesis therefore involves two photosynthetic reaction centers acting in series: photosystem II that oxidizes water and is responsible for oxygen evolution, transferring the electrons to photosystem I which enables the reduction of $NADP^+$. Alternative electron donors used by the different types of anoxygenic phototrophs discussed below are more reduced than water. Such organisms use a single photosystem to drive the photosynthetic processes.

Purple sulfur bacteria phylogenetically belong to the *Proteobacteria* branch of the *Bacteria*. They use sulfide and other reduced sulfur compounds as electron donor for autotrophic CO_2 fixation. They are found in environments in which both light and sulfide are present.

Such habitats include stratified lakes: when sufficient light penetrates through the upper, oxygenated water layer and reaches the anaerobic, sulfide-containing deeper water, dense blooms of purple sulfur bacteria such as *Chromatium*, *Thiocapsa*, and others often develop just below the oxic-anoxic boundary. We also find such organisms in a thin layer below the surface of many shallow marine sediments in which sulfide diffuses upward and meets light coming from above. Such a layer of purple bacteria is often found just below a blue-green layer of cyanobacteria (oxygenic phototrophs) that inhabit the aerobic surface layer of the sediment. A third type of environment in which dense development of purple sulfur bacteria are often observed is in sulfur springs in which geothermal sulfide that emerges from the spring comes in contact with light. The main photosynthetically active pigment in most purple sulfur bacteria is bacteriochlorophyll *a*, a pigment that absorbs light not only in the blue range of the spectrum but also in the infrared range (800–860 nm), Other members of the group have bacteriochlorophyll *b*, a pigment that in vivo shows an absorption peak in the far infrared at 1,020 nm. These are wavelengths not used by oxygenic phototrophs, as these do not absorb light above 700 nm. As a result, anoxygenic and oxygenic phototrophs can coexist in stratified systems such as marine sediments as each group selectively absorbs wavelengths not used by the others. In deep water bodies the ability to use infrared light to drive anoxygenic photosynthesis is of little practical value, as such long-wavelength radiation does not penetrate deeply into the water column, which preferentially transmits light of from around 480 nm (clear "blue" waters) to about 550 nm (turbid "green" waters). The sulfur used as electron donor is oxidized to sulfate. Elemental sulfur formed as intermediate is stored as intracellular sulfur granules in many species (*Chromatium*, *Thiocapsa*, and others), but other members of the group such as *Ectothiorhodospira* and relatives – purple sulfur bacteria that are often found in saline and in hypersaline environments, excrete the sulfur into the medium, to be taken up later to be further oxidized to sulfate.

The green sulfur bacteria (*Chlorobium*, *Prosthecochloris*, and related genera) resemble the purple sulfur bacteria in many aspects. They also oxidize sulfur to sulfate and produce elemental sulfur as intermediate, which is excreted from the cells. However, phylogenetically they are unrelated to the Proteobacteria, and they form a separate lineage within the *Bacteria*. Their absorption spectrum is different with an in vivo absorption maximum at 750–760 nm attributable to bacteriochlorophyll *c*, and the structure of the photosynthetic system differs from that of the purple bacteria in many features. Moreover, they do not use the Calvin cycle for autotrophic fixation of CO_2, but use an alternative pathway, based on reversal of the reactions of the tricarboxylic acid cycle (see Sect. 3.1). Like the purple sulfur bacteria, the green sulfur bacteria are often found to accumulate in stratified lakes at those depths just below the aerobic-anaerobic boundary where sulfide is available and sufficient light is present. Some species are very efficient at growing at extremely low light intensities, such as the case of the Black Sea shows: a species of *Chlorobium* was found developing there at depths between 80 and 100 m, at light intensities as low as 1/500,000 of full sunlight.

Not all anoxygenic photosynthetic bacteria are autotrophs. Photoheterotrophic growth is found in purple nonsulfur bacteria such as *Rhodobacter* and *Rhodospirillum* (Proteobacteria) and green nonsulfur bacteria such as *Chloroflexus* (which forms a separate lineage within the domain *Bacteria*). In such organisms, light provides the energy, while most of the carbon

needed for growth is derived from organic carbon sources (see Sect. 2.2). *Rhodobacter* and relatives are among the metabolically most versatile of all prokaryotes. They can live as photoheterotrophs under anaerobic conditions, as photoautotrophs using hydrogen as electron donor, or even (in spite of their designation as purple "nonsulfur" bacteria) on sulfide when present in very low concentrations. Photoautotrophic growth using divalent iron as electron donor, which is oxidized in the process to trivalent iron, has also been documented in some representatives of the group. Furthermore, they can live aerobically as chemoheterotrophs by aerobic respiration on a variety of organic compounds or as chemoautotrophs on a mixture of hydrogen, oxygen, and carbon dioxide. During aerobic growth, these cells do not synthesize bacteriochlorophyll and other components of the photosynthetic machinery, and the cells do not obtain the red color of light-grown cells. Dark anaerobic growth by fermentation is possible as well. In spite of this metabolic versatility, the purple nonsulfur bacteria are not among the most abundant organisms in nature, and they are never seen to accumulate in high density in any ecosystem. The green nonsulfur bacteria are often found in coastal microbial mats in shallow sediments, and also in microbial biofilms that cover the bottom of many thermal springs. Recently, the presence of photoheterotrophic representatives of the Gram-positive branch of the *Bacteria* (e.g., the genus *Heliobacterium*) has also been documented. Such organisms appear to be common in soil.

Generally spoken, it is true that oxygenic phototrophs (plants, algae, cyanobacteria) inhabit the aerobic world, and the anoxygenic phototrophs are restricted to anaerobic environments. However, exceptions exist. Certain species of cyanobacteria can shift from their oxygenic type of photosynthesis to an anoxygenic photoautotrophic metabolism, using sulfide as electron donor. In anoxic sulfide-containing habitats, such cyanobacteria repress the activity of the water-splitting photosystem II. Electrons from sulfide are donated to photosystem I, and the sulfide is oxidized to elemental sulfur in the process.

It has also become clear that anoxygenic, bacteriochlorophyll *a* containing photoheterotrophic bacteria exist that inhabit aerobic environments. Occurrence of such bacteria (genera *Erythrobacter*, *Roseobacter*, and others) in the oxic marine environment was first documented in the early 1990s. It was recently documented that such photoheterotrophic organisms are very abundant in the ocean, and there are even indications that they may be responsible for a significant part of the photosynthetic electron transport occurring in the marine environment (19, 20).

4.3. Retinal-Based Phototrophic Life

A completely different way of using light as energy source for photo(hetero)trophic growth, not depending on chlorophyll derivatives as the photoactive pigments, is by using retinal-containing proteins. The best known case of retinal-based photoheterotrophic growth is documented in *Halobacterium salinarum*, a representative of the extremely halophilic *Archaea*. *Halobacterium* is an aerobic heterotrophic archaeon that requires at least 150 g/l salt for growth, and grows at salt concentrations up to saturation. *Halobacterium* and related genera develop in hypersaline lakes, saltern evaporation and crystallizer ponds, and on heavily salted meat, fish, and hides. These extreme halophiles are colored red due to a high content of carotenoid pigments. Under the proper conditions (availability of light, low

oxygen concentrations), *Halobacterium* produces in addition to the red carotenoids a purple pigment named bacteriorhodopsin, which is located in patches in the cytoplasmic membrane ("purple membrane"). Bacteriorhodopsin is a 25 kDa protein that carries retinal as a prosthetic group. It absorbs light with an absorbance maximum at 570 nm. In many of its properties, it resembles rhodopsin, the visual pigment of the human eye. When bacteriorhodopsin is excited, protons are transported from the cytoplasmic side of the membrane to the outer medium, thus establishing a transmembrane proton gradient that can be used for the generation of ATP mediated by the membranal ATP synthase. Bacteriorhodopsin-containing cells can use light energy to drive photoheterotrophic growth in the absence of oxygen. Photoautotrophic growth has never yet been demonstrated in the halophilic *Archaea*. A second retinal pigment present in *Halobacterium* and in many other halophilic *Archaea* is halorhodopsin (absorbance maximum 580 nm), which uses light energy to pump chloride ions from the medium into the cells.

Until recently, it was assumed that photoheterotrophy based on retinal proteins is restricted to *Halobacterium* and a few other extremely halophilic *Archaea*. However, genes that encode bacteriorhodopsin-like proteins were first detected in DNA extracted from marine bacterioplankon in 2000 (21). The presence of a 16S rRNA gene in the same genome fragment showed that the DNA fragment carrying the bacteriorhodopsin-like gene belongs to a yet uncultured representative of the Proteobacteria. Cloning and expression of this gene in *Escherichia coli* led to the formation of a functional light-driven proton pump, now termed proteorhodopsin, when the prosthetic group retinal was supplied in the medium. Subsequently, it was shown that functional proteorhodopsin in present in large amounts in the membranes of marine bacterioplankton (22). Different varieties of proteorhodopsin are now known, with absorbance maxima from around 530 nm – abundant in ocean (surface water) to around 490 nm – in the deeper waters (75 m) in the oligotrophic ocean where light of 480 nm penetrates deepest. Genes for proteorhodopsin have now been found in different representatives of the α- and the γ-branch of the Proteobacteria, all yet uncultured. It is now estimated that light energy absorbed by proteorhodopsin contributes significantly to the energy household of the bacterial community in the sea.

5. CHEMOHETEROTROPHIC LIFE: DEGRADATION OF ORGANIC COMPOUNDS IN AEROBIC AND ANAEROBIC ENVIRONMENTS

Chemoheterotrophic bacteria are by far the most abundant among the known species of prokaryotes. They display a tremendous diversity in the range of organic compounds they degrade (see also Sect. 2.2) and in their way of life: aerobic, anaerobic, or facultative aerobic, degrading organic compounds by fermentation or by respiration using a range of electron acceptors. The following sections provide an overview of the ways organic compounds are degraded in nature. Complete aerobic degradation of commonly found organic compounds to carbon dioxide can generally be accomplished by a single type of microorganism. However, complete degradation under anaerobic conditions (to CO_2 when external electron acceptors are present, to a mixture of CO_2 and methane when no suitable electron acceptors are supplied), generally requires a collaborative effort involving a variety of metabolic types of

microorganisms that often maintain complex interactions that have to be closely coordinated to enable anaerobic mineralization of organic carbon in nature.

5.1. Aerobic Degradation

As long as oxygen is available as electron acceptor, nearly any naturally occurring organic compound can be degraded to CO_2. As explained in Sect. 2.2, the strategy of aerobic heterotrophic microorganisms – bacteria as well as fungi and other microorganisms – is to convert the available compounds to intermediates of the central metabolic pathways of the cell such as the glycolytic pathway and the tricarboxylic acid cycle (see Fig. 5.3). These intermediates can then be oxidized to CO_2 through the major metabolic pathways. The electrons released in the process (mostly in the form of NADH) are transferred to oxygen through the cytochromes and other components of the electron transport chain present in the cytoplasmic membrane (in the case of prokaryotes) or in the mitochondria (in the case of eukaryotic microorganisms), coupled with the formation of transmembrane proton gradients that are subsequently used to generate ATP.

As explained before (see Sect. 3.1), the number of different carbon compound that can be degraded by prokaryotes, aerobically as well as anaerobically, is extremely great. The list contains many compounds of importance in environmental engineering such as pesticides and other toxic or harmful chemicals. Biodegradation and bioremediation processes are based on the existence of degradation pathways for such compounds. An overview of 190 metabolic pathways identified to be involved in the breakdown of 1206 compounds (as of November 18, 2009), can be found in the University of Minnesota Biocatalysis/Biodegradation Database (http://umbbd.msi.umn.edu). An in-depth discussion of all these pathways is outside the scope of the present chapter.

The degradation of certain organic molecules, such as hydrocarbons and many aromatic compounds, also involves a direct participation of molecular oxygen. Such molecules can only enter the central metabolic pathways after oxygen molecules have been introduced derived from O_2 by enzymes such as dioxygenases (enzymes that introduce both oxygen atoms of O_2 into the organic compound to be degraded) and monooxygenases (enzymes that add one of the two atoms of O_2 to the organic carbon chain, and reduce the second oxygen atom to water with electrons derived from NADH). A well-known case of the activity of monooxygenases in the initial stage of microbial degradation of organic compounds is the aerobic breakdown of oil hydrocarbons. The first reaction toward the degradation of straight-chain aliphatic hydrocarbons is generally the introduction of an –OH group at one end of the carbon chain, mediated by a monooxygenase. The alcohol group is further oxidized to a carboxyl group, whereafter the long-chain fatty acid formed is degraded stepwise to release two-carbon units in the form of acetyl-CoA, and these are further degraded through the reactions of the tricarboxylic acid cycle. Aerobic oxidation of methane is also initiated by a monooxygenase reaction, in which methane is oxidized to methanol. Such a requirement for molecular oxygen in the initial steps of the aerobic breakdown of aliphatic and aromatic hydrocarbons and derivatives does not imply that such compounds cannot be broken down anaerobically as well. Alternative pathways often exist (not all of them understood in detail) that enable mineralization of such compounds also under anaerobic conditions (23, 24).

5.2. *Anaerobic Respiration: Denitrification*

It commonly occurs in nature that oxygen is not available as electron acceptor to allow aerobic degradation of biodegradable organic material. Examples are plentiful: deeper layers in marine and lake sediments, swamps, the hypolimnion of permanently stratified lakes and other water bodies such as the Black Sea, the digestive tract of animals, sewage treatment systems, etc. Even in well-aerated soils, anaerobic microenvironments are commonly found in which the local rate of oxygen consumption exceeds its supply by diffusion.

When oxygen becomes depleted, one of the first processes to occur is anaerobic respiration using nitrate as electron acceptor (25). The main product is generally gaseous nitrogen (N_2). When nitrate respiration leads to the loss of gaseous nitrogen from the system in the form of N_2 or other gases such as nitrous oxide (N_2O) and nitric oxide (NO), the process is known as denitrification. Dissimilatory reduction of nitrate proceeds in a number of steps in which the nitrate (NO_3^-, N^{5+}) is reduced through nitrite (NO_2^-, N^{3+}), nitric oxide (NO, N^{2+}) and nitrous oxide (N_2O, N^{1+}) to gaseous nitrogen (N_2, N^0). There are also cases known in which nitrate is reduced in a dissimilatory process to ammonium (NH_4^+, N^{3-}) ("nitrate ammonification"). From the point of view of bioenergetics, the amount of free energy released when organic material is oxidized with nitrate as electron acceptor is only little, less than what is obtained during aerobic oxidation with molecular oxygen as electron acceptor, for example:

$$\text{Glucose} + 4.8NO_3^- + 4.8H^+ \rightarrow 6CO_2 + 2.4N_2 + 8.4H_2O \quad \Delta G^{o\prime} = -2666\,\text{kJ} \qquad (11)$$

as compared to:

$$\text{Glucose} + 6O_2 \rightarrow 6CO_2 + 6H_2O \quad \Delta G^{o\prime} = -2822\,\text{kJ} \qquad (12)$$

Such a high energy yield can also be predicted from the relatively high standard reduction potentials of the different redox couples involved (NO_3^-/NO_2^-; NO_2^-/NO; NO/N_2O; N_2O/N_2, see Fig. 5.3). However, the amount of ATP gained by the cells during denitrification is significantly lower than during aerobic oxidation of the same substrates due to mechanistic constraints of the cellular metabolism.

Many bacterial species, belonging to highly diverse phylogenetic groups (including the *Archaea*), can live anaerobically by denitrification. They are nearly all facultative aerobes that use aerobic respiration as long as molecular oxygen is present. Only when oxygen is depleted and nitrate is available do the cells induce the special enzymatic machinery necessary for dissimilatory nitrate reduction. The number of organic compounds that can be degraded while using nitrate as electron acceptor is also very large. However, many compounds that require prior activation by molecular oxygen through the action of monooxygenases and dioxygenases cannot be broken down by denitrification, unless alternative pathways exist for their degradation that bypass the need for molecular oxygen.

Dissimilatory reduction of nitrate is found widespread in nature, and the process is responsible for great losses of biologically available nitrogen with the formation of nitrogen gas. Sometimes the occurrence of denitrification is favorable from the point of view of the environmental engineer, for example when denitrifying bacteria decrease the amount of nitrate-nitrogen during wastewater treatment. In other cases, denitrification causes economic losses,

such as the loss of nitrogen fertilizer (nitrate or ammonium that had been oxidized to nitrate by nitrifying bacteria), from poorly aerated soils (see Sect. 6.1).

5.3. Fermentation

When neither molecular oxygen nor oxidized nitrogen compounds are available as electron acceptors, degradation of organic material cannot generally proceed through anaerobic respiration processes. Potential alternative electron acceptors may still be available; sulfate is present in most environments, and abundantly so in the marine ecosystem. Other potential electron acceptors that are generally present in small amounts only are oxidized forms of iron (Fe^{3+}) and manganese (Mn^{4+}). However, sulfate-reducing bacteria do not use sugars, amino acids, and most other common organic compounds available in nature as electron donors, and the range of compounds that can serve as their electron donors/energy sources is extremely restricted. The list includes short-chain aliphatic acids (acetate, propionate, butyrate, and longer-chain fatty acids of up to 16–18 carbon atoms), other acids such as lactate, and a few other compounds such as ethanol and molecular hydrogen. Also, those bacterial species known to grown anaerobically while reducing trivalent to divalent iron (*Geobacter*, for example) prefer energy sources such as acetate over more complex compounds. As a result, the most important process responsible for further degradation of organic material after the possibilities of aerobic respiration and denitrification had been exhausted is fermentation, a process that does not depend on the presence of external electron acceptors.

Energy generation in fermentative organism (see also Sect. 2.2 and Fig. 5.5c) is based on substrate-level phosphorylation performed by cytoplasmic enzymes. No electron transport through membrane-bound electron carriers such as cytochromes is involved. A common characteristic of nearly all fermentation processes is that in the course of the reactions leading to the formation of ATP (often but not always reactions of the glycolytic Embden–Meyerhof pathway, see the upper part of Fig. 5.2), oxidation reactions occur that generate NADH or other reduced coenzymes. To obtain a sustainable process, these reduced coenzymes need to be reoxidized. The great diversity in fermentative pathways and the accordingly great diversity in fermentation end products are the result of the many different ways in which the cell disposes of this reducing power with the generation of reduced fermentation products (1, 4, 5, 8).

Many microorganisms combine the ability to grow anaerobically as fermenters with other modes of energy formation, including aerobic respiratory metabolism. Well-known examples are the enteric bacteria such as *Escherichia* and *Salmonella*, and yeasts that perform alcoholic fermentation. *Escherichia coli* is commonly grown in the laboratory under aerobic conditions. Organic substrates are then completely oxidized to CO_2. However, the natural habitat of *E. coli* is the human intestine, an environment devoid of oxygen, and there the organism leads an anaerobic life, fermenting carbohydrates to a mixture of products that include ethanol, lactate, acetate, hydrogen, succinate, CO_2, and also formate ("mixed acid fermentation"). In addition, *E. coli* is able of anaerobic respiration using electron acceptors such as nitrate (which is reduced to nitrite), fumarate (which is reduced to succinate), and dimethylsulfoxide (which is reduced to dimethylsulfide). Many fermentative bacteria are sensitive to oxygen. Others are oxygen-tolerant, and can these not only survive but also grow in an aerobic atmosphere, even when they do not use oxygen as electron acceptor. The lactic acid bacteria

(*Lactobacillus*, *Streptococcus*) are well-known examples of such aerotolerant obligatory fermentative bacteria.

The list of products excreted by fermentative microorganisms is long indeed, as the following examples show:

- One-carbon compounds: CO_2, formate
- Two-carbon compounds: ethanol, acetic acid
- Three-carbon compounds: lactic acid, propionic acid, isopropanol, acetone
- Four-carbon compounds: butyric acid, *n*-butanol, 2,3-butanediol, succinic acid
- Molecular hydrogen

No single fermentative microorganism produces all the above products. Some types produce only a small number of products in a fixed stoichiometry. One example is the homolactic fermentation, in which sugars such as glucose are fermented by *Lactobacillus* (some species) and by *Streptococcus* and related organisms to lactic acid as the sole product:

$$\text{Glucose} \rightarrow 2\,\text{Lactate}^- + 2\text{H}^+ \quad \Delta G^{o\prime} = -198\,\text{kJ} \tag{13}$$

These bacteria play important roles in the production of cheese, yogurt, and other milk products, as well as sauerkraut and other fermented vegetables. A second example is the ethanol fermentation of yeasts:

$$\text{Glucose} \rightarrow 2\,\text{Ethanol} + 2\text{CO}_2 \quad \Delta G^{o\prime} = -218.4\,\text{kJ} \tag{14}$$

A third example is the heterolactic fermentation of *Leuconostoc* and certain species of *Lactobacillus*:

$$\text{Glucose} \rightarrow \text{Lactate}^- + \text{Ethanol} + \text{CO}_2 + \text{H}^+ \quad \Delta G^{o\prime} = -208.2\,\text{kJ} \tag{15}$$

In other cases, the stoichiometry of the products formed depends to a large extent on the environmental conditions, among others on the pH and on the concentrations of different fermentation products that have accumulated in the medium. This can be exemplified by the case of the butytic acid forming members of the genus *Clostridium* such as *Clostridium acetobutylicum*, *Butyrivibrio* in the rumen of the cow and *Eubacterium* in the human intestinal flora show a similar type of metabolism. Typical fermentation products excreted are acids such as acetate and butyrate, together with neutral products such as *n*-butanol, acetone, and isopropanol, and the gases hydrogen and CO_2. Fig. 5.9 presents a schematic overview of the reactions occurring during this type of fermentation. This fermentation scheme illustrates a number of principles important for the understanding of the nature of anaerobic degradation processes in nature, and therefore it deserves to be discussed here in some depth.

In the initial stages of the process (Fig. 5.9, part A), sugars are degraded through the glycolytic pathway to pyruvate with the net formation of 2 molecules of ATP and the release of 4 electrons in the form of NADH (compare the upper part of Fig. 5.2). The pyruvate is then further oxidized to acetyl-CoA and CO_2. In contrast to the situation during aerobic respiration (Fig. 5.2) where the electrons are released as NADH as well, here molecular hydrogen is excreted from the cell.

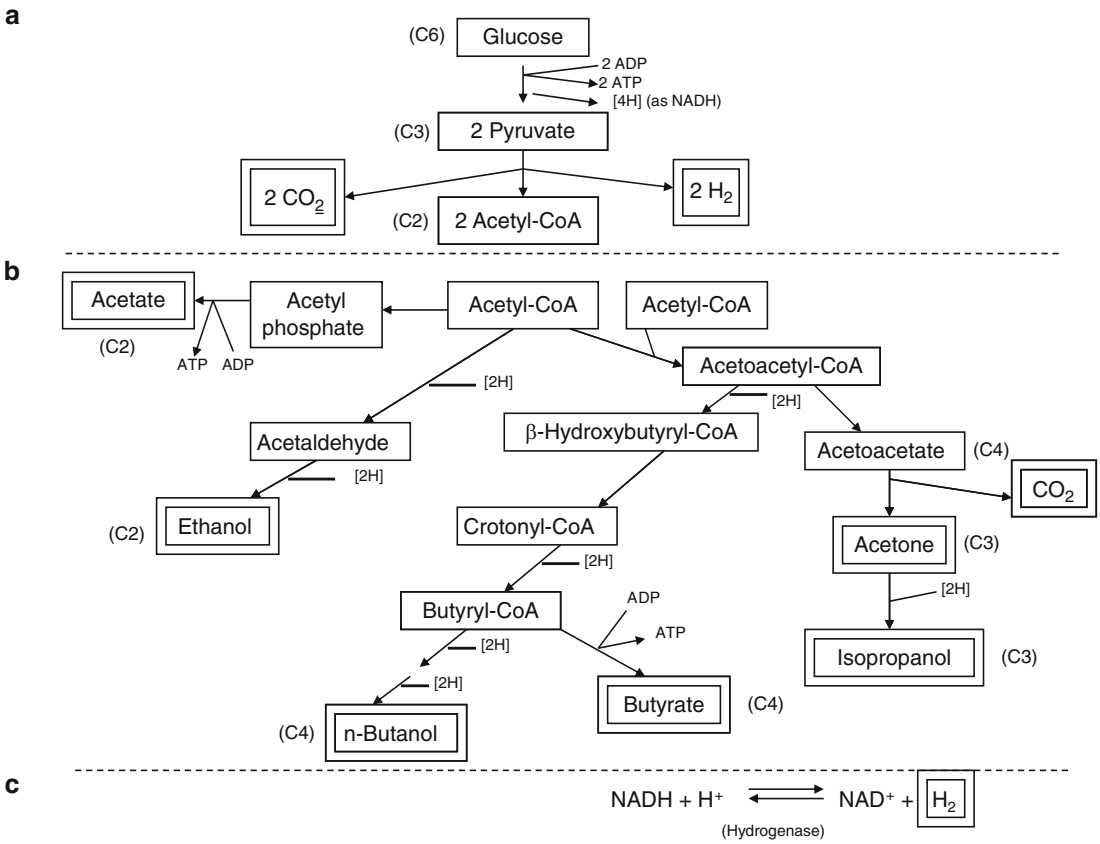

Fig. 5.9. The butyric acid fermentation of *Clostridium acetobutylicum.*

Starting from acetyl-CoA, there are several possibilities as shown in part B of Fig. 5.9. The different pathways presented are used by the cells according to the environmental conditions and the thermodynamic possibilities. Acetyl-CoA is a high-energy compound (see Table 5.2), and the energy of the bond between the acetate group and the coenzyme A moiety is sufficient to enable the production of ATP. The cells exploit this possibility by first exchanging the CoA for a phosphate group with the formation of acetyl phosphate, another high-energy compound (Table 5.2). The phosphate group is then transferred to ADP to form ATP, and acetate is released as the end product. The result is that for each acetate formed in this pathway, the cell gains an additional molecule of ATP. ATP is also formed when butyrate is produced from butyryl-CoA in a similar mechanism, but as each butyryl-CoA is derived from two molecules of acetyl-CoA, the amount of energy to be gained in this way is half of that could have been obtained when only acetate was formed. It is therefore in the interest of the cells to form as much acetate as possible. In poorly buffered environments, the acetic acid excreted causes acidification of the medium to pH values too low for growth. Under such conditions, the cells will shift their metabolism toward the formation of neutral fermentation products such as

n-butanol, ethanol, acetone and isopropanol. There is, however, a much more compelling reason why *Clostridium acetobutylicum* and other bacteria with a similar type of metabolism have to divert part of the acetyl-CoA toward the formation of reduced end products rather than producing only acetic acid; the NADH formed during the first steps of the fermentation has to be reoxidized. For that purpose, the different reaction chains leading to ethanol, isopropanol, and butanol/butyric acid all contain reduction reactions that reoxidize NADH. The aim of the cells is thus to find a balance between maximizing ATP production, i.e., excreting as much acetate as possible, while getting rid of excess electrons (in the form of NADH) by diverting part of the acetyl-CoA toward reactions that lead to the formation of more reduced end products.

Clostridium and many other fermentative anaerobes have an additional mechanism to get rid of excess electrons, and that is by producing molecular hydrogen. The enzyme hydrogenase catalyzes the following reaction (Fig. 5.9, part C):

$$\mathrm{NADH} + \mathrm{H^+} \rightleftarrows \quad \mathrm{NAD^+} + \mathrm{H_2} \tag{16}$$

If this option exists, then theoretically the following fermentation reaction should be possible:

$$\mathrm{Glucose} + 2\mathrm{H_2O} \rightarrow 2\,\mathrm{Acetate^-} + 4\mathrm{H_2} + 2\mathrm{CO_2} + 2\mathrm{H^+} \quad \Delta G^{o\prime} = -198.8\,\mathrm{kJ} \tag{17}$$

with the formation of no less than four molecules of ATP per glucose fermented – two during the degradation of glucose to two molecules of pyruvate, and one more for each acetate produced. However, as explained above (see Sect. 2.1), the true amount of free energy needed by the cell to produce a mol of ATP under, including allowance for the unavoidable loss of energy as heat, is around 70 kJ. Accordingly, Eq. (17) under standard conditions does not provide sufficient energy to drive the synthesis of 4 ATP per glucose. Another way to understand the problem is by considering the hydrogenase reaction (Eq. (16)). As the standard reduction potential of $\mathrm{H^+/H_2}$ is more negative than that of $\mathrm{NAD^+/NADH}$ (see Fig. 5.3), the reaction in the direction of hydrogen evolution is endergonic:

$$\mathrm{NADH} + \mathrm{H^+} \rightarrow \mathrm{NAD^+} + \mathrm{H_2} \quad \Delta G^{o\prime} = +18.1\,\mathrm{kJ} \tag{18}$$

Accumulating molecular hydrogen will tend to reverse the reaction, forcing the cell to find alternative ways of disposing of electrons, i.e., formation of reduced fermentation products at the expense of potential gain of ATP while excreting acetic acid. However, it must be realized that all these calculations were made under standard conditions, and that the true free energy yield or requirement of reactions depends not only on the $\Delta G^{o\prime}$ values that can be calculated from Table 5.1, but also on the concentrations of the reactants and the reaction products, all according to Eq. (4). The implications of this fact will be made clear later in this chapter (see Sect. 5.7).

Some of the compounds formed as end products of the fermentative processes described above can be fermented further by other anaerobes with the gain of additional energy. For example, lactate can be further fermented to propionate, acetate, and $\mathrm{CO_2}$, according to the equation:

$$3\,\mathrm{Lactate^-} \rightarrow 2\,\mathrm{Propionate^-} + \mathrm{Acetate^-} + \mathrm{CO_2} + \mathrm{H_2O} \quad \Delta G^{o\prime} = -161.4\,\mathrm{kJ} \tag{19}$$

Such a reaction is performed by *Propionibacterium* in the course of the manufacturing of Emmenthaler and similar cheeses; the acids provide the characteristic taste, and the CO_2 evolved causes the formation of the big holes in the cheese. A similar fermentation, albeit the biochemical pathway involved is different, is catalyzed by bacteria such as *Megasphaera elsdenii* in the bovine rumen. Propionate can also be formed by fermentation of succinate, a compound formed, e.g., during the mixed acid fermentation of *Escherichia coli* and other enteric bacteria:

$$\text{Succinate}^{2-} + \text{H}^+ \rightarrow \text{Propionate}^- + \text{CO}_2 \quad \Delta G^{o\prime} = -17.1\,\text{kJ} \tag{20}$$

This type of fermentation is of considerable theoretical interest, not only because of the very low gain in free energy associated with the reaction, but also from a mechanistic point of view. None of the compounds involved in the reactions contains any high-energy bonds that can give rise to the formation of ATP by substrate-level phosphorylation (compare Table 5.2). It is now known that the decarboxylation reaction of succinate to yield propionate is coupled with the extrusion of sodium ions from the cell. The sodium gradient thus established can be converted to ATP by a Na^+-driven membranal ATP synthase.

Sugars are not the only compounds that can be anaerobically degraded by fermentation. Amino acids can be fermented as well. Some representatives of the genus *Clostridium* grow anaerobically on glutamate in a type of reaction analogous to the butyrate fermentation of *Clostridium acetobutylicum* (Fig. 5.9); glutamate is converted in a complex series of reactions to acetate, pyruvate, and ammonium ions. The pyruvate is then further fermented to products including acetate, butyrate, CO_2, and H_2.

Another amino acid that can be fermented by many anaerobes is arginine:

$$\text{Arginine}^+ + 2\text{H}_2\text{O} + 2\text{H}^+ \rightarrow \text{Ornithine}^+ + \text{CO}_2 + 2\text{NH}_4^+ \quad \Delta G^{o\prime} = -70\,\text{kJ} \tag{21}$$

Carbamyl phosphate, a high-energy compound (see Table 5.2) is an intermediate in this reaction, and its hydrolysis to carbon dioxide and ammonium ions is coupled with the formation of ATP.

Many other amino acids are anaerobically degraded in an interesting type of fermentation in which some amino acids serve as the electron donor (e.g., alanine, tryptophan, proline, arginine) and others as the electron acceptor (e.g., glycine, valine, histidine, leucine, isoleucine) (1, 10). This process is known as the Stickland reaction. An example of such a pair-wise fermentation of amino acids is:

$$\text{Arginine} + 2\,\text{Glycine} + 2\text{H}_2\text{O} \rightarrow 3\,\text{Acetate}^- + 3\text{NH}_4^+ + \text{CO}_2 \quad \Delta G^{o\prime} = -144.9\,\text{kJ} \tag{22}$$

5.4. Anaerobic Respiration: Dissimilatory Iron and Manganese Reduction

The short fatty acids, alcohols, and other compounds formed as the end products of fermentation, as explained in the previous section, can be further degraded by anaerobic respiration with a variety of electron acceptors. They can be broken down by denitrification (see Sect. 5.2), as well as by respiration with electron donors such as sulfate (see Sect. 5.5), trivalent iron, or tetravalent manganese (26).

Trivalent iron is seldom abundantly found in anaerobic environments, and it is generally assumed that anaerobic respiration by reducing Fe^{3+} to Fe^{2+} is not of great quantitative importance in nature. Still, specialized iron-reducing anaerobes exist, and the understanding of their metabolism has greatly increased in recent years. Members of the genera *Geobacter* and *Shewanella* oxidize acetate and other short fatty acids, alcohols, hydrogen, and even some aromatic compounds to CO_2 with the reduction of trivalent to divalent iron or tetravalent manganese to divalent manganese.

5.5. Anaerobic Respiration: Dissimilatory Sulfate Reduction

A quantitatively much more important process than iron reduction is dissimilatory reduction of sulfate to sulfide. Sulfate is found at a concentration of around 23 mM in the world oceans, and is generally available as potential electron acceptor in freshwater environments as well.

Most known sulfate-reducing bacteria belong to the δ-Proteobacteria, but we also know species such as *Desulfotomaculum* (which produces heat-resistant endospores) that phylogenetically cluster with the Gram-positive bacteria. In addition, dissimilatory sulfate reduction also occurs in a few thermophilic *Archaea* such as *Archaeoglobus*. Most sulfate-reducing bacteria are obligate anaerobes, although some species are to some extent aerotolerant. The range of electron donors that can be used by sulfate reducers is quite limited. Complex organic material cannot be degraded, and only a few types can use a few sugars or amino acids, and they do so at a low rate. The substrates preferred as electron donors for dissimilatory sulfate reduction are fatty acids from formate and acetate up to a chain length of 16–18 carbon atoms, lactate, pyruvate, alcohols with 2–5 carbon atoms, and hydrogen. A few species can even use aromatic compounds such as benzoate.

We can divide the sulfate-reducing bacteria into incomplete oxidizers and complete oxidizers. The former only partially oxidize their substrates, and they excrete acetate as the main product, accompanied by CO_2 when the substrate had an odd number of carbons. Examples of such incomplete oxidizers are *Desulfovibrio*, some species of *Desulfotomaculum*, and *Desulfobulbus*. The first two preferentially oxidize lactate, ethanol, and H_2, the latter degrades propionate to acetate $+CO_2$. The acetate formed may become available as substrate for complete oxidizers such as *Desulfobacter*, *Desulfobacterium*, *Desulfococcus*, *Desulfonema*, *Desulfosarcina*, and *Desulfotomaculum acetoxidans*. *Desulfococcus*, *Desulfonema*, and *Desulfosarcina* can also oxidize longer fatty acids up to a chain length of 12–14 carbons. The thermophilic archaeon *Archaeoglobus* oxidizes lactate completely to CO_2 in a unique biochemical pathway that shares many unusual coenzymes with the methanogenic *Archaea* (see Sect. 5.6).

Before sulfate can be reduced, it has to be activated in an ATP-dependent reaction to form adenosine-5′-phosphosulfate (APS). As a result, the amount of energy that can be gained by dissimilatory sulfate reduction is limited. The APS is directly reduced to sulfite, without prior additional activation to PAPS, as is the case during assimilatory reduction of sulfate (see Sect. 3.4). Sulfate is not a strong oxidant as appears from the relatively low standard reduction potentials of the redox couples APS/AMP + HSO_3^- and HSO_3^-/HS^- (see Fig. 5.3). The free energy change associated with the dissimilatory reactions performed by the sulfate reducers

is accordingly small, as the following examples prove:

$$4H_2 + SO_4^{2-} + H^+ \rightarrow 4H_2O + HS^- \quad \Delta G^{o\prime} = -152.3\,\text{kJ} \tag{23}$$

as compared to:

$$4H_2 + 2O_2 \rightarrow 4H_2O \quad \Delta G^{o\prime} = -948.8\,\text{kJ} \tag{24}$$

$$4H_2 + 1.6NO_3^- + 1.6H^+ \rightarrow 0.8N_2 + 4.8H_2O \quad \Delta G^{o\prime} = -896.8\,\text{kJ} \tag{25}$$

or:

$$\text{Acetate}^- + SO_4^{2-} + 2H^+ \rightarrow 2CO_2 + 2H_2O + HS^- \quad \Delta G^{o\prime} = -40.7\,\text{kJ} \tag{26}$$

to be compared with:

$$\text{Acetate}^- + 2O_2 + H^+ \rightarrow 2CO_2 + 2H_2O \quad \Delta G^{o\prime} = -837.2\,\text{kJ} \tag{27}$$

or:

$$\text{Acetate}^- + 1.6NO_3^- + 2.6H^+ \rightarrow 2CO_2 + 0.8N_2 + 2.8H_2O \quad \Delta G^{o\prime} = -785.2\,\text{kJ} \tag{28}$$

Another type of reaction performed by some species of sulfate reducers is energy generation by disproportionation of thiosulfate or sulfite. In this process, a part of the substrate is oxidized to sulfate, and the electrons released are used to reduce the remainder to sulfide:

$$S_2O_3{}^{2-} + H_2O \rightarrow SO_4^{2-} + HS^- + H^+ \quad \Delta G^{o\prime} = -21.7\,\text{kJ} \tag{29}$$

$$4SO_3^{2-} + 2H^+ \rightarrow 3SO_4^{2-} + HS^- \quad \Delta G^{o\prime} = -235.5\,\text{kJ} \tag{30}$$

The process of dissimilatory sulfate reduction has great environmental and also economic impact. Massive production of sulfide under anaerobic conditions leads to bad smelling waters and sediments. Sulfide-containing sediments are generally colored black due to the reaction of sulfide with divalent iron to yield FeS. Not only is sulfide, the end product of sulfate reduction, highly corrosive to metals, the activity of sulfate-reducing bacteria may also directly contribute to corrosion of metal pipe lines and other metal structures. Spontaneous oxidation of metals establishes a thin layer of molecular hydrogen ($Fe + 2H^+ \rightarrow Fe^{2+} + H_2$) that protects the metal surface from further oxidation. Sulfate-reducing bacteria effectively oxidize the hydrogen as expressed in Eq. (23), a process known as "cathodic depolarization," opening the way to further oxidation of the metal.

Elemental sulfur can also be used an electron acceptor in anaerobic respiration. For example, the obligatory anaerobic *Desulfuromonas acetoxidans* is unable to use sulfate as electron acceptor, but grows on acetate as electron donor and sulfur as electron acceptor:

$$\text{Acetate}^- + 2H_2O + 4S^o \rightarrow 2CO_{2(\text{gaseous})} + 4HS^- + 3H^+ \quad \Delta G^{o\prime} = -16.7\,\text{kJ} \tag{31}$$

5.6. Methanogenesis

As shown above, all common fermentation products can be mineralized to CO_2 when sulfate is present as electron acceptor, either by a one-step complete oxidation or by a collaboration of incomplete oxidizers, which produce acetate and complete oxidizers that take

care of the further oxidation to CO_2. When also sulfate is not available, the only remaining way for the further anaerobic degradation is by methanogenesis.

Methane production is known to occur only in a few groups of obligatory anaerobes, all belonging to the *Euryarchaeota* branch of the domain *Archaea*. We find methanogens in a wide range of anaerobic environments such as sediments and marshes, as well as in the digestive tracts of many animals, notably in ruminants. They are also important components of the microbial community of anaerobic digestion systems in water purification plants and in other systems in which organic material is degraded under anaerobic conditions.

The range of substrates used for energy generation by methanogens is even more limited than that available to the sulfate-reducing bacteria (see Sect. 5.5). In fact, most biologically formed methane is produced by the following two reactions:

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \quad \Delta G^{o\prime} = -139.2\,\text{kJ} \tag{32}$$

$$\text{Acetate}^- + H^+ \rightarrow CH_4 + CO_2 \quad \Delta G^{o\prime} = -27.6\,\text{kJ} \tag{33}$$

Reactions expressed by Eqs. (32) and (33) thus remove hydrogen and acetate, two of the common fermentation products formed in many fermentative pathways (see Sect. 5.3). The first reaction is performed by many methanogenic species (genera *Methanobacterium*, *Methanococcus*, and others). As these bacteria derive both their energy and their cellular carbon from inorganic compounds, they should be classified as autotrophs (see also Sect. 3.1). The second reaction provides little energy, and we know only two genera of methanogens able to grow on acetate as carbon and energy source: *Methanosarcina* and *Methanosaeta*. The acetate-using methanogens are notoriously slow growers, with doubling times in the order of days. In spite of this slow growth they are responsible for more than half of the biologically produced methane in the biosphere. The unusual biochemical pathways and the principles of energy conservation in the methanogens were discussed earlier in Sect. 2.2.

There is a second group of obligate anaerobes that grow by reducing CO_2 with electrons derived from molecular hydrogen. These are the homoacetogens, and they produce acetate instead of methane:

$$4H_2 + 2CO_2 \rightarrow \text{Acetate}^- + H^+ + 2H_2O \quad \Delta G^{o\prime} = -111.6\,\text{kJ} \tag{34}$$

Examples of bacteria that perform this process are *Acetobacterium*, *Acetogenium*, and certain members of the genus *Clostridium*. The acetate formed can be further converted to methane and carbon dioxide, according to Eq. (33).

A third reaction performed by a number of methanogens that also can grow autotrophically on hydrogen $+CO_2$ is the disproportionation of formate:

$$4\text{Formate}^- + 4H^+ \rightarrow CH_4 + 3CO_2 + 2H_2O \quad \Delta G^{o\prime} = -120\,\text{kJ} \tag{35}$$

As we have seen above, formate is one of the products of the mixed-acid fermentation of *E. coli* and other enteric bacteria.

There are only a few additional reactions that can provide the energy for growth of methanogenic *Archaea*. These include the degradation of methylated amines such as trimethylamine – $(CH_3)_3N$ – to methane, carbon dioxide and ammonium ions, and the

formation of methane, carbon dioxide and sulfide from dimethylsulfide (CH_3–S–CH_3):

$$4\text{Trimethylamine} + 6H_2O + 4H^+ \rightarrow 9CH_4 + 3CO_2 + 4NH_4^+ \quad \Delta G^{o\prime} = -668.7\,\text{kJ} \tag{36}$$

$$2\text{Dimethylsulfide} + 2H_2O \rightarrow 3CH_4 + CO_2 + 2H_2S \quad \Delta G^{o\prime} = -221.4\,\text{kJ} \tag{37}$$

Trimethylamine and other methylated amines are not among the major products formed during degradation of biomass. These unpleasantly smelling substances can expected to be formed when compounds that contain tertiary amine groups, such as choline and notably glycine betaine, are degraded in anoxic environments. Glycine betaine (trimethylglycine) is accumulated as an osmotic stabilizer by many microorganisms (cyanobacteria, other photosynthetic, and nonphotosynthetic prokaryotes) that grow in highly saline environments. Dimethylsulfide (DMS) is formed for example during the degradation of dimethylsulfoniopropionate (DMSP), accumulated by marine micro- and macroalgae as an osmotic stabilizer (27). DMS at low concentrations is responsible for the characteristic smell of marine algae; at high concentrations, it is one of the worst smelling substances known.

5.7. Proton-Reducing Acetogens and Interspecies Hydrogen Transfer

As shown in Sect. 5.6, the variety of substrates used by methanogens is extremely limited. Hydrogen and acetate, two of the end products of fermentative processes, can be metabolized with the formation of methane, but many other compounds formed as fermentation end products cannot. The question, thus, remains how other fermentation products such as ethanol, propionate, and butyrate, are further metabolized in the absence of electron acceptors such as nitrate and sulfate.

Further degradation of the above-mentioned fermentation end products and others is possible by their oxidation to acetate and molecular hydrogen, which both can further be metabolized by methanogens. A calculation of the standard free energy change associated with the formation of acetate and hydrogen from ethanol, propionate, butyrate, and similar compounds shows that these reactions are endergonic:

$$\text{Ethanol} + H_2O \rightarrow 2H_2 + \text{Acetate}^- + H^+ \quad \Delta G^{o\prime} = +9.8\,\text{kJ} \tag{38}$$

$$\text{Propionate}^- + 2H_2O \rightarrow \text{Acetate}^- + CO_2 + 3H_2 \quad \Delta G^{o\prime} = +80.1\,\text{kJ} \tag{39}$$

$$\text{Butyrate}^- + 2H_2O \rightarrow 2\text{Acetate}^- + H^+ + 2H_2 \quad \Delta G^{o\prime} = +48.4\,\text{kJ} \tag{40}$$

Therefore, bacteria cannot make a living by performing these reactions under standard conditions (all reagents and products being present in concentrations of 1 M or 1 atmosphere, see also Sect. 2.1), as the equilibrium of the reactions is to the left, at the side of the reagents rather than of the products. However, it must be remembered that the true free energy change associated with chemical reactions depends not only on the standard free energy change, but also on the actual concentrations of the reagents and the products, in accordance with Eq. (4). When the products of the reaction (i.e., hydrogen and acetate) are effectively removed by the activity of methanogenic bacteria in the ecosystem or by other bacteria, the equilibrium of the reactions (38–40) shifts to the right in accordance with Le Chatelier's principle. When the concentrations of the end products are kept at very low levels, the $\Delta G'$ may become

sufficiently negative to allow the formation of ATP. It should be realized that the total amount of free energy that becomes available during anaerobic degradation does not increase by removal of hydrogen and acetate, as the methanogens will have to cope with low substrate concentrations, lowering the $\Delta G'$ of their dissimilatory reactions below the standard values given in Eqs. (32) and (33). For example, the energy available during the oxidation of ethanol to acetate coupled to the reduction of carbon dioxide to methane:

$$2\text{Ethanol} + CO_2 \rightarrow CH_4 + 2\text{Acetate}^- + 2H^+ \quad \Delta G^{o\prime} = -119.6\,\text{kJ} \tag{41}$$

is now shared between the ethanol-oxidizing bacterium (often referred to as the "S-organism) (Eqs. (38) and (42)) and the methanogenic *Archaea* that reduce CO_2 with hydrogen as electron donor (Eq. (32)).

$$2\text{Ethanol} + 2H_2O \rightarrow 2\text{Acetate}^- + 2H^+ + 4H_2 \quad \Delta G^{o\prime} = +19.6\,\text{kJ} \tag{42}$$

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \quad \Delta G^{o\prime} = -139.2\,\text{kJ} \tag{32}$$

The cooperation between the two types of microorganisms is based on "interspecies hydrogen transfer," and organisms, such as the "S-organism" (Eq. (38)), *Syntrophobacter* (Eq. (39)), and *Syntrophomonas* (Eq. (40)), are often designated "proton-reducing acetogens," i.e., organisms that produce acetate while excreting molecular hydrogen (9, 10, 28, 29). We even know proton-reducing acetogens such as the species of the genus *Syntrophus* that degrade benzoate and other aromatic compounds to acetate and molecular hydrogen in reactions that are endergonic under standard conditions, but become feasible for energy production when the end products are efficiently removed:

$$\text{Benzoate}^- + 6H_2O \rightarrow 3\text{Acetate}^- + CO_2 + 2H^+ + 3H_2 \quad \Delta G^{o\prime} = +49.5\,\text{kJ} \tag{43}$$

There are many more cases, based on the same principle, of reactions that under standard conditions are endergonic, but participate in the anaerobic breakdown of organic matter when the concentrations of the products are kept low thanks to the action of methanogenic *Archaea*. To name just one more interesting example: methanogenesis from acetate yields very little energy (see Eq. (33)), and those methanogens that perform the reaction grow very slowly. It is therefore surprising that the reaction may also be performed by a thermophilic consortium of microorganisms that divide the little energy available among each other; one organism that oxidizes acetate to CO_2 with the evolution of hydrogen in a reaction that is highly endergonic, and a methanogen that reduces CO_2 with hydrogen as electron donor (30).

The above-documented principle of interspecies hydrogen transfer that drives otherwise energetically unfavorable reactions is not restricted to cases of obligatory syntrophic associations such as those involving bacteria such as *Syntrophomonas* and *Syntrophobacter*. As discussed in Sect. 5.7, many clostridia and other fermentative anaerobes can choose between gaining more energy by excreting acetate and producing more reduced fermentation products while disposing of excess reducing power that had accumulated in the form of intracellular NADH. The balance between these two options is determined by the ability of hydrogenase to excrete the excess electrons as molecular hydrogen. The hydrogenase reaction is endergonic in the direction of hydrogen production (Eq. (18)), but removal of the accumulating

hydrogen, e.g., by methanogenic or by sulfate-reducing bacteria will shift the equilibrium so that the reaction becomes favorable. The theoretical yield of four molecules of ATP per glucose fermented now becomes feasible as the true $\Delta G'$ of the reaction presented in Eq. (17) will be more negative than the –215.6 kJ calculated under standard conditions, so that the requirements for the production of the maximum amount of ATP can be met. This also means that in anaerobic ecosystems in which fermentative microorganisms live in close association with hydrogen oxidizers (sulfate reducers, methanogens, homoacetogens), and in which acetate is removed as well (both by sulfate reducers and methanogens), the need for the production of reduced fermentation products such as alcohols is largely abolished. The mixture of fermentation products excreted by an organism such as *Clostridium acetobutylicum* in pure culture (Fig. 5.9) is therefore very different from that produced in mixed cultures in which a hydrogen-oxidizing anaerobe is present as well.

The cooperation of fermentative bacteria and methanogens, if necessary with the assistance of syntrophic proton-reducing acetogens, enables the anaerobic mineralization of organic material to carbon dioxide and methane in the absence of any external electron acceptors:

$$\text{Glucose} \rightarrow 3CO_2 + 3CH_4 \quad \Delta G^{o\prime} = -393.2\,\text{kJ} \tag{44}$$

The yield in free energy is only a small fraction of the amount obtained during aerobic oxidation of glucose (−2822 kJ/mol, see Eq. (12)). It should also be noted that during aerobic respiration, all the energy becomes available to a single organism, while anaerobic breakdown to methane and carbon dioxide involves a number of partners, who thus have to share the relatively small amount of free energy released in the process. The remainder of the energy is stored in the methane, and can become available when the methane reaches an aerobic environment and is there oxidized by methanotrophic bacteria:

$$3CH_4 + 6O_2 \rightarrow 3CO_2 + 6H_2O \quad \Delta G^{o\prime} = -2428.8\,\text{kJ} \tag{45}$$

Methane is generally considered to be the end product of anaerobic degradation of organic material. However, the concept that methane cannot be further metabolized under anaerobic conditions needs revision. In recent years, it has become clear that anaerobic oxidation of methane is possible with sulfate serving as the electron acceptor (31):

$$CH_4 + SO_4^{2-} + H^+ \rightarrow CO_2 + HS^- + 2H_2O \quad \Delta G^{o\prime} = -13.1\,\text{kJ} \tag{46}$$

This reaction is performed by a consortium of two organisms, one being a sulfate-reducing eubacterium, and the other an archaeon, which is responsible for oxidation of the methane. No full information is yet available on the mechanism of the anaerobic methane oxidation or on the nature of the reaction intermediate that is transferred between the partners (15, 32) and couples the two partial reactions that lead to the products shown in Eq. (46).

From the information provided earlier, it is obvious that the two molecules that take a central place in the anaerobic degradation process are hydrogen and acetate. When these become available in the course of fermentation processes, there are several possibilities for their further metabolism. Methanogens, sulfate-reducing bacteria, and homoacetogens all compete for hydrogen. Acetate is used both by certain methanogens (*Methanosarcina*, *Methanosaeta*) that convert it to CO_2 and CH_4 (see Sect. 5.6) and by sulfate-reducing bacteria such as

Desulfobacter (see Sect. 5.5). When sulfate is present, little methanogenesis will occur, and the sulfate-reducing bacteria will oxidize the hydrogen and the acetate. This is due to the higher affinity of the sulfate-reducing bacteria for these substrates. Methanogenesis takes over when insufficient sulfate is present. In freshwater environments, such as lakes and marshes, the sulfate concentration is often low, and methanogenesis is therefore the most important terminal process of anaerobic degradation. Sulfate reduction dominates in the marine environment in which sulfate is abundantly present. There are, however, other substrates that are converted to methane and or which sulfate-reducing bacteria do not compete, such as the methylated amines and dimethylsulfide, as discussed in Sect. 5.6.

The ability of microorganisms to bring about complete mineralization of complex organic material to mixtures of CO_2 and CH_4 is exploited in anaerobic sludge digestors. The long retention times employed enable the reactions to come close to completion. Processes very similar to those occurring in anaerobic sludge digestors occur in the rumen of ruminant animals. Also, their organic materials (starch, cellulose, proteins) are fermented to organic acids, CO_2, and hydrogen. In this case, however, the anaerobic degradation is incomplete, and the main end products are acetate, propionate, and butyrate, which are taken up into the blood stream of the animal and serve as the main carbon and energy source of the ruminant. The fact that acetate is here not converted into methane and carbon dioxide by *Archaea* such as *Methanosarcina* (Eq. (33)), and propionate and butyrate are not broken down to acetate by syntrophic proton-reducing acetogens (Eqs. (39) and (40)) is due to the short retention time of the ingested food in the rumen. Those methanogens that use acetate for energy generation have very long generation times (in the order of days), and so have syntrophic bacteria such as *Syntrophobacter* and *Syntrophomonas*. As a result, such microorganisms cannot maintain stable populations in the system in which the retention time is considerably shorter than their generation time. All the methane formed in the digestive system of ruminants – the considerable amount of about 200 l per day in an adult cow – is formed by rapid-growing species (*Methanobrevibacter ruminantium*, *Methanomicrobium mobile*) that use the hydrogen evolved during the sugar fermentations as energy source by reducing CO_2 (Eq. (32)).

6. THE CHEMOAUTOTROPHIC WAY OF LIFE

The third way of energy generation, which is unique to the prokaryote world, is the use of reduced inorganic compounds as energy source to drive autotrophic CO_2 fixation. Chemoautotrophic (chemolithotrophic) microorganisms can thus grow on inorganic compounds only, without being dependent on light energy (13, 14). The general principles behind chemoautotrophic metabolism have been previously explained in Sect. 2.2 and in Fig. 5.6. Chemoautotrophic life is possible in aerobic as well as in anaerobic environments. The different modes of energy generation by chemolithotrophs and their importance in the environment are discussed below in further depth.

6.1. Reduced Nitrogen Compounds as Energy Source

Most organically bound nitrogen in nature exists in the reduced form as $-NH_2$ groups in amino acids. Reduced nitrogen also occurs in the purine and pyrimidine bases of the nucleic acids. Upon degradation of these organic nitrogen-containing compounds, the nitrogen

is released as ammonium ions. This process of ammonification occurs both under aerobic and under anaerobic conditions. Aerobically, the amino acids and other nitrogen compounds are converted to intermediates of the central metabolic pathways such as the tricarboxylic acid (see Fig. 5.2). Anaerobically, fermentation reactions release ammonium ions as well, as exemplified in Eqs. (21) and (22).

The ammonium released not only becomes available for assimilatory uptake by microorganisms and plants (see Sect. 3.2), but can also be used as energy source for chemolithotrophic bacteria that perform the process of nitrification (33). Since the pioneering studies of Sergei Winogradsky around 1890, we know that nitrification is an autotrophic process that proceeds in two steps. First, ammonium is oxidized to nitrite with the release of six electrons (Eq. (7)), and in the second step, the nitrite is further oxidized to nitrate by a different group of bacteria with the gain of two more electrons (Eq. (8)). As explained earlier, the amount of energy that can be obtained from these reactions is relatively small. As considerable amounts of energy are needed to drive the uphill transport of electrons to form NADPH, the reductant needed for autotrophic CO_2 fixation, cell yields are low. Large amounts of substrate are therefore, transformed to products by a small biomass (see the calculations in Sect.2.2). As the equations show, nitrification is associated with net production of protons, leading to an acidification of the environment in which the process takes place.

The two groups of microorganisms involved in autotrophic nitrification have phylogenetically different affiliations. The aerobic ammonium oxidizers (genera such as *Nitrosomonas*, *Nitrosolobus*, *Nitrosococcus* and others) are all Proteobacteria (β- or γ-branch) or belong to a recently discovered group of *Crenarchaeota* (*Candiatus* 'Nitrosopumilus maritimus' and relatives). Most nitrite oxidizers (*Nitrobacter*, *Nitrococcus*, *Nitrospina*) are Proteobacteria as well (α-, γ-, and δ-branch, respectively), but the genus *Nitrospira* forms a separate deep lineage within the domain *Bacteria*. The process of nitrification occurs only under aerobic conditions, as oxygen is the terminal electron acceptor both for ammonium- and for nitrite-oxidizing bacteria. Moreover, the first enzymatic step in the oxidation of ammonium with the formation of hydroxylamine (NH_2OH) as intermediate, catalyzed by ammonium monooxygenase, uses molecular oxygen as co-substrate.

Nitrification occurs everywhere in nature where ammonium ions and molecular oxygen occur together. As explained above, ammonium is the form in which nitrogen is released during the degradation of amino acids and other nitrogen-containing cellular components. In spite of this, nitrate is present in much higher concentrations than ammonium in most aerobic environments as the result of its rapid oxidation to nitrate by nitrifying bacteria. Nitrification occurs in aquatic as well as in terrestrial ecosystems. Nitrogen fertilizer applied as ammonium salts is rapidly oxidized in the soil to nitrate. Nitrification is exploited in many wastewater purification systems; the nitrogen load of the wastewater is reduced in a two-step procedure in which the ammonium is first aerobically oxidized to nitrate (nitrification), which is subsequently reduced in an anaerobic process to gaseous nitrogen (denitrification, see Sect. 5.2).

A completely different process of chemolithotrophic oxidation of ammonium, but this time under anaerobic conditions, was discovered a few years ago. The reaction involves the oxidation of ammonium using nitrite as electron acceptor with the formation of gaseous

nitrogen (see Eq. (9) and Sect. 2.2 for additional details). This anammox process, as it is generally called, was first documented to occur in an anaerobic laboratory-scale wastewater purification system. New processes of wastewater treatment are now under development to exploit the anammox reaction in an attempt to design a one-step process for nitrogen removal to replace the conventional two-step process of aerobic nitrification followed by anaerobic denitrification (34). The process of anaerobic ammonium oxidation with nitrite as electron acceptor is ecologically important in stratified water bodies such as the Black Sea, in which significant concentrations of oxidized nitrogen compounds (nitrate, nitrite) are present in the anoxic zone. It was estimated that up to 20–40% of the N_2 formed in such environments may be derived from the anammox reaction rather than from dissimilatory nitrate reduction – denitrification (35, 36), and recent estimates even indicate that up to 30–50% of the nitrogen evolved from the world ocean may originate from the anammox process rather than from denitrification, mainly in the continental slope and hemipelagic sediments where the majority of the marine nitrogen loss takes place.

6.2. Reduced Sulfur Compounds as Energy Source

Reduced sulfur compounds are excellent electron donors for chemoautotrophic growth (37). Due to the fact that sulfide, elemental sulfur, thiosulfate, and other similar potential electron donors are stronger reductants than ammonium and nitrate (see Fig. 5.3), the amount of energy gained by their oxidation to sulfate with oxygen as electron acceptor is much higher than that available to the nitrifying bacteria:

$$H_2S + 2O_2 \rightarrow SO_4^{2-} + 2H^+ \quad \Delta G^{o\prime} = -796.3\,\text{kJ} \tag{47}$$

$$S^o + 1.5O_2 + H_2O \rightarrow SO_4^{2-} + 2H^+ \quad \Delta G^{o\prime} = -587.0\,\text{kJ} \tag{48}$$

$$S_2O_3^{2-} + H_2O + 2O_2 \rightarrow 2SO_4^{2-} + 2H^+ \quad \Delta G^{o\prime} = -818.2\,\text{kJ} \tag{49}$$

These reactions are all associated with the production of sulfuric acid, a strong acid. In some environments, this may cause severe acid pollution problems. The best-known case is that of acid mine drainage, formed by the aerobic oxidation of pyrite (FeS_2) associated with coal and metal ores to form sulfuric acid and trivalent iron by bacteria such as *Acidithiobacillus ferrooxidans* (see also Sect. 6.3). The formation of acids during chemoautotrophic sulfur oxidation can be exploited when acidification is desirable, e.g., in highly alkaline soils. Addition of elemental sulfur efficiently leads to acid production according to Eq. (48). Many of the chemoautotrophic bacteria that oxidize sulfur compounds are highly acid-tolerant or even obligatory acidophilic. Some grow optimally at pH 2–3, and growth below pH 1 is not unusual.

Chemoautotrophic oxidation can also be coupled to the reduction of nitrate, instead of oxygen with the formation of gaseous nitrogen:

$$5H_2S + 8NO_3^- \rightarrow 5SO_4^{2-} + N_2 + 4H_2O + 2H^+ \quad \Delta G^{o\prime} = -3722\,\text{kJ} \tag{50}$$

The autotrophic bacteria that oxidize reduced sulfur compounds include members of the β- and γ-Proteobacteria such as *Thiobacillus*, *Acidithiobacillus*, *Geothiobacillus*, and *Thiomicrospira*. In addition to obligate autotrophs, the group also contains species that prefer a

mixotrophic way of life, in which reduced sulfur compounds provide the energy, but most of the cellular carbon is derived from organic compounds taken up from the medium rather than from CO_2. Within the γ-Proteobacteria, we also find filamentous colorless sulfur bacteria such as *Beggiatoa*, *Thiothrix*, and *Thioploca*. These organisms are among the largest prokaryotes extant; some *Beggiatoa* types have cells of 100 μm or more in diameter, and filaments as long as several millimeters being not uncommon. The filamentous sulfide-oxidizers are sediment-dwelling bacteria that generally locate themselves at the boundary between the anaerobic sulfide-rich sediment layers and the aerobic layer that supplies the oxygen. The filaments can move through the sediment by means of an unusual mechanism of gliding movement, not involving flagella, and they follow the diurnal changes in the location of the aerobic–anaerobic boundary. Sulfide is oxidized by the filamentous sulfur bacteria with elemental sulfur as intermediate, which is stored within the cells. Sergei Winogradsky defined the concept of chemoautotrophy in 1887 on the basis of his observations of the appearance of elemental sulfur in *Beggiatoa* filaments after feeding with sulfide and their disappearance following starvation. However, *Beggiatoa* filaments are notoriously difficult to grow in the laboratory in pure culture, and it was not done until 1983 when the ability for true chemoautotrophic growth in some *Beggiatoa* strains was first unambiguously demonstrated.

Large masses of the filamentous *Thioploca* occur in the sediments of the continental shelf in upwelling areas near the Pacific coast of South America. Recent studies of the biology of *Thioploca* have shown an unexpected feature: most of the cell volume is taken up by a large vacuole, which contains a very high concentration of nitrate. *Thioploca* accumulates nitrate to serve as electron acceptor during sulfide oxidation when oxygen is in short supply. Rather than producing dinitrogen as the end product of nitrate respiration, ammonium is excreted, so that the amount of biologically available nitrogen does not decrease in the process. A similar type of metabolism was documented in *Thiomargarita namibiensis*, a filamentous organism that lives in upwelling zones off the west coast of Africa, and has the largest cells yet documented in the prokaryotes. *Beggiatoa* can accumulate nitrate in intracellular vacuoles as well.

Also among the *Archaea,* we find chemoautotrophic sulfide and sulfur oxidizers. The best-known genus is *Sulfolobus*, an aerobic thermophilic sulfur oxidizer that lives at high temperatures (optimum at 75°C, maximum at 87°C) in acidic sulfur springs worldwide.

A highly interesting ecosystem in which the chemoautotrophic sulfide-oxidizing bacteria play a key role is found around the deep-sea hydrothermal vents along the spreading zones in the Pacific and the Atlantic Oceans at several kilometers depth. In these regions of intense volcanic activity, the plates that make up the earth crust separate and new crust is added. Springs that emit sulfide-rich water are abundantly found in these areas, some of them with water temperatures of up to 350°C. These areas in which anaerobic sulfide-rich hydrothermal waters mix with the cold oxygenated seawater are ideal habitats for the development of chemolithotrophic sulfur bacteria. The springs are surrounded by dense communities of giant tube worms that can reach a length of several meters and are 10–20 cm thick. Most of the body volume of these worms is occupied by the trophosome, an organ filled with chemoautotrophic bacteria, phylogenetically associated with the γ-Proteobacteria. These bacteria grow at the expense of sulfide, oxygen, and carbon dioxide transported to them by the blood stream of the worm. The organic carbon produced by the bacteria is used as carbon and energy source for

the worm that hosts the cells. Similar symbiotic associations have been documented between clams and mussels that dwell in the sulfide-rich environment of the hydrothermal vents. Sulfide is thus the primary energy source that supports the densely populated hydrothermal vent ecosystem.

6.3. Reduced Iron and Manganese as Energy Source

Reduced iron and manganese can be used as electron donors and energy sources to drive chemoautotrophic growth of specialized bacteria. The reactions involved are:

$$2Fe^{2+} + 0.5O_2 + 2H^+ \rightarrow 2Fe^{3+} + H_2O \quad \Delta G^{o\prime} = -9.0\,\text{kJ (at pH 7)} \quad (51)$$

$$\Delta G^{o\prime} = -65.8\,\text{kJ (at pH 2)}$$

$$Mn^{2+} + 0.5O_2 + H_2O \rightarrow MnO_2 + 2H^+ \quad \Delta G^{o\prime} = -71.2\,\text{kJ} \quad (52)$$

Not much is known about the bacteria that oxidize iron at neutral and alkaline pH, mainly due to the fact that divalent iron is unstable in the presence of molecular oxygen, and chemical oxidation thus competes with the biological process. The best-characterized neutrophilic chemoautotrophic iron oxidizer is *Gallionella*, a bacterium that deposits the iron hydroxides produced in the form of a stalk. *Gallionella* is often observed in environments such as draining bogs and iron-rich springs.

In acidic environments, however, divalent iron is stable, and there chemoautotrophic iron-oxidizing bacteria often have a dramatic impact on the ecosystem. In mining areas where metal ores or coal containing pyrite (FeS_2) are brought to the surface, oxidation of both the iron and the sulfur atoms of the pyrite by *Acidithiobacillus ferrooxidans* results in highly acidic waters and streams, colored orange-brown by the iron hydroxides formed (see also Sect. 6.2). Acid mine drainage is a very severe environmental problem in many parts of the world.

The existence of bacteria that oxidize divalent manganese to the tetravalent form in the presence of oxygen has been documented long ago. However, relatively little is known about the process, and it is not always clear whether the oxidation of the metal ions is coupled with CO_2 fixation. The finding of genes for RuBisCo, the key enzyme of the Calvin cycle (see Fig. 3.1) suggests that in some manganese-oxidizing bacteria the process may indeed enable autotrophic growth.

6.4. Hydrogen as Energy Source

Thanks to the very negative standard reduction potential of the couple H^+/H_2 (Fig. 5.3), oxidation of hydrogen can be coupled with the reduction of many potential electron acceptors with the gain of energy and the possibility to drive chemoautotrophic growth. The aerobic oxidation of hydrogen is energetically highly favorable:

$$2H_2 + O_2 \rightarrow 2H_2O \quad \Delta G^{o\prime} = -474.4\,\text{kJ} \quad (53)$$

A number of aquatic and soil bacteria are known to perform this reaction (the "Knallgas reaction"), including members of the genera *Pseudomonas*, *Paracoccus*, *Ralstonia* (Proteobacteria) and *Bacillus* (Gram-positive bacteria). These can all grow as chemoorganotrophs as well. It is not clear what the importance of the reaction is in soils and in aquatic habitats

of moderate temperature. As documented in Sect. 5.3, hydrogen is one of the major products of bacterial fermentations, but it is used at a very high efficiency in the same environment in which it is produced by sulfate reducing, methanogenic and homoacetogenic bacteria, which keep the hydrogen concentrations at a sufficiently low value so that syntrophic associations between proton-reducing acetogens and methanogenic *Archaea* can exist (see Sect. 5.7). The amounts of molecular hydrogen that will escape from such environments to reach oxygen-rich niches are probably small. There are also thermophilic species that perform the Knallgas reaction. These include species of the genera *Aquifex* and *Hydrogenobacter*, isolated from hydrothermal vent environments where molecular hydrogen is among the substrates present in the hot waters that emerge from the vents. Phylogenetically, these genera belong to deep lineages branching off at the basis of the phylogenetic tree of the *Bacteria*.

Anaerobically, the oxidation of hydrogen can be coupled with a range of electron acceptors. These include nitrate (in facultative anaerobes such as *Paracocccus denitrificans*), sulfate (in some species of *Desulfovibrio*, and in *Desulfonema* and *Desulfosarcina*, Eq. (23)), elemental sulfur (especially in a wide range of extremely thermophilic *Archaea*), or carbon dioxide to form methane by methanogenic *Archaea* such as *Methanobacterium* and *Methanococcus* (Eq. (32)) or to form acetate by homoacetogens such as *Acetobacterium* (Eq. (34)). Some of the organisms mentioned use the Calvin cycle for autotrophic CO_2 fixation, others use alternative pathways such as the reduction of CO_2 to CO, which is then coupled with methyl groups to form acetyl-CoA (see Sect. 3.1).

6.5. Other Substrates as Energy Sources for Chemoautotrophic Growth

In addition to the well-known electron donors such as ammonium, nitrite, sulfide, sulfur, reduced iron, and hydrogen, there are a few additional compounds whose oxidation can drive chemoautotropic growth of certain bacteria. Some can grow autotrophically on carbon monoxide under aerobic conditions:

$$CO + 0.5O_2 \rightarrow CO_2 \quad \Delta G^{o\prime} = -248.8\,\text{kJ} \tag{54}$$

Bacteria that can use this reaction generally can perform the Knallgas reaction (Eq. (53)) as well. Anaerobic growth on CO is also possible according to:

$$CO + H_2O \rightarrow CO_2 + H_2 \quad \Delta G^{o\prime} = -11.6\,\text{kJ} \tag{55}$$

A few thermophilic anaerobes (*Carboxydothermus*, *Caldanaerobacter*) use this reaction. The thermophilic methanogen *Methanothermobacter thermautotrophicus* can grow anaerobically on CO with the production of methane.

There are also indications that the oxidation of arsenite (AsO_3^{3-}) to arsenate (AsO_4^{3-}) can be coupled with autotrophic growth. Little is known yet about the organisms that perform these processes and about their ecological importance. The waters of the highly saline and alkaline Mono Lake (California) contain about 0.2 mM arsenic compounds, and a microbiological arsenic cycle is operative there, including anaerobic respiration that reduces arsenate to arsenite, and aerobic chemoautotrophic oxidation of arsenite to arsenate, as well as anaerobic arsenate oxidation with nitrate as electron acceptor (38).

7. THE BIOGEOCHEMICAL CYCLES OF THE MAJOR ELEMENTS

The preceding sections provide the basis for an understanding of the biogeochemical cycles. A short discussion will suffice to show how the different processes interact to obtain functional cycles of carbon, nitrogen, sulfur, and other elements.

7.1. The Carbon Cycle

We may consider the carbon cycle (Fig. 5.10) as a series of conversions of inorganic carbon (CO_2 from the air or CO_2, bicarbonate, or carbonate dissolved in the water) into organic carbon and vice versa. Autotrophic, energy-requiring processes enable the fixation of inorganic carbon into cell material. Oxygenic phototrophs that use light as energy source (green plants, algae, cyanobacteria) are responsible for most CO_2 fixation on Earth, but in certain ecosystems anoxygenic phototrophs and chemoautotrophs may contribute significantly to the fixation of inorganic carbon.

The pathways that lead to mineralization of organic carbon to CO_2 in any single ecosystem primarily depend on the availability of potential electron acceptors to receive the electrons released when reduced carbon is oxidized. Organisms that obtain the most energy will generally have an advantage, and for respiratory processes the amount of energy involved primarily depends on the standard reduction potential of the electron acceptor; the more oxidized, the more energy can be gained. Thus, the order of the processes is generally: aerobic oxidation > denitrification > dissimilatory sulfate reduction > methanogenesis, all in accordance with the availability of the respective electron acceptors.

Mechanistic constraints complicate the picture to some extent, as processes such as dissimilatory sulfate reduction and even more so methanogenesis function with a limited range of electron donors only. Therefore, additional stages in the anaerobic degradation of organic material are essential, notably fermentation processes that degrade complex organic compounds into a range of smaller molecules that are amenable to further mineralization by the terminal anaerobic degradation processes of sulfate reduction or (in the absence of any other electron acceptors) methanogenesis. An interesting observation is that the small amount of energy available in the anaerobic degradation is often shared between a number of partners, and that the processes performed by each partner have to be carefully coordinated so that each of the organisms can make a living. Although indications now exist that some methane can be oxidized also under anaerobic conditions, it is the aerobic oxidation of the methane formed under anoxic conditions that closes the cycle. The nature of the microorganisms involved and the relative importance of the different processes will vary for each ecosystem, but the principles are universally valid.

The above analysis of the microbial carbon cycle shows that it is closely linked and interrelated with the cycles of nitrogen and sulfur; oxidized forms of nitrogen and sulfur can serve as electron acceptors during the mineralization of organic carbon by anaerobic respiration. Similarly, oxidation of reduced nitrogen and sulfur compounds drives autotrophic fixation of CO_2, both by chemoautotrophic and by anoxygenic photoautotrophic microorganisms (in the latter case based on oxidation of sulfur compounds only; the hypothetic ammonia-oxidizing anoxygenic photoautotrophs envisaged by Broda (16) are yet to be discovered).

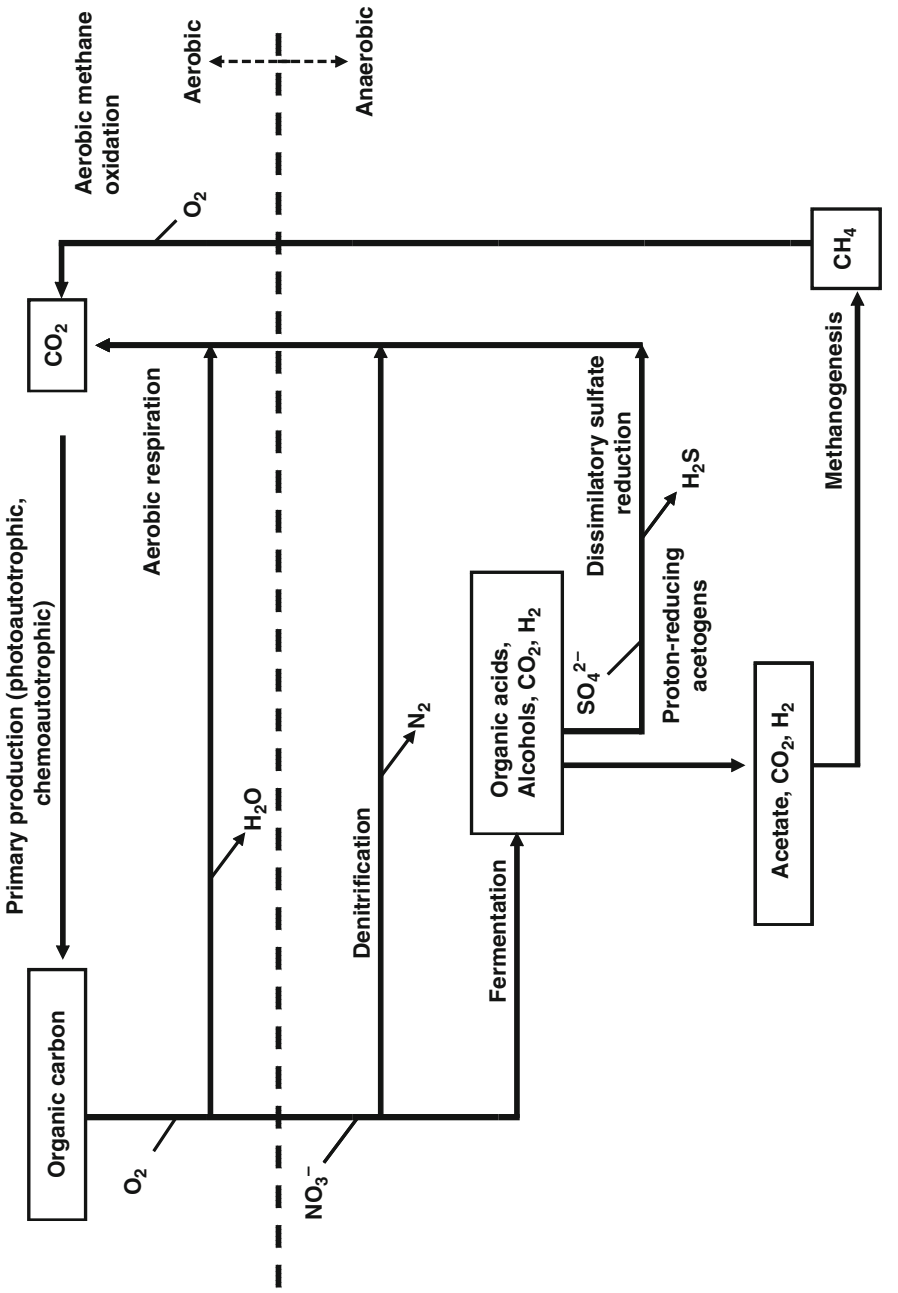

Fig. 5.10. The most important processes in the carbon cycle.

7.2. The Nitrogen Cycle

The nitrogen cycle is particularly rich in transformations of inorganic forms of nitrogen, and almost all possible oxidation states, from ammonium (N^{3-}) to nitrate (N^{5+}), are encountered. An overview of the principal processes in the nitrogen cycle is given in Fig. 5.11.

The same principles shown for the carbon cycle are operative here, such as energy-expensive assimilatory processes – which are extremely energy-costly in the case of fixation of molecular nitrogen, as well as energy sharing between partners that together perform processes that either one alone cannot accomplish – in this case the autotrophic oxidation of ammonium to nitrate, which proceeds with nitrite as obligate intermediate. Also on the assimilatory level, both inorganic and organic forms of nitrogen can be used, the latter mainly bound in the form of amino acids. The recent discovery of the anammox process, including the demonstration that at least in certain environments this reaction is also quantitatively significant, nicely demonstrates that we still may not known all processes in the biogeochemical cycles, and that new insights are continuously being obtained.

7.3. The Sulfur Cycle

Also in the sulfur cycle (Fig. 5.12), alternations in oxidation state abound. Both oxidized and reduced sulfur can be used for assimilatory purposes, reduced sulfur can serve as electron donor for photoautotrophic growth or as electron donor and energy source for chemoautotrophic growth, and oxidized sulfur compounds can be used as electron acceptors in anaerobic respiration.

One aspect of the sulfur cycle that was not discussed in-depth in the sections above but which is of considerable importance, also on a global level, is the formation and transformations of methylated sulfur compounds. DMSP, an osmotic stabilizer produced by marine algae, is degraded among other products to dimethylsulfide (DMS). Other ways DMS may be formed are by anaerobic respiration with dimethylsulfoxide as electron acceptor, a process whose ecological significance is not yet clear, as well as by anaerobic degradation of aromatic methoxylated compounds in the presence of sulfide (39). Part of the DMS dissolved in seawater escapes as a gas to the atmosphere. DMS is thus the main chemical form in which sulfur can be transported from the marine to the terrestrial environment. Oxidation of DMS in the atmosphere leads to the formation of tiny droplets of sulfuric acid, and they act as condensation nuclei for the formation of water droplets and clouds. The net flux of DMS from the oceans to the atmosphere thus directly influences cloud cover and rainfall on a global scale. DMS can also be oxidized aerobically in a process in which both the sulfur and the methyl groups are oxidized with the gain of energy, and can be anaerobically converted to sulfide and methane (Eq. (37)).

7.4. Biogeochemical Cycles of Other Elements

Many other elements are subject to microbial transformations, often associated with changes in oxidation state. We have discussed how divalent iron can be oxidized to drive chemoautotrophic and even anoxygenic phototrophic CO_2 fixation. Trivalent iron can be used as electron acceptor in anaerobic respiration. Similar phenomena have been described for

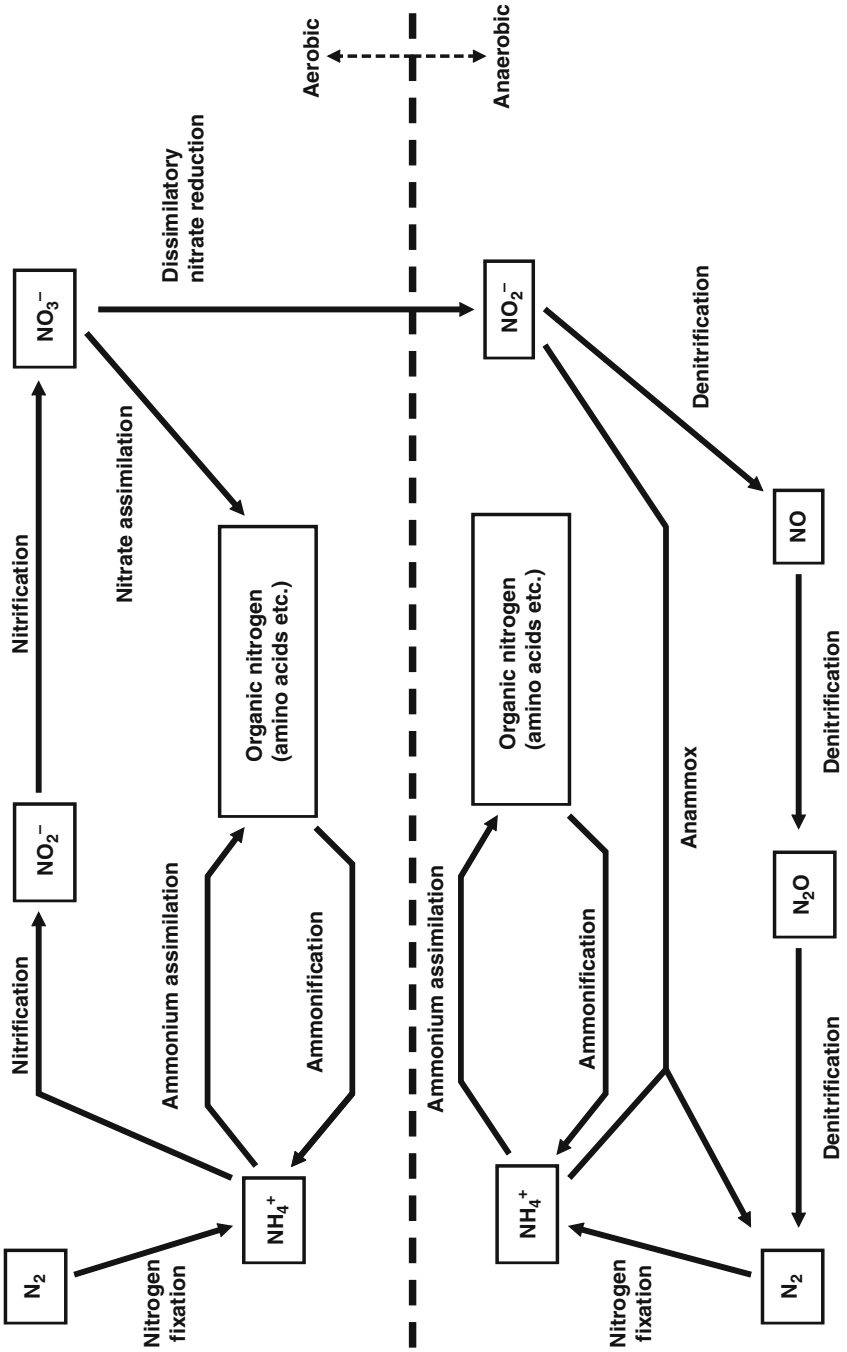

Fig. 5.11. The most important processes in the nitrogen cycle.

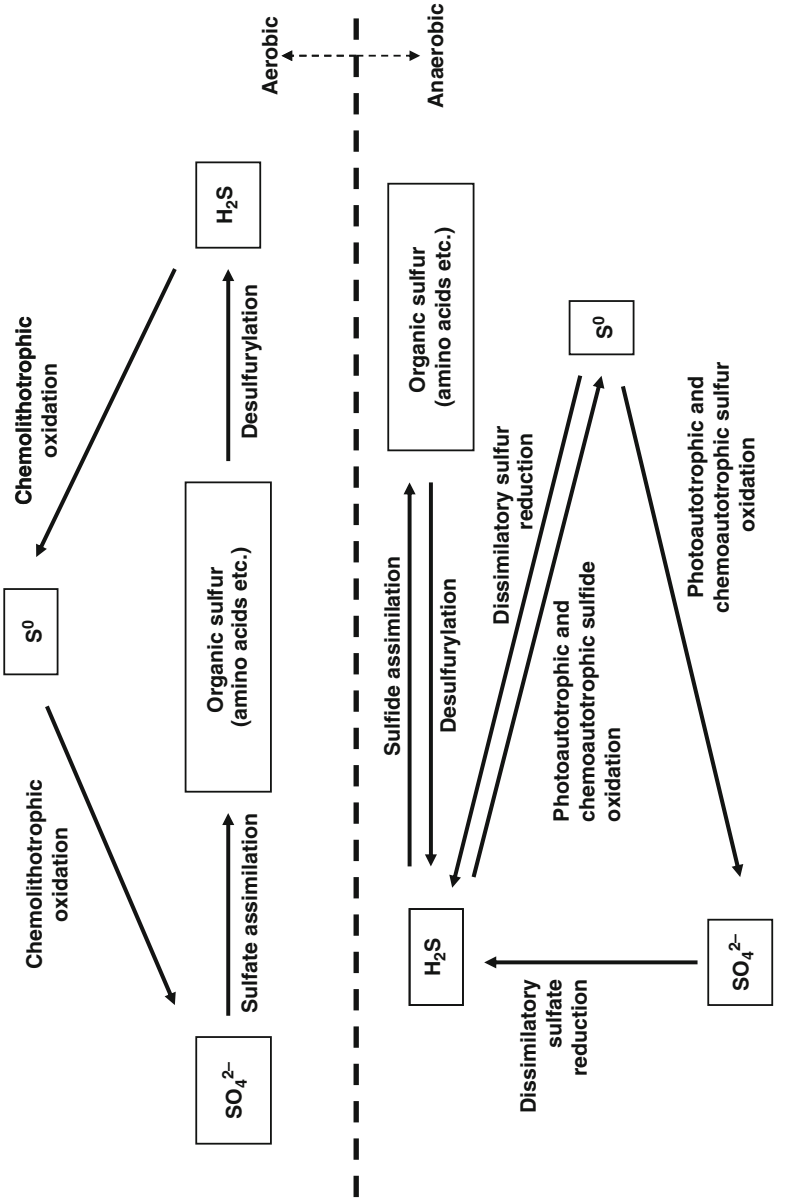

Fig. 5.12. The most important processes in the sulfur cycle.

manganese, and for ions of selenium, chromium, copper, arsenic, and additional elements. The general principle is similar in all cases, when such compounds can participate in an energy-yielding reaction, either as electron donor or as electron acceptor, some bacterium will be found that can exploit the reaction.

8. EPILOGUE

The preceding sections have provided an overview of the tremendous diversity in metabolic types among microorganisms, and especially among the prokaryotes. Hardly any process that is thermodynamically feasible remains unexploited by the microbial world. This metabolic diversity drives the biogeochemical cycles of carbon, nitrogen, sulfur, and other elements. Combination of the different processes – phototrophic and chemotrophic, autotrophic and heterotrophic, aerobic and anaerobic – enables the cycling of the elements, thus sustaining life on Earth. The processes performed by the microbes can also often be manipulated to assist man in exploiting the environment or to solve environmental problems. Understanding the metabolic potential and diversity of the microorganisms is the basis for their successful exploitation to the benefit of mankind.

NOMENCLATURE

ADP = adenosine diphosphate
APS = adenosine-5′-phosphosulfate
ATP = adenosine triphosphate
CoA = coenzyme A
E'_o = standard reduction potential of a redox couple, V
F = Faraday constant, 96.5 kJ/V
FAD = flavin adenine dinucleotide, oxidized form
FADH = flavin adenine dinucleotide, reduced form
G^o_f = free energy of formation, kJ/mol
ΔG = change in free energy during a chemical reaction, in kJ/mol
$\Delta G^{o\prime}$ = change in free energy during a chemical reaction, in kJ/mol at pH 7 under standard conditions
$\Delta\mu^+_H$ = proton electrochemical gradient over a biological membrane, mV
NAD^+ = nicotinamide adenine dinucleotide, oxidized form
NADH = nicotinamide adenine dinucleotide, reduced form
$NADP^+$ = nicotinamide adenine dinucleotide phosphate, oxidized form
NADPH = nicotinamide adenine dinucleotide phosphate, reduced form

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APPENDIX: COMPOUNDS OF ENVIRONMENTAL SIGNIFICANCE AND THE MICROBIAL PROCESSES RESPONSIBLE FOR THEIR FORMATION AND DEGRADATION

Below follows a nonexhaustive list of compounds formed during the dissimilatory metabolism of prokaryotic organisms (*Bacteria* as well as *Archaea*), with special emphasis on those compounds of importance in environmental engineering. Information is also provided on those microbial processes (assimilatory as well as dissimilatory) responsible for the disappearance of these compounds. Reference is made to the appropriate sections in the text above in which the nature of the respective processes was discussed in further depth.

Compounds of Carbon, Hydrogen, and Oxygen

Hydrogen (H_2*)*

Hydrogen is a characteristic end product of fermentation by anaerobic bacteria (representatives of the genus *Clostridium* and many others). It can be formed in ferredoxin-mediated reactions such as the oxidative decarboxylation of pyruvate to acetyl-CoA and/or by action of hydrogenase, using reducing equivalents from NADH (see Sect. 5.3). Hydrogen is also excreted by syntrophic bacteria, such as *Syntrophomonas* and *Syntrophobacter,* in the course of the oxidation of organic acids and other compounds (see Sect. 5.7). Minor amounts of hydrogen are formed also as a byproduct of nitrogenase activity in all nitrogen-fixing prokaryotes.

Hydrogen seldom accumulates at large concentrations in nature as it is effectively used by a variety of sulfate-reducing bacteria (Sect. 5.5), methanogenic bacteria (Sect. 5.6), and homoacetogenic bacteria (Sect. 5.6) (all under anaerobic conditions), and by aerobic chemolithotrophic hydrogen oxidizers ("Knallgas bacteria") (Sect. 6.4).

Oxygen (O_2*)*

Molecular oxygen is formed as a byproduct of photosynthesis by oxygenic prokaryotes (Cyanobacteria) (see Sect. 4.1), eukaryotic microalgae, macroalgae, and terrestrial plants.

Oxygen is the terminal electron acceptor in aerobic respiration, enabling degradation of about every biodegradable organic compound, as well as the chemoautotrophic oxidation of reduced nitrogen and sulfur compounds to nitrate and sulfate, respectively.

Carbon Dioxide (CO_2*)*

CO_2 is the end product of oxidation of organic material by organisms that perform aerobic respiration (animals, fungi, many bacteria) or anaerobic respiration with nitrate or sulfate as electron acceptor (see Sects. 5.1, 5.2, and 5.5). CO_2 is also released in the course of many fermentative processes together with organic fermentation products (Sect. 5.3), and in disproportionation reactions mediated by methanogenic *Archaea,* such as methanogenesis, from formate (Sect. 5.6).

Most assimilation of carbon dioxide occurs through the Calvin cycle, with ribulose bisphosphate carboxylase (RuBisCO) as the key enzyme (Sect. 3.1). This is true both for photoautotrophs and for chemoautotrophs. Alternative modes of autotrophic fixation exist in certain groups of microorganisms such as the green sulfur bacteria, the methanogenic *Archaea*,

and others. Carboxylation reactions, such as the carboxylation of phosphoenolpyruvate to oxalacetate, incorporate carbon dioxide into cellular carbon also in heterotrophic organisms.

Methane (CH_4*)*

Methane is formed only by a specialized group of *Archaea* as the end product of their energy-yielding reactions. The major precursors for methane are acetate, which is split into methane and carbon dioxide, and the reduction of carbon dioxide by molecular hydrogen (see Sect. 5.6). Methane can also be formed from formate, from methanol, from methylated amines, and from dimethylsulfide.

Methane is oxidized aerobically by methanotrophic bacteria. Anaerobic methane oxidation is possible as well in a yet incompletely understood process performed by a consortium of *Archaea* and *Bacteria* in which methane oxidation is coupled with the reduction of sulfate to sulfide (see Sect. 5.7).

Carbon Monoxide (CO)

No microorganisms are known that release CO into the environment. Carbon monoxide is an intermediate in the autotrophic fixation of CO_2 in certain autotrophs that do not use the reactions of the Calvin cycle (some sulfate-reducing bacteria, some methanogenic *Archaea*), and as such remains intracellular (see Sect. 3.1).

Carbon monoxide can be metabolized by a variety of microorganisms, aerobic as well as anaerobic. Some aerobic chemoautotrophs can obtain their energy by the oxidation of CO to CO_2. Anaerobically, CO can be converted to methane. Another anaerobic energy-yielding pathway, performed by a number of thermophilic representatives of the *Bacteria*, is its oxidation to CO_2 with concomitant formation of hydrogen (see Sect. 6.4).

Short-Chain Organic Acids

FORMIC ACID (HCOOH)

Formate is produced by pyruvate:formate lyase in a variety of fermentative processes, including, e.g., the anaerobic degradation of sugars by *Escherichia coli* under anaerobic conditions (see Sect. 5.3).

Formate can be oxidized aerobically by a variety of bacteria. Anaerobically, it can be converted to a mixture of methane and carbon dioxide in a disproportionation reaction performed by methanogenic *Archaea* (see Sect. 5.6). Alternatively, it may serve as electron donor for denitrifying bacteria or for certain sulfate-reducing bacteria.

ACETIC ACID (CH_3–COOH)

Acetate is formed as a major fermentation product by many carbohydrate- and amino acid-fermenting bacteria (see Sect. 5.3) and by proton-reducing acetogens in syntrophic partnerships (see Sect. 5.7). In addition, homoacetogenic bacteria form acetate under anaerobic conditions by reducing carbon dioxide with hydrogen as electron donor (see Sect. 5.6). Moreover, acetate can be formed in incomplete oxidation processes, aerobically as well as anaerobically. Aerobic acetic acid bacteria, such as *Acetobacter,* oxidize ethanol to acetate with molecular oxygen as electron acceptor (Sect. 5.1). Anaerobically, incomplete oxidation

of lactate, propionate, and other organic acids by dissimilatory sulfate reducing bacteria using sulfate as electron acceptor leads to acetate formation (see Sect. 5.5).

Acetate can be oxidized to carbon dioxide using oxygen, nitrate, sulfate, or trivalent iron as electron acceptors. Certain methanogenic *Archaea* split acetate into methane and carbon dioxide (see Sect. 5.6). At high temperatures, acetate can be oxidized anaerobically to carbon dioxide with the release of molecular hydrogen in a process that has to be coupled with hydrogen consumption to be energetically feasible (see also Sect. 5.7). Acetate is also used as an assimilatory carbon source by many aerobic bacteria, by facultative or obligatory photoheterotrophs, and by mixotrophic oxidizers of reduced sulfur compounds.

Propionic Acid (CH_3–CH_2–COOH)

Propionate is a characteristic fermentation product, made from sugars or from lactate by a specialized group of propionic acid bacteria (*Propionibacterium*, *Selenomonas*, *Megasphaera*) (see Sect. 5.3).

Propionate can be degraded aerobically by aerobic respiration, and anaerobically by denitrifying bacteria or sulfate-reducing bacteria such as *Desulfobulbus,* which oxidizes propionate incompletely to acetate + carbon dioxide (see Sect. 5.5). The proton-reducing acetogen, *Syntrophobacter,* converts propionate to acetate + carbon dioxide as well as hydrogen, which has to be efficiently removed for the process to be energetically favorable (see Sect. 5.7).

Butyric Acid (CH_3–CH_2–CH_2–COOH)

Butyrate is a fermentation product excreted by many fermentative prokaryotes growing on sugar or amino acids (see Sect. 5.3).

Butyrate can be oxidized to carbon dioxide by many aerobic bacteria. Under anaerobic conditions, butyrate can be converted to carbon dioxide by denitrification (Sect. 5.2) or by certain sulfate-reducing bacteria (Sect. 5.5). When no electron acceptors are available, *Syntrophomonas* converts butyrate to acetate and hydrogen in process that is thermodynamically favorable only if the hydrogen formed is efficiently removed by a syntrophic partner (see Sect. 5.7).

Lactic Acid (CH_3–CHOH–COOH)

Lactate is a product of fermentation by specialized lactic acid bacteria: homolactic organisms such as *Streptococcus* and many *Lactobacillus* species, and heterolactic species such as *Leuconostoc*, which produce a mixture of lactate, ethanol, and carbon dioxide. It is formed during other fermentations as well, such as the mixed acid fermentation of *Escherichia coli* and relatives under anaerobic conditions (see Sect. 5.3).

Lactate can be degraded by aerobic respiration, by anaerobic respiration with nitrate as electron acceptor, as well as by sulfate-reducing bacteria, such as *Desulfovibrio,* that degrade lactate incompletely to acetate + carbon dioxide (see Sect. 5.5). The sulfate-reducing thermophile *Archaeoglobus* performs complete oxidation of lactate to carbon dioxide using sulfate as electron acceptor. Lactate can also be fermented further under anaerobic conditions to a mixture of propionate, acetate, and carbon dioxide (Sect. 5.3).

SUCCINIC ACID ($COOH–CH_2–CH_2–COOH$)

Succinate is formed as a minor fermentation product in the mixed acid fermentation of enteric bacteria such as *Escherichia coli,* and is also formed by anaerobic bacteria such as *Bacteroides*, *Ruminobacter*, and *Succinomonas* that live in the digestive system of animals (see Sect. 5.3). Succinate can also be formed anaerobically as the product of anaerobic reduction of fumarate used as electron acceptor in respiration.

Succinate can be oxidized aerobically and anaerobically (by denitrification) to carbon dioxide. Moreover, it can be fermented to propionate + carbon dioxide by certain propionic acid bacteria (*Propionigenium*, *Schwartzia*) (Sect. 5.3).

Ethanol ($CH_3–CH_2OH$*)*

Ethanol is formed in many fermentation processes, both in eukaryotes (the alcohol fermentation of yeasts) and prokaryotes (*Zymomonas*, heterolactic fermenters such as *Leuconostoc*, and also as a minor product in the fermentation of enteric bacteria and some clostridia; see Sect. 5.3).

Ethanol can be oxidized aerobically (complete oxidation to CO_2 or incomplete oxidation to acetate by acetic acid bacteria; see Sect. 5.1). Under anaerobic conditions, ethanol can be oxidized to CO_2 while using nitrate as electron acceptor, to acetate by sulfate-reducing bacteria such as *Desulfovibrio* (see Sect. 5.5), or by proton-reducing acetogens under the excretion of molecular hydrogen (see Sect. 5.7).

Isopropanol ($CH_3–CHOH–CH_3$*)*

Isopropanol is formed as a minor product during carbohydrate fermentation by certain species of *Clostridium* and related organisms (see Sect. 5.3).

Isopropanol can be oxidized aerobically to CO_2. In the absence of oxygen, it can serve as electron donor for the reduction of carbon dioxide to methane in certain methanogenic *Archaea*.

n-Butanol ($CH_3–CH_2–CH_2–CH_2OH$*)*

n-Butanol is often formed during fermentation of carbohydrates by *Clostridium* species (see Sect. 5.3).

Butanol can be oxidized aerobically and anaerobically to CO_2 with oxygen, nitrate, or sulfate as electron acceptors.

Acetone ($CH_3–CO–CH_3$*)*

Acetone is a minor product of some fermentation processes, e.g., the fermentation of carbohydrates by *Clostridium acetobutylicum* (see Sect. 5.3). Furthermore, it can be formed by certain methanogenic bacteria from isopropanol that may serve as electron donor for methanogenesis.

Acetone can be oxidized aerobically by oxidation to hydroxyacetone by means of a monooxygenase, followed by oxidation to pyruvate. Anaerobic degradation by certain denitirying bacteria is possible in a pathway initiated by carboxylation to acetoacetate.

Nitrogen-Containing Compounds

Ammonium (NH_4^+)

Ammonium ions are generated as the result of aerobic as well as anaerobic degradation of amino acids and other organic compounds containing reduced nitrogen such as the purine and pyrimidine bases present in nucleic acids (ammonification, see Sect. 6.1). Ammonium ions can also be formed in the dissimilatory process of nitrate reduction, but nitrate ammonification is less common than denitrification with the formation of dinitrogen and nitrous oxide.

Ammonium can be used both aerobically and anaerobically for assimilatory purposes as nitrogen source, and can also serve as energy source in dissimilatory processes: nitrification (under aerobic conditions, where it is oxidized to nitrite), or anaerobic ammonium oxidation (the "anammox" process) in which nitrite serves as electron acceptor (see Sect. 6.1).

Nitrite (NO_2^-)

Nitrite can be formed aerobically as the product of the oxidation of ammonium ions in the first step of autotrophic nitrification by *Nitrosomonas* and related organisms as well as by ammonium-oxidizing *Archaea* (see Sect. 6.1). Anaerobically, nitrite can accumulate as an intermediate in denitrification processes during the reduction of nitrate. Certain bacteria, such as *Escherichia coli,* anaerobically reduce nitrate to nitrite as end product. Minor amounts of nitrite may also originate from $–NO_2$ residues during the aerobic breakdown of organic nitro compounds.

Nitrite can be used as nitrogen source for assimilatory purposes by a variety of photosynthetic and nonphotosynthetic microorganisms. Furthermore, it serves as energy source for autotrophic nitrifiers such as *Nitrobacter* (see Sect. 6.1). Anaerobically, it is reduced via nitric oxide and nitrous oxide to dinitrogen in the process of denitrification (see Sect. 5.2), or it may be used as the electron acceptor in anaerobic oxidation of ammonium ions (the "anammox" reaction, see Sect. 6.1).

Nitrate (NO_3^-)

Nitrate is the end product of autotrophic nitrification in which ammonium ions are aerobically oxidized via nitrite to nitrate. Minor amounts of nitrate may be formed anaerobically by the "anammox" bacteria, which use nitrite as electron donor to provide electrons for autotrophic fixation of carbon dioxide (see Sect. 6.1).

Nitrate can be consumed both in assimilatory processes when it serves as nitrogen source to plants, microalgae, and many aerobic bacteria (Sect. 3.2), as well as in dissimilatory processes: nitrate respiration – denitrification with the formation of more reduced products: nitrite, nitric oxide, nitrous oxide, dinitrogen, or ammonium ions (Sect. 5.2).

Dinitrogen (N_2)

Nitrogen is the major end product of denitrification – the dissimilatory reduction of nitrate and nitrite under anaerobic conditions (see Sect. 5.2) – as well as the product of anaerobic oxidation of ammonium ions with nitrite as electron acceptor in the "anammox" process (see Sect. 6.1).

Nitrogen can be used as a nitrogen source for assimilatory purposes by a limited number of prokaryotes, many of them living in symbiotic associations with higher organisms, in an energy-expensive process catalyzed by the enzyme nitrogenase (see Sect. 3.2).

Nitrous Oxide (N_2O*)*

Nitrous oxide is a product of dissimilatory nitrate respiration – denitrification (see Sect. 5.2), and is generally found as a minor end product besides dinitrogen. There are also indications that activity of nitrifying bacteria may be responsible for the formation of part of the nitrous oxide present in the marine environment.

Nitrous oxide can be further reduced to dinitrogen during denitrification.

Trimethylamine [$(CH_3)_3N$*] and Other Methylated Amines*

Trimethylamine and other methylated amines can be formed during degradation of choline (a component of the lipid phosphatidylcholine) or glycine betaine, a compound found as an intracellular osmotic stabilizer in many halophilic and halotolerant microorganisms inhabiting hypersaline environments.

Methylated amines can be oxidized aerobically by a variety of methylotrophic bacteria. Anaerobically, they can be used as energy source by many methanogenic *Archaea* with the production of methane, carbon dioxide, and ammonium ions.

Putrescine ($NH_2–CH_2–CH_2–CH_2–CH_2–NH_2$*), Cadaverine (*$NH_2–CH_2–CH_2–CH_2–CH_2–CH_2–NH_2$*), Agmatine (*$NH_2–CH_2–CH_2–CH_2–CH_2–NH–C(NH_2) = NH$*), and Related Organic Amines*

Putrescine, cadaverine, agmatine, and related bad-smelling compounds can be formed by decarboxylation of amino acids (ornithine, lysine, and arginine, respectively) by a variety of anaerobic fermentative bacteria.

Little is known about the further metabolism of these compounds in the absence of molecular oxygen. Putrescine can be fermented to acetate, butyrate, hydrogen, and ammonium ions. The amines can all be oxidized to carbon dioxide and ammonium ions under aerobic conditions.

Sulfur-Containing Compounds

Hydrogen Sulfide (H_2S*)*

Hydrogen sulfide may be formed by desulfurylation during anaerobic degradation of amino acids (cysteine, methionine) and other organic sulfur compounds. In addition, major amounts of sulfide are produced as the end product of dissimilatory reduction of sulfate, elemental sulfur, and other oxidized inorganic sulfur compounds under anaerobic conditions (see Sect. 5.5).

Sulfide is unstable under aerobic conditions and is oxidized abiotically in the presence of molecular oxygen. Moreover, it serves as the energy source for chemolithotrophic aerobic sulfide oxidizers such as *Thiobacillus* and *Beggiatoa* (see Sect. 6.2). Anaerobically, sulfide can be oxidized by green and purple phototrophic sulfur bacteria, in which it serves as electron donor for autotrophic fixation of carbon dioxide (Sect. 4.2), or by certain denitrifying sulfide oxidizers, in which it acts both as energy source and as electron donor for autotrophic growth (Sect. 6.2).

Sulfate (SO_4^{2-})

Sulfate is formed as the end product of both photosynthetic (Sect. 4.2) and chemosynthetic (Sect. 6.2) oxidation of sulfide and other reduced sulfur compounds. Photosynthetic sulfide oxidation occurs in anaerobic environments in which sufficient light is available to serve as energy source. Chemoautotrophic sulfur oxidation occurs aerobically, but can also proceed anaerobically in the presence of nitrate as electron acceptor.

Sulfate can be used as source of sulfur for assimilatory purposes by plants, algae, and many bacteria (see Sect. 3.4). Sulfate is also used as terminal electron acceptor for anaerobic respiration by sulfate-reducing bacteria (Sect. 5.5).

Elemental Sulfur (S^0)

Sulfur can be formed both by the abiotic oxidization of hydrogen sulfide and as an intermediate during the oxidation of sulfide to sulfate by green and purple photosynthetic bacteria (see Sect. 4.2).

Under aerobic conditions, elemental sulfur is used as electron donor and energy source by chemolithotrophic bacteria (*Bacteria* of the *Thiobacillus* group; at high temperatures *Archaea* such as *Sulfolobus*), causing acidification of the medium (see Sect. 6.2). Under anaerobic conditions, elemental sulfur can be an electron donor to photosynthetic green and purple bacteria, which oxidize it to sulfate (Sect. 4.2), or as an electron acceptor in anaerobic respiration by *Bacteria* such as *Desulfuromonas* or a variety of thermophilic *Archaea* (Sect. 5.5).

Dimethylsulfide (CH_3–S–CH_3) and Methylmercaptan (CH_3–SH)

Dimethylsulfide and methylmercaptan (methylsulfide) can be produced during the anaerobic degradation of the amino acid methionine and other organic compounds that contain reduced sulfur. A major source of dimethylsulfide in the marine environment is the degradation of DMSP, an intracellular osmotic stabilizer of many marine algae. Dimethylsulfide can also be formed as the product of anaerobic respiration processes with dimethylsulfoxide as electron acceptor. Finally, anaerobic degradation of methoxylated aromatic compounds in the presence of hydrogen sulfide can lead to the formation of dimethylsulfide.

Under aerobic conditions, dimethylsulfide can be oxidized by chemolithotrophic sulfur oxidizers and by methylotrophs, leading to the formation of carbon dioxide and sulfate. In the absence of molecular oxygen, dimethylsulfide can be used as energy source by certain methanogenic *Archaea*.

Other Elements

IRON OXIDES

Oxidized forms of iron (Fe^{3+}) are formed as the result of the chemolithotrophic oxidation of divalent iron by bacteria such as *Acidithiobacillus ferrooxidans* (see Sect. 6.3). Massive accumulations of iron hydroxides [$Fe(OH)_3$ and other forms] are often found in mine drainage waters, accompanied by low pH caused by autotrophic oxidation of reduced sulfur compounds (pyrite and others) present in many ores. Another organism that deposits trivalent iron is the autotrophic *Gallionella*, which produces iron oxide stalks. An intermediate state of oxidation as magnetite (Fe_3O_4) is found intracellularly in magnetotactic bacteria (see Sect. 3.5).

Under anaerobic conditions, trivalent iron can be reduced to divalent iron by iron-reducing bacteria such as *Geobacter* and *Shewanella* (see Sect. 5.4).

MANGANESE (Mn^{2+}, Mn^{4+})

Oxidized forms of manganese (Mn^{4+}) are formed as the result of the chemolithotrophic oxidation of divalent manganese (see Sect. 6.3).

Under anaerobic conditions, tetravalent manganese can be reduced to the divalent form in anaerobic respiration processes.

SELENATE (SeO_4^{2-}), SELENITE (SeO_3^{2-}), AND ELEMENTAL SELENIUM (Se^o)

Selenate can be used as an electron acceptor for anaerobic respiration, and is respired to selenite (SeO_3^{2-}) or to a mixture of selenite and elemental selenium. Furthermore, it can be taken up for assimilatory use by many microorganisms and used in the biosynthesis of selenocysteine, an unusual amino acid that is incorporated into some proteins.

ARSENATE (AsO_4^{3-}) AND ARSENITE (AsO_3^{3-})

Arsenate can be used as an electron acceptor for anaerobic respiration by a number of bacteria, who reduce it to arsenite (AsO_3^{3-}).

Arsenite can be used as an electron donor for chemoautotrophic arsenite oxidizers, both under aerobic conditions and anaerobically, using nitrate as electron acceptor, causing its oxidation to arsenate (AsO_4^{3-}).

6
Microbial Ecology of Isolated Life Support Systems

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CONTENTS

Abstract Microorganisms are the most widely spread form of living matter – occurring in every climatic region and in every zone of water and soil strata. They have a profound role in biogeochemical processes and are crucial for the completion of all biologically important material cycles, which support all life on Earth. Microorganisms can enter into varying interactions with human population – from "positive" forms of symbiosis, known as mutualism, to "negative" manifestations of parasitism, as in lethal generalized infections. Phenotypic adaptations, spontaneous mutations, and the action of natural selection regularly occur in microbial populations and microbial associations. Microorganisms have a significant role to play in all types of systems, which include humans. This is particularly so for enclosed habitats, where they need to be managed, as they are potentially the most hazardous component in the ecosystem. Characteristics, such as their great number, diversity of species, short life cycle, and ability to adapt rapidly to changes in environmental conditions give microorganisms inside the environment and those associated with the higher organisms themselves (humans, animals and plants), the potential to change a healthy system to a state of imbalance and hazard. Microflora can be introduced into a system deliberately to perform functions like photosynthesis, bioregeneration, and the controlled oxidation of organic matter. Such microflora need to be monitored and managed. The conditions to which microfloras are exposed in the closed habitat can differ markedly from those found in nature. In these cases, relationships between humans and internal bodily microfloras, between humans and the

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microfloras of the enclosure, and the exchange of microfloras between members of the crew become significant vectors. Spacecraft and space stations (orbital vehicles or habitats, Mars or Moon stations) operating for prolonged periods, as well as submarines representing a kind of simulated environment with similarities to the natural environment maintained artificially, can be considered as "testbeds" for research on microecological dynamics, function and risk. All materials discussed in the chapter prove that microbial populations and communities are the most active links of every type of closed ecosystems designed for Earth or Space needs. The problems of macro and microorganisms coexistence in different types of LSS, including Biosphere, are regularly discussed at different international conferences, especially COSPAR Scientific Assemblies and are important for environmental engineering design of different ecosystems.

Key Words Microbial populations • life support systems • human microflora • microecological risk • macro and microorganisms coexistence • microbial evolution.

1. INTRODUCTION

As the name suggests, the defining feature of microorganisms is their small size. The term includes viruses, bacteria, lower fungi, microalgae, and protozoa. Microorganisms are the most widely spread form of living matter – occurring in every climatic region and in every zone of water and soil strata. They are characterized by their dense numbers and the wide variety of species, amazing viability, metabolic mechanisms and plasticity, which "help" them to interact with both the abiogenic and biogenic components of environment. Microorganisms have a profound role in biogeochemical processes and are crucial for the completion of all biologically important material cycles which support all life on Earth. Viable microbes have been found in the atmosphere at altitudes over 100 km, in the ocean at depths up to 11 km, in cores brought from depths of 4–5 km, in Antarctic valleys under ice, in cooling water tanks of nuclear reactors, etc.

Microorganisms can enter into varying interactions with the human body – from "positive" forms of symbiosis, known as mutualism, to "negative" manifestations of parasitism, as in lethal generalized infections. Phenotypic adaptations, spontaneous mutations and the action of natural selection regularly occur in microbial populations and microbiocenoses (microbial ecosystems). Microorganisms have a significant role to play in all types of systems, which include humans. This is particularly so for enclosed (materially sealed and isolated) habitats, where they need to be managed, as they are potentially the most hazardous component in the ecosystem. Characteristics such as their great number, diversity of species, short life cycle, and ability to adapt rapidly to changes in environmental conditions, give microorganisms inside the environment and those associated with the higher organisms themselves (humans, animals and plants) the potential to change a healthy system to a state of imbalance and hazard. Microflora can be introduced into a system deliberately to perform functions like photosynthesis, bioregeneration and the controlled oxidation of organic matter. Such microflora needs to be monitored and managed. The conditions to which microfloras are exposed in the closed habitat can differ markedly from those found in nature. In these cases, relationships between humans and internal bodily microfloras, between humans and the microfloras of the enclosure, and the

exchange of microfloras between members of the crew become significant vectors. Spacecraft and space stations (orbital vehicles or habitats, Mars or Moon stations) operating for prolonged periods, as well as submarines representing a kind of simulated environment with similarities to the natural environment maintained artificially, can be considered as "testbeds" for research on microecological dynamics, function and risk.

The review in this chapter is designed to give an overview of the role of microflora in closed habitats and the relationships between micro and macroorganisms, with particular emphasis on microecological problems and health risks.

2. FUNCTIONAL AND REGULATOR ROLE OF MICROBIAL POPULATIONS

2.1. Microalgae and Bacteria Communities as Bioregenerators in Life Support Systems

Microalgae were the best candidates in many experiments as the metabolic counterpart of human metabolism for a variety of reasons: (a) Potential growth rates and multiplication rates of microbes are extremely high. Under optimal conditions, cell division occurs every few hours. (b) Microalgae metabolism varies with environmental conditions and inputs, and therefore the content of their biomass can be controlled. (c) The efficiency of light energy conversion into organic synthesis in photosynthesis can exceed 10% under optimal experimental conditions. (d) Microalgae can uptake carbon dioxide directly from the air. (e) Under optimal growth conditions, microalgae do not require special antibacterial measures, and can be cultivated in nonsterile media, forming algal–bacterial populations serving as quite an effective and useful bioregenerator air and water for life support systems (LSS) (1).

Substantial achievements were accomplished by U.S. and Soviet space researchers with *Chlorella*-based systems. The first such experiments were conducted by U.S. researchers soon after the first spaceflights. Experiments were conducted at the United States Air Force School of Aviation Medicine in 1961, in which monkeys were linked in gas exchange with algae tanks for up to 50 h in 1960–61 (2). Researchers at the Institute of Plant Physiology and Institute of Biomedical Problems in Moscow conducted experiments along the same lines with rats and dogs for periods up to 7 days. Shepelev of the Institute of BioMedical Problems, Russian Ministry of Health, Moscow, was the first human to place himself as an experimental subject in a human/algae system in 1961. The basic oxygen/carbon dioxide gas exchange between Shepelev and his supporting *Chlorella* was successful, although a build-up of odors indicating trace gas contamination was noticed (3).

Later, closures of 15 and 30 days were achieved. In the Soviet 30-day experiment, the human lived in a 4.5 m^3 sealed room, sustained by a 30-L algae apparatus which absorbed his carbon dioxide and supplied his oxygen, going through 15 cycles of regeneration. Two potentially toxic components of the air system stabilized during the course of the experiment: carbon monoxide after 3 days, and methane (generated from the digestive tract of the person) after 12 days. Water was condensed from the air, filtered and reused, as was the urine. There are problems even in the simple air linkup of man and *Chlorella*, because the coefficients of CO_2 and O_2 production and assimilation ratios differ. On average, every liter of carbon dioxide produced by human respiration, when absorbed by *Chlorella* growing tanks, results

in the production of 1.21 of oxygen. So if carbon dioxide levels are maintained, the systems will have an oxygen increase. In the 30-day trial, the oxygen was held constant, resulting in an excess of carbon dioxide, which was removed by chemical filters. It was subsequently discovered that *Chlorella's* respiratory quotient and production of organic gases depend on many factors, including the density of algae, illumination intensity and cycle, and conditions of the nutrient medium (3).

The most extended experiments were made in Siberia (Russian Federation) at the Institute of Biophysics, Krasnoyarsk, in the "human–microalgae" system upon the closing of gas exchange, then process and hygiene water exchange, and, later, drinking and cooking water exchange. (Installations: Bios-1, Bios-2 and Bios-3) (1)

A number of experiments on direct gas exchange between humans and a microalgal cultivator were conducted. The duration of these experiments increased gradually from several hours to 90 days, and the accumulated data proved that the regenerated atmosphere is not toxic for humans. The first experiments with humans in the Bios-1 were performed in 1964. Their duration was 12 h (I. Gitelson was the test subject) and then 24 h (the test subject was Yu. Gurevich). No health reasons to prevent long-duration experiments were found. During 1965–66, a series of experiments of increasing duration: 5, 14, 30, and 90 days were carried out. Healthy men and women of 20–33 years of age took part in the experiments.

During the course of the experiments, the participants kept a regime of maximum activity; physical exercises were done according to schedule, and active movements in the cabin were encouraged. Special attention was paid to possible signs of sensitivity to substances of algal origin (The gas composition of the closed atmosphere was investigated: continuously for CO_2 and O_2 content, and once a day for CO, ammonia, hydrogen sulfide, mercaptans, nitric oxide, indole, and skatole. The cabin bacterioflora was sampled daily).

In the course of the experiments, no accumulation of deleterious contaminants in dangerous quantities was recorded in the cabin atmosphere; their concentration in the system was kept at a safe, stable level. No penetration of the cultivator bacterioflora into the cabin was detected either, though the cultivation was not sterile.

The results of the experiments suggest the following conclusions:

1. It has been experimentally proven that the atmosphere for human respiration can be maintained with the help of continuous algal cultivation, and the atmospheric O_2 and CO_2 concentrations can be kept constant.
2. Contrary to some previous results, humans and microalgae (*Chlorella vulgaris*) prove to be biologically compatible with regard to gas exchange; their off-gas products are not toxic to each other.
3. The discrepancy between microalgal AQ (Assimilation Quotient) and human RQ (Respiration Quotient) can be eliminated by correction of the diet. The composition of the food ration needed for complete gas balance of the system is within the range of the physiological optimum (1).

It was noted that long-term nonsterile microalgal culture was able to form an algal–bacterial symbiosis. Dynamic equilibrium between phototrophs and heterotrophs was attained ecologically; no specific measures were needed to maintain sterility. This was far more reliable and incomparably simpler, technically, than to use sterile culture. No toxic or allergenic gases were detected in the regenerated atmosphere in long-term experiments.

Experiments with 3 – link systems (human–microalgae–higher plants) and 4 – link systems (human–microalgae–higher plants–microbial cultivator, utilizing human solid wastes) proved that stable and reliable operation of algal–bacterial community function as an active bioregenerator. However, the problem of biological compatibility is very complicated and must be studied thoroughly for every kind of recommended LSS (1).

The biocenosis of algae in association with dozens of bacterial species exchanging metabolites has a rather complex metabolic network. Trophic relations between algae and the totality of associated bacterial species can be described as symbiotic. To illustrate, algae cannot assimilate directly the organic matter of human urine, but they can utilize the biogenous elements contained in it after it has been mineralized by bacteria. In the same manner, bacteria mineralizes the organic substances excreted by algae, and algae consume biogenous elements excreted by bacteria during mineralization. Organic matter in the algal–bacterial cultivator of a multilink biological life support system, besides metabolites of the cenosis proper, includes various metabolites entering it from other links. Human liquid metabolites consist of fully oxidized organic substances, some of which can be utilized by algae directly, e.g., urea; others first have to be mineralized by bacteria: uric and hippuric acids, creatine, creatinin. The microbial fermenter processing human solid waste supplies organic matter the composition of which differs from the composition of organic matter present in the microalgal link.

Bacteria of the microalgal link can be subdivided into *autochthonous*, constituting their own microflora that is well adapted to the ecological conditions of the algal–bacterial cenosis, and *allochthonous*, which enter the microalgal link from other links of the ecosystem in the course of interlink water and gas exchange. More than 4,000 bacterial strains belonging to 12 families and 17 genera were extracted from the microalgal link on peptone agar. Fungal flora included representatives of ten genera belonging to the family *Moniliacea*. Yeast-like fungi of the family *Sacchromycetaceae*, genera *Rhodotorula* and *Candida*, were extracted sporadically. Bacteria of the genus *Pseudomonas* dominated. Bacteria of the genera *Flavobacterium* and *Achromobacter* also occurred frequently.

Among bacteria persistently accompanying *Chlorella*, a numerous group of bacteria close to corynebacteria (of the genus *Arthrobacter*) were especially prominent. In the algal–bacterial cenosis of a closed ecological system (CES), *Chlorella* is permanently accompanied by bacteria of the genus *Caulobacter*, earlier found in algal cultures and often promoting lysis of *Chlorella* cells. The bacterial population of the algal–bacterial cenosis becomes more diverse when a microbial cultivator and a phytotron are included in the system. The algal cultivator receives bacteria of the genera *Enterobacter*, *Escherichia*, and *Alcaligenes* with the fluid from the microbial cultivator. When the phytotron is added to the system, the biocenosis of the microalgal link receives microorganisms typical of epiflora and rhizospheric microflora, such as *Achromobacter* agile, *Achromobact*. sp., *Enterobacter cloacae*, *Proteus vulgaris*, *Brevibacterium liquefaciens*, and *Alcaligenes aquamarines*. These microorganisms may be classed with allochthonous microflora, representatives of which are not dominant in the biocenosis of the algal cultivator. As soon as water exchange stops, they are eliminated from the biocenosis of the algal cultivator or die out. The number of fungal spores in the culture liquid constitutes just a small fraction of the total number of microorganisms. The main source supplying fungi in the CES is the higher plant link. Conditions in the phytotron are highly favorable for the

multiplication of fungi. *Chlorella* was permanently accompanied in the system by fungi of the genus *Aspergillus*, *Penicillium* and *Mucor* were recorded somewhat less frequently. With the higher plant link in the system, the fungi characteristic of plant microfloras (*Fusarium*, *Botrytis*, *Cladosporium*, *Cephalosporium*) are detected in the microalgal cultivator (1, 4).

In the closed ecological systems described above, the designed regulation of biosynthesis keeps the principal parameters within the range of values optimal for the producing species. Such stability of parameters results in steady-state *Chlorella* photosynthesis and, consequently, a steady level of bacterial numbers. In the course of water exchange, other links of the system supply the algal cultivator not only with organic matter, allochthonous to the organic matter formed in the microalgal link, but also with a great number of microorganisms constituting allochthonous microflora. However, under these conditions, instead of the expected sharp increase in the total number of bacteria in the biocenosis, an overall numerical reduction of microorganisms, accompanied by an enrichment of species composition was recorded Fig. 6.1. An illustration of this is also the death of *E. coli*, entering from the microbial cultivator, and of epiphytic microflora, primarily spores of microscopic fungi supplied by the higher plant link (Fig. 6.2).

Analysis of the specific structure, physiological groups, and trophic relations has shown that the naturally formed microbial biocenosis used as the bioregenerative link in the CES contains a great many bacterial and fungal species, having numerous enzymatic systems. The *Chlorella* culture medium contains macroelements and trace elements, products of algal and bacterial metabolism, lysed cells, amino acids, carbohydrates, proteins, fats, cellulose, humic substances, etc. Against this background, the algal–bacterial cenosis develops, in which the bacterial component numerically approaches that of the primary producer.

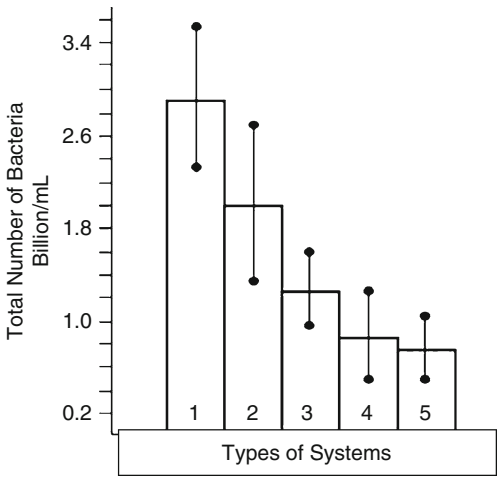

Fig. 6.1. Total number of bacteria in the microalgal link in different variants of the ecosystem. 1 – two-link (human–microalgae); 2 – three-link (human–microalgae–microbial cultivator); 3 – three-link (human–microalgae–phytotron with vegetables, 30 days); 4 – three-link (human–microalgae–phytotron with wheat, 30 days); 5 – four-link (human–microalgae–microbial cultivator–phytotron, 90 days). The *vertical line* segments show the confidence range.

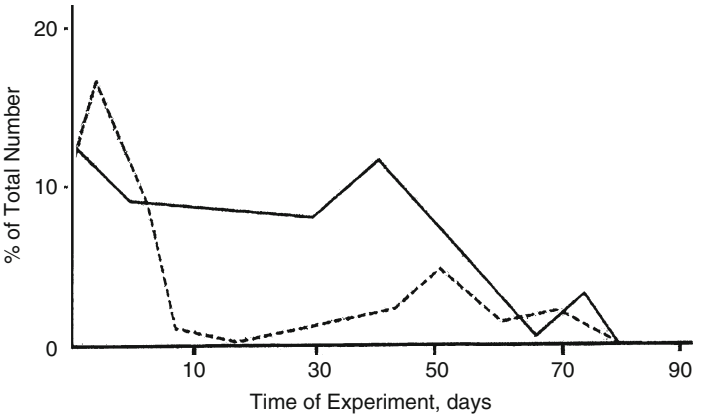

Fig. 6.2. Dynamics of *E. coli* (1) and fungi (2) in the microalgal link in the 90-day experiment with the four-link system.

The transformation of nitrogen, sulfur, phosphorus, and iron, involves a wide range of nonspecific heterotrophic and specific microorganisms. Bacteria, in the process of their life activity, decompose compounds difficult to oxidize, making them accessible to *Chlorella*. This is the principal environment-forming function of microflora. The cultivation of algae and bacteria together precludes an inhibitory effect of metabolites, thanks to an ecological balance between these groups of microorganisms, which is of crucial importance in effecting cyclic water exchange in the system.

2.1.1. Special Waste Treatment Systems for LSS

The waste management strategy for future piloted space missions should meet the standards of crew member safety, and respect the principles of planetary ecology. Biological treatment is based upon the biodegradation of organic substances by various living organisms. Microbial degradation systems have been used for centuries to treat human waste (sewage). Usually, microbial systems used in waste management are energy-efficient and generally less expensive than conventional chemical or physical methods (The solid waste treatment strategies of spaceflights are nowadays based on the principals of isolation, compression and storage. This mainly concerns feces, vomit, plastic, soft paper and cellulose swabs. This technology was implemented on Orbital Stations Salyut, Mir, Freedom, Gemini, Apollo, Skylab, and on the Space Shuttle. Now, it is used on the International Space Station).

A complex approach to waste treatment based on the biodegradation technologies of waste treatment of humans was made by ESA/ESTEC specialists (Project MELISSA – Micro-Ecological LSS Alternative). It was based on microbial ecosystems and dedicated to understanding the peculiarities of artificial ecosystems, and also for the development of new biological LSS for piloted spacecraft and lunar bases. The concept of MELISSA is presented in Fig. 6.3.

In the framework of the MELISSA project, five compartments of an aquatic ecosystem are being developed, from the anaerobic fermenter to the photosynthetic link made up of

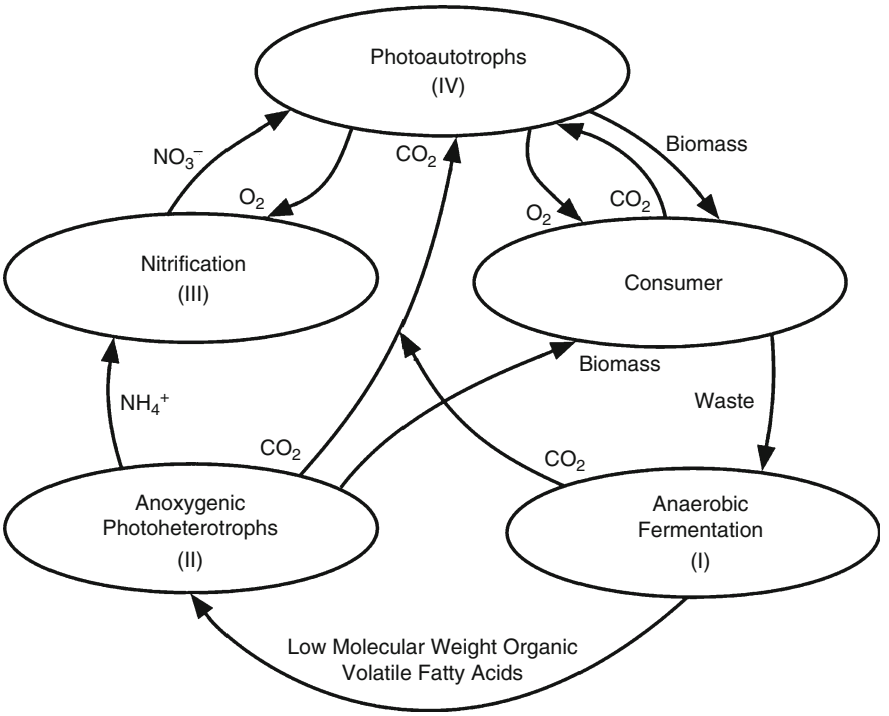

Fig. 6.3. Concept of MELISSA.

microalgae and higher plants. The general goal of the project is to recover edible biomass from wastes and carbon dioxide. The five compartments are colonized by thermophilic bacteria, photoheterotrophic bacteria, photosynthetic bacteria, higher plants, and the crew. The starting compartment of MELISSA is a Liquefying Compartment, designed for biodegradation of human fecal material and other wastes. The microalgal link is represented in the MELISSA project by the microalga *Spirulina*. An important innovation was to introduce into the culture *Rhodospirillum rubrum* as an absorbent for volatile fatty acids (5). As it is obviously impossible to close the food chain without regenerating traditional vegetable foods, the MELISSA project included a higher plant link.

After 14 years of research, the feasibility of each MELISSA compartment has been demonstrated, and a recycling level of better than 70% has been shown by simulation. The combination of advanced biotechnology processes and the requirements of space missions have resulted in a number of applications suitable for bioremediation processes on Earth (More information on the topic can be found at http://www.estec.esa.nl/ecls/default.html). In the MELISSA project, much attention has been concentrated on the development of algorithms of control and process modeling.

Closing the loop on waste recycling in the Biosphere 2, closed ecological system facilities were based on a soil-based system with microorganisms and higher plants. The use of a soil-based system enabled Biosphere 2 to achieve complete regeneration of human and animal

waste products (6). This is accomplished by an in-vessel composting system for inedible crop residues and animal manure, and by a constructed wetlands system for handling human wastes.

The wastewater treatment system operates in two steps. Initial decomposition occurs in anaerobic holding tanks. Then, batch treatment occurs in aerobic constructed wetland lagoons, which recirculate the water, exposing it to the aquatic plants (water hyacinth, canna, aquatic grasses and reeds) and their associated microbes which continue the regeneration process. These systems handle all human wastes from the Biosphere 2 habitat (bathrooms, kitchen, laundry) and the wash-down water from the animal barn. The plants used in these systems are fast growing and are periodically cut for fodder or used in composting. After passing through the constructed wetland wastewater treatment system, the water is added to the irrigation supply for the agricultural crops, thus utilizing the remaining nutrients. A similar constructed wetland wastewater system is employed for any chemical effluent that may occur from internal workshops and laboratories, taking advantage of the fact that aquatic plants can concentrate heavy metals, thus isolating them from soil and water contamination. An additional advantage of this type of soil-based waste water regeneration for space life-support systems is that the high rates of transpiration of aquatic plants make them valuable sources for quality potable water, which can be condensed from air humidity (6, 7). Constructed wetland systems are relatively low maintenance and energy processes, with valuable byproducts. As Schwartzkopf and Cullingford note in a study of technologies for a lunar base: "Many previous CELSS concepts have incorporated high energy methods of waste degradation such as wet oxidation or super critical wet oxidation. In the process, all of the energy stored in the chemical bonds of the waste materials is lost. By using either bioregenerative technologies or appropriate physiochemical technologies some of the chemical bond energy can be provided to the system by converting wastes into low complexity materials which can be used as food stocks for bacteria, algae or higher plants" (8).

Microorganisms also played an important role in a soil-based agriculture of Biosphere 2 facilities.

The requirements of the agricultural system for Biosphere 2 included three major elements: it had to be nonpolluting, intensive, and sustainable. Initial research began with trials of hydroponic and aeroponic cropping techniques. A variety of reasons underlay the subsequent switch to soil-based agriculture. One, of course, is that hydroponics depends on chemical nutrient solution inputs that would be difficult to produce in a space-setting. Another is that the related question of developing waste recycling for animal and human wastes and inedible portions of crops is much more difficult to resolve without the ability to compost or utilize plant/microbe systems for wastewater regeneration. Composting and marsh wastewater systems are far less energy consumptive than alternatives like wet oxidation or incineration. The criterion for a nonpolluting agriculture is required because in a small, tightly sealed environment, the use of chemicals which might cause toxicity in air or water poses extreme and immediate hazards. Even in the $180,000\,m^3$ volume of a facility like Biosphere 2, water, soil, and air buffer capacities are so small, that there is no way of introducing pesticides and herbicides without serious health hazards. Thus, no conventional biocides are employed. A variety of disease/insect controls are used, including introduction of beneficial predator and

parasitic insects, safe sprays, having microbial populations as regulator agents (sulfur, soap, *Bacillus thuringensis*), use of an extensive culture and rotation of crops, selection of resistant varieties, and environmental manipulations of temperature/humidity (9).

3. MICROECOLOGICAL RISKS FOR HUMAN LIFE SUPPORT SYSTEMS

3.1. Man and His Microflora as a Single Ecosystem

Usual microflora of humans in open systems. Humans and their microflora can be considered a single ecological system where the microflora acts as the most active link of the ecosystem. The challenge for each human is learning how to help manage and, if necessary control the activity, adaptation and evolution of this ecosystem.

Intestinal microflora. Under normal conditions, the relation between humans and their internal microflora is symbiotic, with both living in dynamic balance. The upper segments of the human intestine are colonized by a few species of microorganisms. The main species are acid-resistant and include Streptococci, *Lactobacilli* and yeasts. The large intestine is the most densely inhabited area with every gram of material estimated to contain 3×10^{11} bacterial cells. These constitute some 30% of the total volume of feces and more than 1,000 species of microorganisms belonging to eight to ten different families have been isolated from the intestinal duct of humans. 95–99% of these are sporeless *obligate anaerobes* – the *Bacteroides* and the *Bifidobacteria.* On average, both types of bacteria amount to 10^9–10^{11} cells per gram. Other species are present in smaller amounts. *Aerobic floras* in the human intestine include the *Enterobacteria, Enterococci*, and the *Lactobacilli*. These represent less than 1–5% of the total population of the gut. Lactobacilli are essential to normal microbial functioning and have been found in all persons examined. Transitory microflora, spore carrying anaerobes, *Staphylococci*, yeasts, proteus, and other microorganisms, amount to 0.01% (10–14).

The microflora performs several functions essential to the macroorganism. This includes balanced microbiocenosis of the intestinal duct, which is very important for the human's natural resistance to infection. Microfloras also produce antibiotic compounds that prevent pathogens penetrating and reproducing in organs open to the environment. This protective function is restricted not only to antagonistic effects – many bacteria also stimulate immunological responses within the organism, enhancing the bactericidal effect of the blood serum, and promote a faster response to antigen stimulation (15, 16). Intestinal microflora synthesize vitamins, some enzymes, and organic acids. They also produce acid media that suppress the growth and development of pathogenic and potentially pathogenic microbes in the intestine. Microbial associations have an essential influence on the hepatic-intestinal transformation of bile salts, cholesterol, and bile pigments (17). Microfloras also participate in the metabolism of lipids. Normal microfloras influence gas exchange, enzyme metabolism, enterokinase, and alkaline phosphotase (18, 19). Factors affecting microbial cenosis are classified conventionally as endogenous and exogenous. Thus for example, the composition of microfloras in the intestine is regulated by mechanical factors, e.g., peristalsis, by the chemical secretion of acid, bicarbonates and enzymes, and by the antagonistic and symbiotic relationships that exist between the different microorganisms. Exogenous factors affecting the composition and the vital activity of flora include diet, standards of hygiene within the habitat, and

microorganisms in the food consumed. Medical preparations (serums, antibiotics, etc.) will also affect microbial populations. Provided it is fully nutritional, an altered diet has been shown to have little effect on the microflora of healthy people. Analysis of the literature shows considerable variations in the number of microorganisms found in the feces of individuals living on changed diets. At the same time, similar variability has been noted in studies of different people having the same diet (20–22).

Microflora of the skin. The human skin provides a favorable environment that a variety of microorganisms can penetrate, lodge, and multiply on. The outer layers of dead keratinized cells form ridges and furrows that are pierced by sweat glands. Serum, salts, urea, oils, and proteins (largely keratin) from dead epidermal cells provide all the nutrients required by microorganisms. Although many fatty acids on the surface of the skin exhibit antimicrobial properties, a number of successful inhabitants can metabolize these compounds (23). The population of the skin includes aerobic and anaerobic bacteria. The dominant species occur in relatively high numbers and are gram-positive. These include *Staphylococcus* spp. and *Micrococcus* spp. Less abundant microorganisms include *Corynebacterium* spp., *Brevbacterium* spp., and *Propionibacterium* spp.Viruses, fungi, and possibly protozoa, may be present as transient or resident organisms on the skin of healthy people. Those that are resident or autochthonous, are found on the surface of the skin and are non evasive. Routine washing will keep the microbial population of the skin within a limited range, irrespective of changes in the environment (24). Sometimes, it may be desirable or more convenient to use germicides. Antimicrobial activity of the skin has been used to indicate immune response of the organism. A fall in response increases the number and activity of microorganisms. At the same time, the skin's index of bactericidicy decreases (25).

The human mouth can be considered as an open cultivator for different species of microorganisms. There is usually a high proportion of *Streptococcus* spp. and *Veillonella* spp., that are often abundant. There are usually a few *Lactobacillus* spp., *Actinomyces* spp., *Pseudomonas* spp., and *Bacteroides* spp. that may be present (26). The yeast Candida *albicans* is commonly found, and the protozoan, *Entamoeba* spp. and *Trihomonas* spp., may be present.

The respiratory tract can serve as a "harbor" for variety of microorganisms. *S. pyogenos* can cause pharungites, tonsillitis, and scarlet fever. *S. aureus* can cause abscess of the larynx and pneumonia. *Streptococcus pneumoniae* can cause pneumonia. *Proteus* spp., *Pseudomonas* spp., *Kiebsiella* spp., and other coliforms can cause nonspecific membranous laryngitis, chronic sinusitis, pneumonia, and abscess of the larynx. Thrush, bronchitis, and pneumonitis, can be caused by *Candida albicans* (27). *Proteus* sp. is highly pathogenic when it is found outside the gastrointestinal tract, often acting as a secondary invader in dermatitis, especially of the feet (28). Many of the above microorganisms implicated in the appearance of disease are normally found in small numbers in association with healthy humans. They are usually pathogenic only when they occur in large numbers and/or when the host organism has characteristics of reduced resistance.

Human microflora in closed habitats and the interchange of microflora. Many experiments with people living in closed habitats have been carried out over the last 40 years, involving analysis of the associated microflora. The experiments have had different goals, tasks, and conditions, covering the following common features: limited exchange with the external

environment; restricted volume and mobility; small number of occupants; restricted means of personal hygiene; altered diet (partly sterile); modified composition of the atmosphere; limited capacities for storage and reproduction of microorganisms; psychological isolation.

Objectives of the microbiological studies conducted have included the following: to measure the degree of microbial exchange between individuals; to measure the influence of sanitation and hygiene on the microflora of humans and the enclosed environment; to investigate the microbial composition of aerosols, air, and surfaces within the habitat; to evaluate the composition of fecal microflora in response to changes of clinical significance and the influence of diet.

With the development of astronautics, the exploration of ocean depths, as well as the Arctic and Antarctic zones of Earth, researchers meet new challenges, microflora of closed spaces being one of them. Many researchers report that under the influence of extreme conditions – nervous and physical overwork, severe climate, feeding on specially developed diets, etc. – the composition of gut microflora can be shifted (29).

The amount of gut microflora of humans staying in a closed space is reduced (30). When the ecological balance is upset, protective mechanisms may be depressed, which may allow the introduction of foreign microfloras and the activation of potentially pathogenic microorganisms always present in the organism (31–35). For instance, by the end of their stay on the Antarctic Continent, members of an expedition had *E. coli* vegetating in their mouth cavities.

After a 2-week flight by astronauts, a 50% reduction in the total number of the detected microorganism species was recorded (36). Disturbance of the microflora composition may prove detrimental for astronauts' health and working ability in long-duration space missions (37). When people stay long in a hermetically sealed space (during a year-long experiment), significant shifts in their gut microflora composition are recorded: bifidobacteria and lactic-acid bacilli sharply decrease in numbers. As for the risk of "microbial shock" that can be experienced by astronauts after a long-duration flight, it has been mentioned by several authors, e.g., Luckey (38). Meanwhile, a wide variety of microflora in isolated conditions and a decreased ability to inhibit the growth of potentially pathogenic microorganisms may constitute a serious threat of infection to astronauts in long-duration space flights.

Humans staying in isolation for a long time exchange their microfloras (39). Moreover, it seems that it is mostly pathogenic microflora that is exchanged. Inhabitants of Antarctic polar stations often contract intestinal and other infectious diseases caused by potentially pathogenic microbial species.

After a 96–140-day flight (*Salyut-6*), it was found that cosmonauts mutually exchanged pathogenic staphylococci. No accommodation of nonpathogenic staphylococci was recorded (40). Mutual exchange of microorganisms in a closed space creates the problem of biological compatibility. Conditionally pathogenic microbes are exchanged in any group of people, but in a hermetically sealed space, such exchange occurs more frequently (34, 41–43).

The most detailed experiments pertaining to the aspects listed above were carried out by the Institute of Biomedical Problems (IBMP) in Moscow using a physical–chemical life support system and by the Institute of Biophysics (IBP) in Krasnoyarsk, who used series of biological–technical systems. Experiments at the respective institutes were conducted in 1967–68 and

between 1970 and 1984. Researchers at the IBMP, dealing with physical–chemical LSS observed the following results (44):

- Significant simplification of intestinal microflora, particularly in respect to the number of bifidobacteria and lactobacilli.
- Interchange of intestinal microflora. This was studied by monitoring the colicinogenic and hemolytic activities of *E coli*. Interchange included strains of staphylococci and those of an intestinal bacterium.
- The number of strains resistant to selected antibiotics increased.
- *E. coli*, *Candida albicans*, and *Candida tropicalis* were detected in the pharynx and oral cavities. The presence of *E. coli* in these locations and the numbers of Candida measured, were abnormal.

Some results are described here on the basis of the data obtained with Biological LSS in the course of several long-duration experiments in Bios-3 lasting 180 and 120 days each (4, 45, 46). Experiments with autonomous biological ecosystems yielded a great number of data on the effect exerted by these systems on human gut microflora. Feces samples for analysis were collected for 3 days in succession every month.

In experiments, the highest and the most stable numbers were recorded for bacteroids. The total amount of microorganisms per g of feces also varied: from 9.1 to 10.2 (lg of number per gram). For the so-called "transit microflora" – staphylococci and yeasts – wide numerical variations were recorded in the samples analyzed – from 0 to 10^4 cells/g. Such variations in "transit microflora" can also be registered under ordinary conditions in healthy people. Though in some periods the gut microflora was unstable and tended to simplify itself, the total number of microorganisms in 1 g of the sample was relatively constant for all test subjects. Bacteroides were the most stable while staphylococci and yeasts the least stable and numerous groups.

In these experiments, no essential shifts were recorded in the gut microbial community unlike in the hermetically sealed space-simulating space flights, isolated Arctic and Antarctic polar stations, and in the previous versions of LSS. No shifts in gut microflora were recorded in the 4-month experiment (120 days), while in the 6- and 12-month medical–engineering special experiments they were evident (45). Biological LSS systems are, in many ways, essentially different from a closed space or physical–chemical LSS, particularly the systems in which the principal environment-forming link is represented by higher plants.

In a biological system, humans consume food and fresh vitamins regenerated in the system; only part of the food is stocked in a lyophilized (freeze-dried) form. As we have mentioned before, one of the exogenous factors influencing the formation of the gut microbial cenosis is food (its quality and diversity). The ecosystem includes natural reservoirs (phytotrons) for the storage and multiplication of microorganisms. Moreover, they are the microorganisms which are supported by their association with plants in their natural habitat. This must account for the fact that in Bios-3, no negative changes were observed in microflora similar to the adverse changes seen in isolated habitats with no plants.

The described system – Bios-3 – is similar to other closed systems in one respect: Bios-3 is isolated, essentially materially-sealed, from its surroundings. Special measures were taken to achieve this isolation, and to preclude the introduction of microflora from the outside during the experiment, i.e., gnotobiotic conditions were created. On the other hand, the Bios-3 facility

differs from previous closed systems, in that the crew staying in it contacts various plants and eats their fresh produce. It would appear that a rather small phytotron can serve to ensure regular self-regeneration of human gut microflora. Comprehensive medical examinations of test subjects during the half-year experiment, and for a long time afterwards revealed neither any worsening of their health nor any deviations of their physiological parameters from the original state. A conclusion was drawn that the habitat generated in Bios-3 was adequate for human physiological and ecological requirements, and a healthy human can stay in this biological life support system for quite a long time (4).

The general conclusion from experiments conducted with physical–technical life support systems is that the microflora of surfaces, aerosols, and the air is represented by bacteria that is expelled by humans. In the case of a biological LSS, additional microflora is introduced from the biological components of the system. Sanitation and hygiene procedures used in different life support systems have a significant influence on the microflora of the human skin and the microflora of the habitat as a whole. The European Space Agency (ESA) has completed some experiments with groups living in closed habitats. Exchange and simplification of skin flora was observed. Human bacterial flora (typically, gram positive cocci) also came to dominate the environment after a few weeks. Later experiments indicated a trend towards uniformity of skin microbial flora and similar colonization of the environment by human microorganisms (43, 47). The stress of flight may increase the hazard of exchange and interaction between man and microbes during a space mission.

Results obtained during the years of operation of the Russian (Soviet) “Salyut” and “Mir” orbital stations suggest that, during flight, in the status of human internal microbial ecosystem, there were, as a rule, signs of activation of the potentially pathogenic component represented by staphylococci and gram-negative bacteria. The staphylococcal flora showed an increase of already existing foci of pathogenic staphylococci or formation of new foci as a result of colonization of exogenic cultures originating from other members of the original or visiting crews (48). This process was manifested as formation of transient carrying of these cultures in new hosts, and may be classified as an unmanifested form of staphylococcal infection. Appearance of clinical symptoms of the disease could be provoked by an additional effect upon the microorganism of a nonspecific factors of a physical or chemical nature.

Besides this typical process, a characteristic change in the status of human microbiocenoses aboard space vehicles was colonization of upper respiratory tract mucosa (more rarely of skin) by gram-negative bacteria that are not typical for these biotopes – *Proteus, Klebsiella, Enterobacter, Citrobacter, Escherichia* sp., etc. – the role of which, as pathogenic factors of opportunistic infections in human is well known.

In some cases in the intestinal microflora, there was an increase in the number of potential pathogenic enterobacteria, an exchange of less virulent serovares for more virulent, and an increased number of cultures capable of generating pathogenic enzymes. As a rule, this was associated with a reduction of normal inhabitants – a diminished number of bifidobacteria and lactoflora.

In parallel with these changes of the status of the cosmonauts’ automicroflora, physical parts of the space station environment, e.g., on nonmetallic interior and hardware materials, there was observed formation of specific reservoirs of accumulation and

propagation of microorganisms, especially gram-negative bacteria, Bacillus bacteria, and mould micromycetic fungi. This process was one of resident colonization of the pressurized cabin environment as a kind of anthropogenic technological niche by potentially pathogenic microorganisms, biodestructive and micromycetic bacteria probably forming trophic bonds with polymeric materials accumulating on them (condensate of atmospheric moisture, etc.)

The changes listed above became still more marked when the artificial environment, in a number of characteristics, significantly differed from a normal one. Thus, in hermochambers (hyperbaric) that were characterized by high pressure (25 ATA) and a humidity increased to 90% (submarine diving complexes and their simulators), there were clearly marked, widely spread, and expansive processes of colonization of "open" human biotopes (nasal, and mouth cavities, pharynx outer auditory channels, skin) by gram-negative bacteria, as well as formation of massive reservoirs of the microorganisms on various segments of the interior, hardware, and life support systems (48).

3.2. Environmental Microflora in Different Types of LSS

In every day life, as we mentioned, microbes colonize every surface and volume of the animate and inanimate environment. No single macroorganism is free from microorganisms and in closed conditions they form an integral part of the habitat and its life support system, be it physical–chemical, biological, or mixed. No source of air, water or fresh food or surface is sterile. Here are briefly presented some data on environmental microflora dynamics in the atmosphere and surfaces of closed habitats. A variety of processes contribute to the environmental conditions found within a closed habitat. These include its life support system, as well as the activities of its human and other biological occupants. In the case of extended space flight, factors such as microgravity and variations in magnetic fields will also have a role to play. In a closed space, man and the medium surrounding him develop a relationship different from that found under natural conditions. In normal situations, it is the environment that determines the processes of human vital activity. In the case of sealed or closed habitats, it is human activity that affects the environment, particularly chemical composition of the air and microbial contamination of air, water, and physical surfaces. The degree of contamination in a habitat using a physical–chemical system of life support, will depend on the number of crew members present, the duration of their stay, the degree of hygiene practice applied, and the filtering and sterilizing capabilities of the air, water and waste management systems. The microflora found in closed habitats are mainly "human" in origin. They include epidermal species, as well as those of the respiratory tracts and gastroenteric duct. When a system accommodates plants and animals, the number and diversity of microorganisms increases considerably. A large number of physical, chemical and biological factors affect the ability of microorganisms to survive and reproduce. These include: (a) Physical – temperature, osmotic pressure, surface tension, visible radiation, UV-radiation, ionizing radiation, gravity, absorption phenomena and viscosity. (b) Chemical – structure and activity of bound water in solid wastes or food, pH, inorganic nutrients, gaseous contaminants, organic nutrients, hormones, growth regulators, metabolic control substances, poisons, inhibitors, nutrient analogs and redox potential. (c) Biological – duration and type of life cycle of the organism, the presence or

absence of other organisms of the same species and their interactions. Many microorganisms recovered from sealed volumes adapt rapidly to changes in the above factors. Microorganisms also develop resistance to germicides and disinfectants.

Surfaces. All surfaces in a closed habitat will be sites for microbial growth and any contact by the crew will contaminate them (24, 25). The type of surface and the microenvironment can also influence the reproduction of microbes. A study measuring aerobic, mesophilic and heterotrophic microorganisms, showed a greater number of viable microbes on nonmetallic surfaces. The largest numbers of microbes will be found on surfaces most in contact with the crew. This includes walls, meal areas, and hatches, etc. A great diversity of microorganisms such as *Enterobacter*, *Staphylococcus*, *Aeromonas*, *Bacillus*, *Corynebacterium*, *Micrococcus*, *Klebsiella*, the fungi *Penicillium*, *Streptomyces*, *Aspergillus*, and *Fusarium*, have been found (49). High humidity, the presence of organic contaminants, and elevated room temperatures increase the activity of microorganisms and enhance the formation of metabolites capable of inflicting damage on structural materials. For example, fungi produce amino acids and such organic acids as citric, fumaric, and gluconic acids. Microbes and the types of bacterial cenosis occurring in biological systems of life support are reported to have damaged materials and metallic structures. Fungal degradation of electronic parts has also been documented. Some authors have concluded that microbial populations growing on solid materials should be known accurately so that their contribution to the total contaminant load of the spacecraft can be assessed (49, 50). In addition, it will be necessary to consider the potential risk of contamination when missions finally reach other planets. The processes associated with the biodegradation of materials cause the atmosphere in closed habitats to change. In particular, volatile toxic substances accumulate. The key classes of volatile metabolites released by bacteria, fungi, and algae, on different substrates are presented in the work of (51). The work (51, 52) also identified many volatile organic compounds. These included carbonic acid, nitrogen, ammonia, oxides of nitrogen, hydrogen, ethane, butane, propane, and methane. The release of toxic substances from structural materials as a consequence of biocorrosion does not appear to depend on the association of microbial species present. The growth of pathogenic and other microorganisms on materials can also reduce sanitary and hygiene conditions within a closed habitat.

Biodamage is not only seriously influenced by the environment, but it in its turn influences the latter, altering everything in the closed environment (48). In some cases, biodamage can provoke appearance of new biocenoses. In particular, polymeric materials, as an ecological niche, support many species of microorganisms that are capable, under certain conditions, of developing in them. In each case, the structural stability and life span of the newly formed microbiocenoses are different, but on the whole, they are characterized by a connection with the environment around the polymers.

The problem of microecological risk associated with the creation and utilization of high technologies goes beyond purely health and medical aspects, important as they are. The experience of prolonged operation of orbital stations can be a vivid example confirming this conclusion. Thus, one of the main crews of the “Salyut-6” orbital station found a “white coat” on some segments of the interior, rubber bands of the training device for physical exercise, and

some other zones in the habitable modules. Analysis of samples of this "coat" on the ground revealed growth of micromycets of the *Aspergillus*, *Penicillium*, *Fusarium* species (52, 53).

During work of one of the crews on board the "Salyut-7" orbital station, the cosmonauts reported visible growth of mold in some areas of the frame, connectors, and wires in the operation module. Fragments of these materials were selected by the crew and brought to the Earth for laboratory analysis. A visual investigation of the fragments received, demonstrated that mycelium covered 25–50% of the surface of the samples. This suggested that the materials could be a source of nutrition for the microorganisms. A microscopic analysis of the samples revealed changes in the structure of the materials, while on some segments of the protective tape there were holes. When samples taken from the materials were cultured, mould fungi were found representing the *Penicillium* species (in most cases, *Penicillium chrysogenum*), as well as *Aspergillus*, *Cladosporium*, *Mucor*, and actinomycets.

Of particular interest is the situation related to the zonal window of one of "Soyuz" transport vehicles that functioned for 6 months connected to the "Mir" station. The crew of this station noted a progressive deterioration of optical characteristics of the view window of the recoverable module. After its return to the ground, analysis were performed, which revealed the following. On the central and most peripheral windows of the windows made of a superstrong quartz glass, as well as on the enameled titanium frame, there was mycelium of mould fungi; in one of the zones, a growing micromycete colony was clearly seen. When samples from the damaged sites were cultured, a microorganism association was found containing spore-forming bacteria, mainly of the *Bacillus Polymyxa* species, and mould fungi *Penicillium chrysogenium* combined with representatives of the *Aspergillus* species. The visual impression was that the growth originated from the gasket used for fixing the glass in the titanium frame. Also of importance is the fact that isolated cultures (using *Aspergillus versicolor* as an example) presented certain morphological differences to the standard strain. The data obtained on the development of microbiological damage to construction materials during flight were the basis for extended systemic research of microorganisms forming the microsphere of space vehicles.

Years of observation within one space station revealed changes in the typical species organization of its microecosphere. Thus, during operation of the "Salyut-7" orbital station (using the status of the fungal flora as an example), the following was observed: when prime crew (PC) 1 was flying, 13 species of micromycets were found, when PC 2 was flying, there were only 4. Subsequently (PC 3), the number of species again increased to 8. In the latter case, appearance of "new" species was the cause of the broadening of the spectrum, rather than the reappearance of those primarily found during the flight of PC 1. Only one species – *Penicillium chrysogenium* – remained predominant at all times in the various zones of the station. Thus, though during the second stage of operation of the vehicle the spectrum of species in the environmental microflora decreased, this trend was not maintained. Probably the interaction of microorganisms with elements of the station environment with polymeric construction materials was a wave-like process manifested as periodical successions of the micro ecosystem. In any case, there's reason to believe that what happened was resident colonization of the space vehicle environment by a microorganism association as an anthropogenic technological niche in which a sort of evolution of the microflora occurs.

In submarine hyperbaric diving complexes, *Pseudomonas aeruginosa* most actively colonized water reservoirs of the water supply system and other moisture – containing zones of the interior and hardware. In such niches, microorganisms are known to develop at the borderlines between fluid and solid phase. Due to adhesion, this is associated with the formation of a specific biofilm including bacteria and their produce – a polysaccharide complex (glicocalex), which involves organic and nonorganic components of the aquatic medium. This film is exceptionally stable and nonpermeable to many biocides and antibiotics. In such cases, use of antimicrobial drugs results in death only of the so called "floating" forms of microbes. Bacteria contained in the interior portions of the bio-film survive easily, and after some time reproduce, and again contaminate the environment.

From the cumulative data, the microfloras of different space vehicle and space station environments (including orbital station MIR, the International Space Station) include more than 250 species of bacteria and microscopical fungi (48).It was discovered, that in considering long-term space habitation, the space vehicle environment should be considered as a potential niche for the development of particular groups of associations of "microorganisms–bacteria–fungi." The potentially pathogenic microorganisms, and bacteria and fungi – biodestructors (so-called technophiles) can be involved in this process, using as a substrate the construction materials of the interior, as well as equipment and technical devices of the habitat and provoking, in some cases, their biodamage.

It has thus been shown, that the condition of the microflora, its quantitative and the qualitative characteristics depend on abiogenic (physical and chemical) environmental factors of the long-term operational space environment, including such important factors as temperature and humidity, chemical composition of atmospheric trace gases, condensate, water, the level of ionizing and reionizing radiation, physical properties and chemical composition of polymeric materials, techniques of regeneration of vital waste products of the human beings, sanitary/hygienic measures, etc.

Research revealed that the quantitative dynamics of the microbial populations of the space object environment in conditions of long-term operation does not have linear behavior, but it has the form of an undulating process of alternation of phases of activation and stagnation of the microflora. This is accompanied with periodic change in which species and types of microorganism are abundant and dominate the microbial ecosystem within space environments. This indicates that both cooperative and competitive relationships can operate between microbial species during their assimilation into the environment of the orbital complex. The long-term observation of the dynamics of the fungal component onboard the "MIR" station showed that some representatives of fungi have the ability to adapt and survive in the space station environment. It was shown, for example, that the particular strains of *Penicillium chrysogenum*, – the dominating kind in the composition of microflora of the orbital complex, – were present for a period of at least 8 years. The capability of a resident population of the mycosis forms of fungi has great importance, as it was discovered that during orbital flight, their aggression (colonizational and biodestructive activity) damaged constructional materials of the space station. This destruction was in excess of what would be expected when compared with reference strains of similar kinds of fungi. At the same time, numerous cases of damage of constructional materials and equipment by other microorganisms were observed.

Air. The majority of microorganisms found in closed habitats with a physicochemical system of life support are human in origin. Vegetation of fungal spores can also occur, e.g., air in an American submarine was found to contain spores of the fungi *Penicillium*, *Cladosporium*, and *Aspergillus.* Concentrations were reported to amount to 200–240 cells/m^3. Strains found in the air of space stations and spacecraft have included *Staphylococci*, *Pseudomonas*, and *Corynebacterium*. The degree to which microorganisms can contaminate such atmospheres depends on the number of crews, working conditions, and the efficiency of the air purification system. It has been shown that variations in the microflora of the upper respiratory tract can be related to the environment in which the crew lives. For example, the number of spores of the fungi *Penicillium* and *Aspergillus* increased in the air of BIOS-3. This habitat contained experimental plants (4). In McDonnell Douglas' 90-day Space Station simulator test of 1970, most atmospheric fungi were resistant, nonfastidious and ubiquitous in soil and air. These included *Aspergillus* spp., *Penicillium*, *Pullaria*, and *Rhodotorula* (a yeast). Scientists at the Institute of Biophysics investigating closed ecological systems have demonstrated that microorganisms release volatile substances into the atmosphere that are biologically active. These agents are retained for many days and can be toxic to plants and possibly people (51).

Water. The water facilities of spacecraft and other closed installations provide an ideal habitat for microorganisms. For example, the potable and wash water systems of Apollo flights 7–17 were contaminated by such environmental bacteria as *Pseudomonas* and *Flavobacterium*. At the same time, postmission examination of potable water revealed no correlation between these microorganisms and human microflora found on the skin, clothes and surfaces of the habitat. (This shows the effectiveness of containerization to prevent cross contamination). Although not directly related to the vehicle's potable water system, an experiment on Shuttle Flight STS-8 was contaminated by *Pseudomonas* and *Flavobacterium*. This occurred in spite of extensive preflight sterilization. *Pseudomonas* is an adaptive organism that can contaminate distilled water, chlorinated swimming pools, and public supplies of water (54–57). It is associated with a number of human, animal and plant diseases, including the urinary tract infection which affected a crew member on Apollo mission 13. *Flavobacterium* has been cultured from distilled water and drinking faucets, etc. It is sensitive to heat and has been implicated in hospital deaths. The microflora of potable water reclaimed from cabin condensate on Mir has been studied (58, 59), and 1 ml of condensate was found to contain 10^2–10^4 microbes. After purification, the number of microbes decreased to 100 cells/ml. Strains isolated included *Micrococci*, *Staphylococci*, *Citrobacter*, *Aeromonas*, *Alacaligenes*, and yeast. The most resistant forms after purification of water by sorption, were *Alcaligenes faccalis* and *Citrobacter freuendii*. In a review of reclaimed water quality, an American author, Janic (60), discusses the flight experiences of NASA, the former Soviet Union and others. His analysis showed potable water to contain species of *Pseudomonas*, *Flavobacterium* and *Achromobacter*, as well as fungi – *Asperigillus*, *Cephalo-sporium* and *Candida.* The researcher (name) notes that the following parameters in the US und Russia are used to define/estimate the quality of water: (a) total organic carbon as an indicator of organic chemical toxicity and (b) coliform as indicators of microbial contamination. These criteria are considered insufficient. Furthermore, the paper notes that quality specifications are usually formulated in terms of the methods available for testing. These include influent, effluent and "tap" standards with special emphasis

on inorganic, organic, bacterial, viral, and fungal contaminants. However, it is preferable to use the following parameters as a better measure of drinking water quality:

- Indicators for low molecular weight (MW) organics that may be carried over with water during its reclamation.
- High MW organics (e.g., steroids, antibiotics).
- Key bacterial contaminants including *Pseudomonas* and *Flavobacterium*.
- Viruses and fungi.

Food. People living in closed habitats using physical–chemical systems of life support have often consumed lyophilized (freeze-dried) food. Nominally sterile, such rations contain microorganisms. In mixed systems (biological–physical–chemical), like the Biosphere 2 experiment, a complete diet of fresh food can be grown within the habitat. Although more suited to human needs, such food also contains microorganisms. Unbalanced and sterile diets cause various changes to the intestinal microflora and different researchers have their own experimental results after having examined the effects of various diets (21, 22). The presence of microbes in food should be considered as a further factor influencing the performance of humans and their microflora in a closed space. Pathogenic and potentially pathogenic microflora found in food includes *Salmonella* spp., *Campylobacter* spp., *Staphylococcus aureus* and *Clostridium perfringens*.

Clothing. The clothing, especially when it is wet, can harbor many microorganisms some of which are potentially pathogenic. Some 30 different species have been isolated from laundry wash water. These include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Paracolbacterium* sp., *Escherihia* sp., *Aerobacter* sp., *Proteus* sp., and *Alcaligenes* spp. The main source of contamination includes skin microflora, the remains of food, urine and feces (32) performed experiments to estimate the extent of microbial transfer from the skin of humans to their clothes. Sample discs, three layers thick, were applied to the skin of volunteers in a sealed room. The inner layer of fabric tissue was found to have more microbes on it than the skin, whilst the outer and intermediate layers contained approximately 80 and 16%. This demonstrated the importance of skin microflora in contaminating clothing. It is common knowledge that clothes and bed linen are a constant source of secondary air contamination. Some authors think certain types of fabric can decrease the microbial contamination of clothes (33).

3.3. Unsolved Problems and Prospects

The foregoing considerations have illustrated the complexities of the coexistence between man and microbes. There are no clear answers to many of these questions, and it is not surprising that much published literature is either inconclusive or too specific and narrow in its observations. Moreover, examples of contradictory results exist. This can be explained because many observations and experiments have been conducted under different conditions with different individuals and microbes. The problem is compounded by the tendency of microorganisms to change their characteristics during experiments of long duration. Nevertheless, the task is not unsolvable. A number of practical recommendations can be formulated from the body of accumulated data. Some of these involve typical sanitary-hygiene procedures, whilst others may have a more radical impact on the "man–microbes system."

Correlating these actions with theoretical predictions provides an opportunity for further advances to be made.

Drug therapy and microbial resistance to drugs. Some publications have presented data purporting to show an increase in the drug resistance of microorganisms exposed to the real or simulated conditions of space flight (61, 62). The reasons for these phenomena are not clear. It may be associated with changes in the rate of microbial proliferation or with changes in the structure of microbial cell walls and their permeability to drugs. Even if the effect was substantial (for example, a twofold increase in the resistance of *E. coli* to colicine is reported), it cannot be a big danger to future activities. Theoretical biology predicts that a spontaneous increase in drug resistance cannot be very large or stable when it is not required for survival of the microorganism. If it does appear spontaneously, it should be eliminated over a number of generations by the action of natural selection.

The situation will markedly change if the drug is used against infectious diseases. In this case, the apparent increase in resistance of the drug can be explained. Soviet researchers have also noted the appearance of resistance in *E. coli* (63). Perhaps, by using a rotation of different types of drug, it possible to avoid the problem of quick adaptation. The hope is that natural selection should eliminate those strains with resistance to a particular drug, if the latter is not used for a long period of time. In other words, sensitivity to a drug can be renewed. This is the so-called law of stabilization from the action of natural selection.

The danger of opportunistic infections. This problem is similar to the one described previously. Until now, it has not been studied properly, but it becomes an issue of importance for the future of manned life support systems. Normal associated microorganisms could become pathogenic for a number of reasons. These include:

- The effect of mutation. The probability of this occurrence is low because a benign microbe would need to acquire many new features to become pathogenic.
- The effect of excessive drug (antibiotic) therapy. In this case, useful and protective forms of microorganisms will be killed.
- Weakening of the immune system due to stress.

3.3.1. Use of the Organism's Protective Microorganisms to Defend Against Pathogens

There are reports that cosmonauts on Salyut and Mir have suffered disbacteriosis of the intestinal flora to varying degrees. The proportion of potentially pathogenic microbes then rose, increasing the danger of infection. It is not certain if the problem of disbacteriosis is caused by the ecology within the closed habitat or whether such factors as dietary restrictions have a role to play. Soviet researchers have developed bacterial preparations containing lacto and bifidobacteria for consumption by cosmonauts (62). These have been found to be highly effective in helping to reestablish a normal balance of intestinal flora. The task could be made easier by preparing inoculums for each individual using his or her own microbes taken and stored before the mission.

Maintenance of the immune system's activity. Activity of the immune system is one of the key problems on manned missions because of the stressful conditions that people will be exposed to in space habitats. In this respect, stress related suppression of the immune system

will last for the duration of the mission. It has been detected in Soviet cosmonauts returning from long missions on the Mir station with symptoms remarkably similar to those of aging. Bones have decalcified and weakened, blood production declines and the immune system slowly decays (64). The mechanisms involved have yet to be elucidated. From the point of view of the interactions between man and microbes, weakening of the immune system can be the reason for unpredictable behavior of the man–microbe system. To sum up the results of the previous considerations, we can divide all space missions into three types by which the behavior of man–microbe interactions may be categorized (65).

Short missions. Special and dangerous changes in man–microbial interactions should not occur, especially if adequate preflight quarantine procedures have been adhered to. Note however, that conditions of extreme stress can occur in an emergency. In such circumstances, hygienic provisions may become inadequate and opportunistic infections may occur rapidly.

Long missions with visiting crews. Many of the problems described in this report can occur on such missions because of changes to both the people and microbial constituents of the system. Measures such as the consumption of bacterial preparations or immune system stimulants may be pursued in the short term. Visitors to a space habitat can bring new foreign microbes into a weakened system. At the same time, it seems likely that visiting crew will have similar associations of microflora simply because they will have had to train and live in the same places on Earth as the occupying crew. Another problem concerns the long term hygiene of the space vehicle and its internal equipment. For example, many surfaces on Mir were reported to be colonized by associations of bacteria and fungi (66). Pathogenic and biodestructive forms of microorganisms were present in these associations. Some of these microbes may have developed super tolerance to the new conditions making it impossible to sterilize sites completely under flight conditions.

Long term settlements on other planets. Problems mentioned above will become severe and stronger on such missions. Visiting expeditions will destabilize interactions between the system of macro and microorganisms. Problems to be solved will be many. One of note can be taken from the history of contact between isolated groups of people living in small settlements and the rest of the world. There is a high risk that such groups could be the victims of infections or opportunistic diseases caused by normal microflora brought by visitors transforming into pathogens. Special and new types of procedures would have to be developed for such encounters.

4. THE INDICATOR ROLE AND MONITORING OF MICROORGANISMS IN LSS

Examination and insights into the dynamics of microorganisms as indicators of the health of closed systems (67, 68) require new developments and modifications to techniques that are presently used to measure microbial activity and their numbers. Preference should be given to methods that can be automated. Modern monitoring techniques include: Laser Light Scattering, Primary Fluorescence, Secondary Fluorescence, Bioluminescence, Electronic Particle Detection, Monoclonal Antibody or DNA Probes, Measurement of ATP, Remote Microscopy-Telemycology.

4.1. Microbial Diagnostics Method

A new method is needed, one which will function as a kind of "Microbial Diagnostics" (69, 70). Microbial Diagnostics would be a diagnosis of infection, inflammation and loss of normal flora in the human body by detection of specific microbial substances – markers – especially fatty acids, sterols and other lipid compounds and microbial metabolites using gas chromatography–mass spectrometry, single ion monitoring (GC–MS–SIM) analysis, and lipid compounds profile balance. These methods allow the quantitative determination of genera and species composition of mixed infection and microbial communities in body fluids or environment. The features of such a diagnostic test would include simultaneous quantitative determination of more than 100 markers (35 microorganisms) during a single equipment run. Detection of bacteria and microscopic fungi, viruses, protozoa, and slow or fast growing, aerobes or anaerobes, nonfermenters, noncultivating needs to be accomplished as well. Ideally, a uniform sample preparation would prove suitable for detection of all the microorganisms mentioned above. The analytical procedure should require less than 5 h, with computerized analysis and calculations. Desirable sensitivity of $n \times 10^{-12}$ g of marker substances or 1,000 microbial cells per 1 ml of sample. The test should be able to identify at the species level. Direct probe analysis of the biological sample without precultivation is also valuable.

The method doesn't require biochemicals, i.e., cultural media, antigens, ferments, primers, etc. This short-term analysis (less than 5 h) should be a universal microbial test, which does not require cultivating and excludes biological test specimens. Local database was created by one of the authors (LAS), which consists of standard fatty acid profiles of pure cultures, the microorganisms of clinical value, and algorithm of marker control. Analysis is based on determination of trace chemical markers in biological fluid. Occurrence of branched and odd acids, hydroxy acids, metabolized sterols, and certain aldehydes, in body fluid is chemical evidence of bacterial presence. Microbial cells contain more than 200 fatty acids (FA) that differ from human ones. Only 10–20 FA from the list of 200 is typical for a single bacterial genus. Thus, characteristic FA markers and profiles allow one to distinguish bacterial genera and species from another and from human cells. There are a number of environmental criteria that have been used to define the quality of air and potable water in closed living quarters, such as a space station. These criteria are basic and do not differ markedly from nation to nation.

4.2. The Use of Skin Bacteria and Bactericidal Activity to Estimate Immune Responsiveness

There are simple, quick and informative methods for estimating immune responsiveness of the macroorganism and thus its health. Microbial resistance of the macroorganism can be estimated by the following parameters:

- The total quantity of microorganisms on an area of skin.
- Bactericidal activity of the skin.
- The number of hemolytic strains of microorganisms on the skin.
- Presence of *E. coli* in the mouth.

To study skin microflora, a technique of inoculating the nutrient medium has been proposed (70). In this method, selective nutrient medium is applied to the slide plate, the plate is pressed against the surface of the skin and then incubated in a thermostat. A healthy person has from five to eight microbial cells on a square centimeter of skin surface. The typical number of deep skin microflora is 8–12 cells/cm^2. When immune responsiveness of the organism is disturbed, the number of microorganisms on the skin increases. The proportion of hemolytic strains gives an indication of their pathogenicity. Not more than 10% of hemolytic strains are found in a healthy person. The main indicator of inhibited immune responsiveness is lowered bacterial activity of the skin. Bacterial activity can be estimated by applying a specific dose of a test microbe to a portion of the skin. A selective nutrient is then applied some minutes later. If bactericidal activity is high (e.g., a healthy person), the test microbe will fail to grow and dies. (In a similar manner, *E. coli* dies on the skin or in the mouth of healthy men and animals. When there is a decrease in immune responsiveness, *E. coli* is found both on the skin and in the mouth). Such a method is simpler and quicker to use than other techniques (71).

4.3. The Use of Microecosystem Response to Indicate Human Health

According to the approach of theoretical ecology, it is possible to distinguish four ecosystem's level (types) to different impacts: organismic, population, communities, and ecosystem as a whole (72):

(a) The organismic level is the most sensitive and rapid, driven by the reconstruction of microorganisms' physiology within the limits of the genetically determined reaction standard. The response takes place within the normal range of physiological adaptation. At this level, the introduced impact can be overcome without irreversible changes within the system.
(b) Population (species) level of response manifests through redistribution of available populations (species) by their domination. This level corresponds to the stress range of the microbiocenosis, and results in a change of the ratio of microbial species present.
(c) Communities level of response manifests through decrease of species diversity of microbes, simplification of the species composition of the microbiocenosis (microbial ecosystem). At this level, there is increased probability of invasion of "foreign species" to this system, and a replacement of previously dominant species takes place. This magnitude of impact is high and difficult to overcome.
(d) The level of ecosystem response is marked by suppressed growth and development of indigenous organisms and explosive growth of organisms not normally found in this community. A particularly extreme intensity of impact can destroy the ecosystem.

For example, the response of a microecosystem of the intestinal duct of a healthy person living in a space vehicle can be considered. Under normal conditions, the intestinal duct would be occupied by a stable community of microorganisms. Moving to a more extreme environment would affect the viability of the community and cause the physiology and biochemistry of its microorganisms (level a) to change. The structure of the microbial population (level b) and the numbers of dominating microbes might also change. At the trophic level (level c), certain species might be eliminated and a number of populations specific to the microbiocenosis of unhealthy people emerge. These can include resistant pathogens and saprophytes of the type *Enterobacter* and *Citrobacter*, etc. Evidence from the literature indicates the microbial

response in people exposed to extreme living conditions will be characterized by changes at population and community level (b and c). The response at the organismic and population levels corresponds to the normal functioning of microflora. When the stressing influence is removed, microbiocenosis will probably return to its former state. The response at the community level corresponds to stress impact and is hard to overcome. The final level (d) corresponds to collapse of the ecosystem. Exactly this approach was used to estimate changes in the microbial ecosystem of the intestinal system of the crew members in different versions of a closed man-made ecological system (Bios-3). In 6 months long experiments with three-component "man–algae–higher plants" system, the intestinal tract microecosystem responds in the first months as the organismic level response, in the months to follow – as the population level response. In the experiment with two-component "man–higher plants" system, the intestinal duct microecosystem responds, as in the first type (a) (organismic level response), with the biochemical activity of microorganisms changed in the first month of experiment (72).

4.4. The Estimation of the "Health" and Normal Functioning of LSS and Its Links

Any bioregenerative LSS, with a large living component, shares certain features of a large organism. However, a biological LSS differs from it in its "immortality," its ability to exist for an indefinite period, i.e., to maintain true homeostasis. It possesses such general integrated parameters as productivity, light utilization, production and utilization of carbon dioxide, oxygen, essential nutrients, water, etc. Along with the dynamics of indicator microorganisms, these parameters may indicate normal functioning or the health of a LSS considered as a single whole. The correlation between prokaryotic cells (P) and eukaryotic cells (E), the E/P ratio, can provide a common indicator for the state of practically all links in a biological LSS (73). The point is that when environmental conditions or the health of a link changes, prokaryotes can adapt and multiply very quickly (within hours or even minutes). The associated E/P ratio will fall rapidly, more so as the numbers of eukaryotic organisms decrease because of their higher sensitivity to unfavorable conditions. Formation of a large number of spores by fungi (eukaryote) under unfavorable conditions can also be measured as a seeming increase in the P number. This can be detected easily if an automated system is used to measure the size distribution of small prokaryotes and eukaryote spores and the larger size of truly unicellular eukaryotes. In general, every location in the internal volume of an LSS can be mapped in terms of its normal state and deviations from the norm. This is one of the tasks facing those developing LSS and is particularly true of air samples, water samples, (potable and utility) and any surface within the LSS, including living macroorganisms and those of the human occupants. As to potable water and the air in living compartments, they must meet the requirements of practical hygiene. The producer link in a biological LSS carries the main load, generating oxygen and biomass for other links of the system. Fortunately, these parameters are closely related to one another, and any increase in biomass can be measured directly by measuring the release of oxygen. This is a well developed technology. Additional information on the health of this link can be obtained by examining the status of the algal

reactor and higher plants, or of both when they are both part of the biological LSS. The E/P ratio is useful in assessing the algal reactor, since the slightest decrease in the growth of algae causes a concomitant increase in the number of prokaryotic bacteria. Real-time monitoring can be performed by measuring the ratio between green pigments and yellow ones. In evaluating higher plants in the LSS, valuable information about the health of a system can be obtained by monitoring the dynamics of microorganisms living on the leaves of the higher plants (74).

5. CONCLUSION

In conclusion, we should like to emphasize, that the problem of interaction between man and microorganisms in a closed habitat is an inextricable part of the whole problem of co-existence between macro and microorganisms. Manned space flight has provided the incentive to carry out a series of excellent experiments and observations. Now, we understand that the task of maintaining the health of a human being under conditions of stress is not only a question of sanitation and hygiene, but also a problem of the ecological balance within the habitat.

Another significant regularity of these man-made ecosystems is that despite intensive exchange between links no shifts in their microflora occur, and microbial landscapes remain specific for each link, even in a Biological LSS. Thus, the ecological factor remains the dominant one in controlling microflora in the man-made ecosystem. This circumstance is essential for deciding on the methods for management and control of microbiological conditions in the closed ecosystem. The choice is either to establish sterile intrasystem barriers or to allow nearly free exchange limited only by hygienic measures precluding the seeding of living quarters with fungal spores and bacteria that may be hazardous to humans.

The results of the experiments to date conducted in closed ecological systems and in space-flight conditions argue for the ecological method of microflora control, i.e., for controlling the composition and numbers of microflora by maintaining proper environmental conditions. The alternative method – the establishment of sterile barriers between ecosystem links – is difficult to realize technically in a life support system with size and energy constraints. Sterile barriers may also be more dangerous than an ecological balance: if the barrier is broken by accident, which can hardly be avoided, the microflora, which has been developing in isolation until this moment, can change quickly and profoundly (1).

Conventionally, concerning the support of human life in closed habitat, we can divide microorganisms, acting in LSS into three groups: useful, neutral and harmful. The tasks for human beings for optimal coexistence with micro habitants are simple yet challenging: (a) to increase the activity of useful forms, (b) decrease the activity of harmful forms, and (c) not allow the neutral forms to become harmful and even to help them to play a positive role in the overall engineered ecosystem. As we mentioned above, the problem of macro and microorganisms coexistence is becoming more and more fundamental, and different types of closed ecological systems can serve as experimental testbeds to support our sustainable and healthy development. The readers are referred to the literature (75–78) for recent development in microbial ecology and LSS.

As we could see in all materials discussed in the review, microbial populations and communities are the most active links of every type of closed ecosystems designed for Earth or Space needs. The problems of macro and microorganisms' coexistence in different types of LSS, including Biosphere, are regularly discussed at different international conferences, especially COSPAR Scientific Assemblies (Section F: Life Sciences). The authors of the review are scientific organizers of F 4.1 Session (Closed Ecosystems for Earth or Space applications). Coming Scientific Assemblies (COSPAR-38, Germany, July 2010; COSPAR-39, India, July 2012) are of interest for Environmental Engineers of different qualifications.

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7
Environmental Solid-State Cultivation Processes and Bioreactors

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CONTENTS

Abstract Solid-state cultivation involves the growth of microorganisms in beds of moist solid particles that have a minimum of free water between the particles. The chapter describes environmentally-related solid-state cultivation processes. For example, some processes use substrates that are residues of agriculture, forestry, or food-processing, thereby reducing

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the environmental impact of the residue. Other processes do not use residues, but produce products that have environmental applications. Still other processes use environmental-friendly biotransformations that have the potential to replace current industrial processes. Finally, some solid-state cultivation processes can be used to remove pollutants from soil or waste streams. Typically, environmental applications of solid-state cultivation involve large-scale processing of organic solids. The current chapter addresses the design and operation of bioreactors for these processes. It shows how the various bioreactor types can be classified according to the aeration strategy, namely whether the bed of solids is forcefully aerated or not, and according to the agitation strategy, namely the frequency of mixing of the bed of solids. It discusses the current state-of-the-art in optimizing the design and operation of the various bioreactor types, showing how mathematical models that combine microbial growth kinetics and heat and mass transfer phenomena are the most powerful tools that we have available for this task. The chapter concludes by highlighting the necessity to convert current mathematical models into user-friendly computer programs that can guide design and operation decisions for large-scale solid-state cultivation bioreactors.

Key Words Bioreactor • solid state cultivation • SSF • solid organic residues • mathematical models • heat and mass transfer.

1. DEFINITION OF SOLID-STATE CULTIVATION PROCESSES

This chapter explores the applications of solid-state cultivation processes within the area of environmental biotechnology. The term solid-state cultivation is used to denote cultivation processes that involve the growth of microorganisms on particles of moist solid substrate particles, with a minimum of free water between the substrate particles. Figure 7.1 shows the essential features of solid-state cultivation systems and how this method of cultivation differs from various other cultivation processes.

Note that in solid-state cultivation systems, the inter-particle spaces (void spaces) may contain thin films and droplets of water, but the majority of water in the system is adsorbed within the moist substrate particles (1). The gas phase within the bed is continuous, with all solid surfaces that are not in contact with each other being exposed to this gas phase. This is the only cultivation method that offers such intimate contact between the microbial biomass, which develops at the surface of the substrate particles, and air. In this respect, it is quite different from various other cultivation systems (Figure 7.1b):

- *Trickling filters*: A liquid containing nutrients flows downwards through a bed of inert solids, with an upward flow of air. A microbial biofilm develops attached to the solid surfaces. Such systems are sometimes used in the aerobic treatment of wastewater. The gas phase may be largely continuous, but there is a significant amount of liquid in the spaces between the solid particles, with many solid surfaces in direct contact with a flowing liquid phase.
- *Submerged cultivation of suspended solids*: Solid particles containing nutrients are suspended in a continuous liquid phase. The liquid phase may already contain soluble nutrients, while other soluble nutrients may leach out of the solid phase into the liquid, and the microbial biomass will typically grow both in the liquid phase and as biofilms at the surface of the solid particles. The gas phase within the culture medium is discontinuous (i.e., present in the form of bubbles). Such systems include high-solids anaerobic digestion.

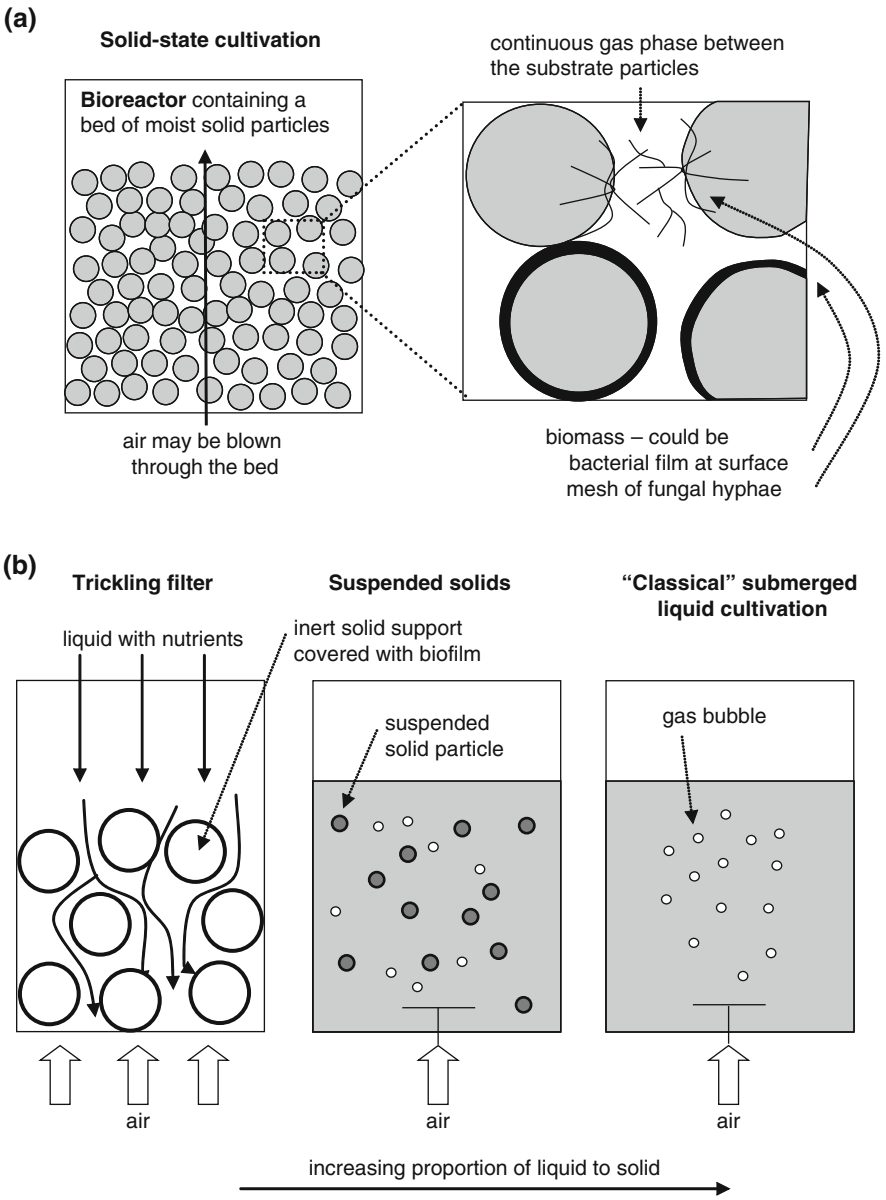

Fig. 7.1. Schematic representation of solid-state cultivation processes, highlighting the main features that define this system and distinguish it from several other cultivation systems (1). (**a**) Solid-state cultivation systems; (**b**) Other cultivation systems, from *left* to *right*: Trickling filter, Solids suspension, and "Classical" submerged liquid cultivation with soluble nutrients.

- *"Classical" submerged liquid cultivation*: the biomass is suspended within a continuous liquid phase, from which it absorbs soluble nutrients, and the gas phase is discontinuous (i.e., present in the form of bubbles).

Solid-state cultivation has a long history of applications, having been used for many hundreds of years in the first stage of soy sauce production, which involves the growth of the fungus *Aspergillus oryzae* on cooked soybeans. During this first stage, the fungus produces a cocktail of enzymes that diffuse into the soybeans. In the second stage, in which the soybeans are steeped in brine, these enzymes work slowly to digest the soybeans. Despite this long history, various technical difficulties with large-scale solid-state cultivation processes mean that submerged liquid cultivation, for which the technology developed significantly over the last half of the twentieth century, is the dominant cultivation method for biotechnological applications. However, in certain situations, solid-state cultivation has advantages over submerged liquid cultivation, especially in various environmentally related applications.

Section 2 classifies various types of environmentally related applications of solid-state cultivation, providing short descriptions of selected examples. Special emphasis is placed on processes that use bioreactors, for example, in situ bioremediation is not considered here. Since the various applications have some significant differences, Sect. 3 draws out some basic features that can be used to classify the nature of the process, and which affect bioreactor design and operation. Section 4 presents the general functions of a bioreactor. Various different bioreactors, which differ in aeration and agitation strategies, can be used for solid-state cultivation processes; Sect. 5 classifies these bioreactors into several groups based on the strategies used. Section 6 then shows the general considerations involved in the design of solid-state cultivation bioreactors and goes on to discuss these more specifically in the context of each different bioreactor type. Section 7 addresses some associated issues that must be considered during the bioreactor design process, namely decisions about the air preparation system and the system for monitoring and control of the bioreactor. This chapter ends with an evaluation of the scope for further improvement of bioreactors and bioreactor design methods.

2. CLASSIFICATION OF ENVIRONMENTAL APPLICATIONS OF SOLID-STATE CULTIVATION PROCESSES

Much of our current interest in solid-state cultivation processes is motivated by environmental concerns. It fits well with the vision proposed by Gunter Pauli in the Zero Emissions Research Initiative (ZERI proposal), in which all wastes of industrial processes are utilized as inputs to other processes, of the same or another industry. This vision requires the integration of various industries with different activities, in which the original wastes undergo a chain of transformations, with the final wastes only being returned to the environment if this can be done without any negative environmental effect (2). Solid-state cultivation has the potential to provide destinations for solid organic wastes from agricultural and forestry activities and from the food-processing industry, often producing products that have environmentally related applications. The following subsections explore several of these applications.

2.1. General Scheme for Classifying Solid-State Processes Used in Environmental Biotechnology

Solid-state cultivation finds several different environmentally related applications that can be divided into several classes based on the nature of the environmental relevance:

1. Class 1: Processes that use as substrates solid residues that would otherwise be discarded as wastes, although the products of these processes do not have environmentally related applications. Utilization of residues in these processes reduces the environmental impact that the wastes would have if they were to be discarded directly.
2. Class 2: Processes that use higher-value solid materials as substrates, for example, grains that could otherwise be used as food for humans or feed for animals, but the products of the process are either applied directly in environmental technology or, upon application, have less negative environmental impact than alternative products.
3. Class 3: Processes that both use solid residues as substrates and produce products that have environmentally related applications.
4. Class 4: Processes that either partially or totally replace other processes for biotransformation of solids, the solid-state cultivation process having a lesser negative impact on the environment than the process that it replaces.
5. Class 5: Pollutant removal processes for treating waste streams or contaminated soils.

Table 7.1 shows a selection of processes that are currently under study, classified according to this general scheme (3–44). In Sect. 2.2, examples of each type of application are briefly explored in order to highlight the environmental importance of these processes.

2.2. *Examples of Environmentally-Related Processes that Use Solid Residues*

2.2.1. Two Examples of Class 1 Processes

There are numerous examples of solid-state cultivation processes that use various solid organic residues as substrates that would otherwise simply be discarded, although the products from these processes do not have direct environmental applications (45). Two brief examples are given here (Fig. 7.2). First, wastes from the vegetable and fruit processing industry can be used for the production of a range of flavor and aroma compounds by solid-state cultivation (46). Note that in this case, a solid waste does remain after the flavor and aroma products are recovered. Second, wheat straw can be treated in a solid-state cultivation by either of two processes:

- Treatment with an actinomycete that preferentially utilizes lignin, having little cellulolytic activity, such that this process serves as a biopulping step, enabling the product to be used for paper manufacture (47).
- Treatment with a white-rot fungus, *Phanerochaete chrysosporium*, producing a product with improved digestibility to be used as an animal feed for ruminants (48).

2.2.2. An Example of a Class 2 Process

In some solid-state cultivation processes, the substrate is not actually a solid waste, but the product does have environmentally relevant applications. One example for this type of process is the production of spore-based fungal biopesticides, using grains such as wheat or rice. The environmental relevance comes from the fact that, in general, the application of a biopesticide in the environment will have a lower environmental impact than the application of a chemical pesticide. Biopesticides tend to have a much greater specificity for the pest than chemical pesticides. For example, in the case of bioinsecticides, it may be possible to target the pest insect without killing other insects, such as predatory insects; the latter are then able

Table 7.1
A selection of recent studies into environmentally related solid-state cultivation processes

Class 1: Processes that use as substrates solid residues that would otherwise be discarded as wastes, although the products of these processes do not have environmentally related applications

(a) Production of enzymes, including lignocellulolytic enzymes from *Trametes gallica* grown on wheat straw (3), pectinase from *Aspergillus niger* grown on sugar beet pulp (4), alkaline protease from *Bacillus* sp. grown on wheat bran and lentil husk (5), α-galactosidase from *Penicillium* sp. grown on soybean meal and beet pulp (6), α-amylase from *Aspergillus oryzae* grown on coconut oil cake (7), cellulase from *Trichoderma* sp. grown on wheat straw (8), lipase from *Penicillium simplicissimum* grown on soy cake (9), laccase by *Coriolus versicolor* grown on rice bran (10), and chitinase by *Trichoderma harzianum* and *Trichoderma longibrachiatum* grown on wheat bran supplemented with colloidal chitin (11)
(b) Production of secondary metabolites, including griseofulvin from *Penicillium griseofulvum* grown on rice bran (12) and tetracycline from *Streptomyces* spp. grown on groundnut shells, corncob, corn pomace and cassava peels (13)
(c) Production of p-coumaric acid (p-CA) and ferulic acid (FA) from *Sporotrichum thermophile* grown on corn cobs (14)
(d) Production of the aroma compound decalactone by various fungi grown on olive and castor oil press cakes (15)
(e) Production of single-cell protein for use as animal feed, growing various microorganisms on citrus residues and cotton stalk (16)
(f) Growth of *Aspergillus niger* on palm kernel cake to produce an animal feed with improved digestibility (17)
(g) Improving potential commercial value of guava wastes by increasing the phenolic antioxidant content using *Rhizopus oligosporus* (18)

Class 2: Processes that use higher-value solid materials as substrates but the products of the process are either applied directly in environmental technology or, upon application, have less negative environmental impact than alternative products

(a) Production of biological control agents, including *Epicoccum nigrum* grown on a vermiculite-based substrate (19) and *Coniothyrium minitans* grown on wheat grains (20)
(b) Production of phytase, which, when added to animal feeds, improves phosphorus absorption and therefore reduces phosphorus excretion in the feces, by *Aspergillus niger* grown on 1upin flour (21)

Class 3: Processes that both use solid residues as substrates and produce products that have environmentally related applications

(a) Production of xylanase, for use in biobleaching of wood pulp, by *Thermoascus aurantiacus* grown on sugar cane bagasse (22) and by *Bacillus coagulans* grown on soybean residue (23)
(b) Production of xylanase, for the enzymatic hydrolysis of corncob and sugarcane bagasse in the production of biofuels, by *Thermomyces lanuginosus* (24)
(c) Production of a biofertilizer by growing *Azotobacter vinelandii* on technical lignin, derived from the Kraft pulping process (25)
(d) Production of inocula for composting processes using various ligno-cellulolytic fungi grown on pepper plant wastes and almond shell residues (26)

(Continued)

Table 7.1
(Continued)

(e) Production of biocontrol agents, including *Beauveria bassiana* grown on rice straw and wheat bran (27) and *Trichoderma harzianum* grown on dried banana leaf (28)
(f) Production of enzymes, for use in decolorization of dyes, by *Pleurotus ostreatus* grown on wheat straw (29), by *Funalia trogii* grown on wheat bran and soybean residue (30), by *Lentinula edodes* grown on corn cob (31) and by *Trametes versicolor* and *Trametes hirsuta* grown on barley bran, a waste from the brewing industry (32)

Class 4: Processes that either partially or totally replace other processes for biotransformation of solids, the solid-state cultivation process having a lesser negative impact on the environment than the process that it replaces

(a) Use of wood-inhabiting basidiomycetes for biokraft pulping of softwood chips (33) and of wood-rotting polypores and corticioid fungi for the biopulping of Norway spruce wood (34)
(b) Delignification of wheat straw by *Streptomyces cyaneus* as a pretreatment to produce handsheets for the pulp and paper industry (35)
(c) Biobleaching of hardwood kraft pulp by the white-rot fungi *Phaneroehaete sordida* and *P. chrysosporium* (36)

Class 5: Pollutant removal processes for treating waste streams or contaminated soils

(a) Bioremediation of solids contaminated with diethylhexyl phthalate (37) and hydrocarbon (38) in reactors
(b) Benzopyrene removal from soil by *Phanerochaete chrysosporium* by mixing sugarcane bagasse and pine sawdust into the soil (39)
(c) Decolorisation of industrial dyes by *Pleurotus pulmonarius* grown on corn cobs (40)
(d) Fungal biofilters for removal of hexane vapor (41) and other solvents (42) from waste gas streams
(e) Solid-state bioconversion process for domestic wastewater sludge as an environmental-friendly disposal technique, using two mixed fungal cultures, *Trichoderma harzianum* with *Phanerochaete chrysosporium* and *T. harzianum* with *Mucor hiemalis* and two bulking materials, sawdust and rice straw (43)
(f) Biodegradation of chromium shavings in tannery waste by *Aspergillus carbonarius* grown under SSF conditions, permitting recovery, and reuse of chromium (44)

to continue their role in keeping the pest population in check. In addition, bioinsecticides tend not to have the same levels of general toxicity to higher animals as chemical insecticides.

Several biopesticides, especially those based on fungal spores, are better produced in solid-state cultivation than in submerged liquid cultivation. For example, some fungi only sporulate when they grow in the environment provided by solid-state cultivation. Or, if they sporulate in both solid-state cultivation and submerged liquid cultivation, the spore yields are much higher in solid-state cultivation. Another important consideration is the robustness of the spores, since this affects their survival upon application in the field. A greater robustness increases the durability of the spores and, therefore, they remain infective for longer periods. Some fungi produce various different types of spores, with different degrees of resistance. As a general rule, less robust spore types are produced in submerged liquid cultivation, while

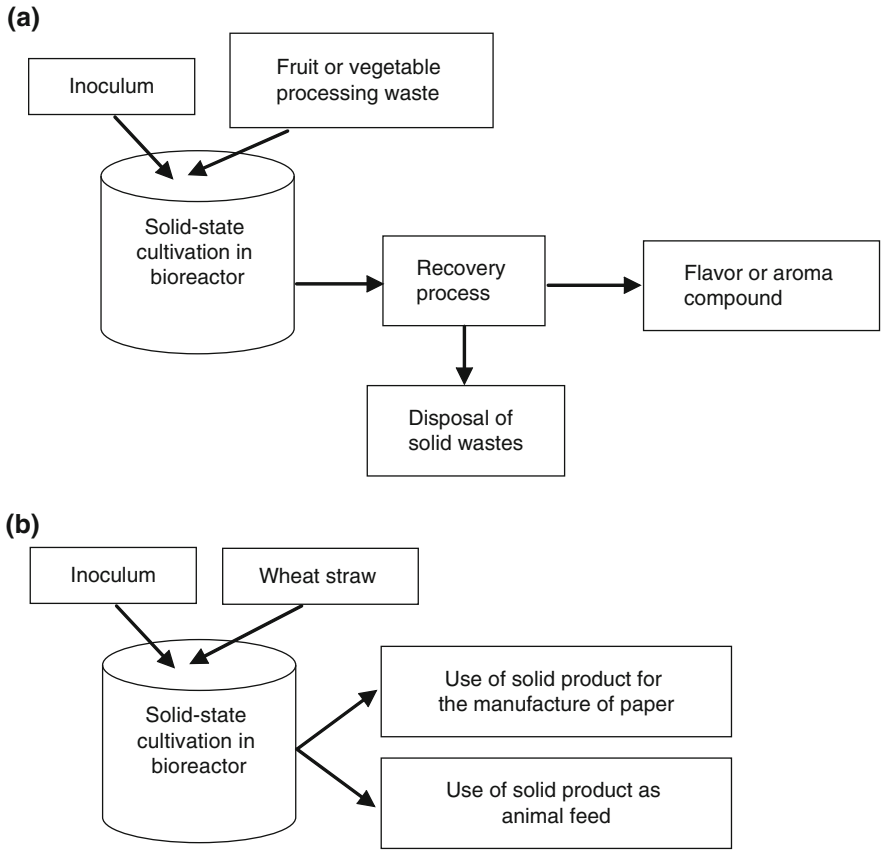

Fig. 7.2. Two examples of solid-state cultivation processes that use solid wastes, although the products do not have environmentally related applications (46–48). (**a**) Production of flavor and aroma compounds from solid fruit and vegetable wastes. In this case, the solid-state cultivation process generates a solid waste. (**b**) Utilization of wheat straw. The solid product from the process is not discarded but rather used either for the manufacture of paper or as an animal feed.

more robust types are produced in solid-state cultivation. A general scheme for the production of fungal spores as biopesticides is shown in Fig. 7.3. As a specific example, spores of the fungus *Metarhizium anisopliae* are produced on rice, as a bioinsecticide against the greyback canegrub, a pest of sugar cane that causes damages worth over $5 million in Australia (49).

2.2.3. Two Examples of Class 3 Processes

One example of a process that uses solid residues as raw material and also generates products that have environmentally related applications is the use of babassu oil cake, a solid residue generated during the industrial production of babassu oil, for the cultivation of the fungus *Penicillium restrictum* and the production of an enzymatic pool rich in lipases, proteases, and amylases. This enzymatic pool can be used to hydrolyze oily wastewaters prior to aerobic or anaerobic treatment (50).

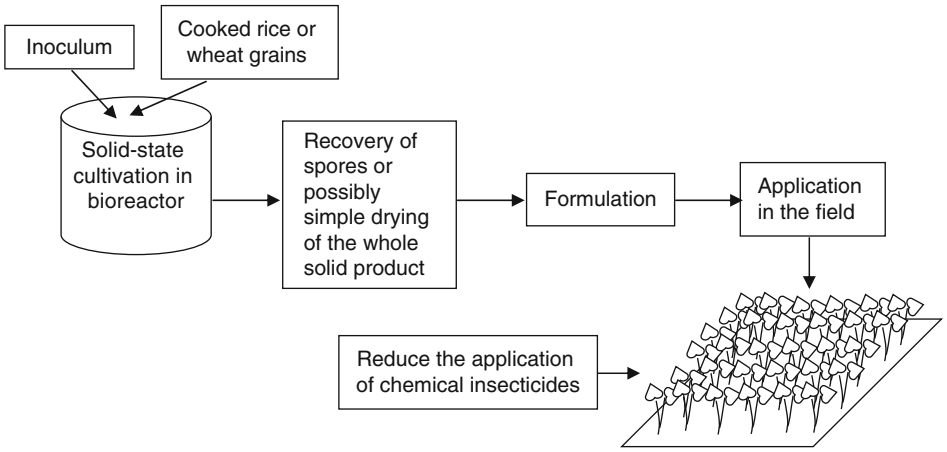

Fig. 7.3. An example of a solid-state cultivation process that uses a higher value solid substrate, but produces a product with environmental benefits (49). In this example, spores of a fungus are produced for application as a bioinsecticide.

Wastewaters with high oil and grease contents, such as effluents of dairy and slaughter plants, present problems to biological treatment systems due to the low biodegradation rate of fats. In anaerobic reactors, fats may cause clogging of the reactor, develop unpleasant odors, cause sludge flotation, and limit the transport of soluble substrates to the biomass, thus reducing the efficiency of removal of COD and BOD. In aerobic systems, the presence of fats leads to the development of filamentous microorganisms of the genera *Sphaerotilus*, *Thiothrix*, *Beggiatoa*, *Nocardia,* and *Microthrix*, which cause an undesirable phenomenon known as "bulking" (51). Furthermore, the presence of oil and grease in aerobic reactors causes the formation of stable foams on the surface of the aeration tanks and generates pellets inside the sludge flocs, hindering biomass flocculation and sedimentation.

Enzymatic hydrolysis of oily wastewaters prior to the biological treatment stages can solve these problems (50). The necessary enzymes can be produced by cultivating fungi of the genus *Penicillium* on babassu oil cake. One to two days after inoculation with fungal spores, fermented solids with high titers of hydrolytic enzymes such as lipases, proteases, and amylases are obtained (52). As shown in Figure 7.4a, the enzymes can be extracted to a liquid buffer and then separated from the solids, resulting in a "liquid enzyme product," which can be added to an oily effluent prior to biological treatment. Alternatively, the fermented solids can be simply dried under mild temperatures and the "solid enzymatic product" that is obtained can be directly added to oily wastewaters, at concentrations of 1 g/L or higher, prior to biological treatment (50).

The benefits of the enzymatic pretreatment step have been demonstrated for both the anaerobic and aerobic treatment of dairy wastewaters, containing elevated fat and grease levels (800 mg/L). Pretreatment of this wastewater with the solid enzyme product increased the efficiency of the subsequent treatment in either an upflow anaerobic sludge blanket reactor (53) or an aerobic batch activated-sludge bioreactor (54).

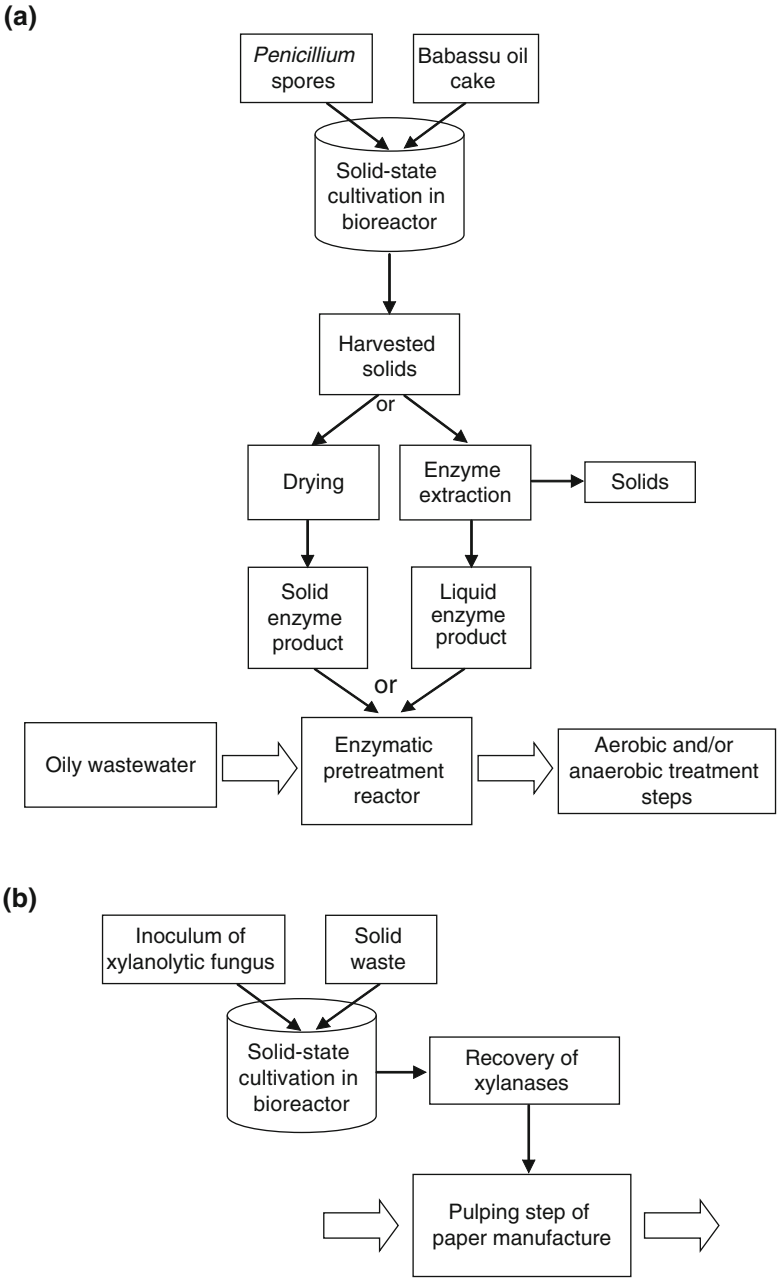

Fig. 7.4. Two examples of solid-state cultivation processes that both use a solid residue and produce a product with environmental applications (50, 56). (**a**) Simplified scheme of the solid-state cultivation of *Penicillium restrictum* on babassu oil cake to produce an enzyme cocktail in either liquid or solid form, and the application of this cocktail in the hydrolysis of oily wastewaters prior to either aerobic or anaerobic treatment. (**b**) Production of xylanases for use in biobleaching.

Comparing the effluent from the anaerobic digester with the enzymatic pretreatment system to one without pretreatment (53), the pretreatment step gave oil and grease removal efficiencies as high as 90% and:

- COD removal was increased from below 50% to as much as 90%;
- Turbidity was reduced by 75% (from 760 nephelometric turbidity units to below 200);
- Volatile suspended solids were reduced by 90% (from 940 mg/L to below 100 mg/L).

In the case of an aerobic batch activated-sludge system (54):

- As the oil and grease concentration in the dairy wastewater increased (400, 600, and 800 mg/L), the COD removal efficiency in the activated-sludge system without pretreatment decreased (86%, 75%, and 0%, respectively) while with the same effluent but with the enzymatic pretreatment step, COD removal efficiency in the activated-sludge bioreactor was maintained at high levels (93%, 92%, and 82%, respectively).
- At an oil and grease concentration of 800 mg/L, the effluent from the activated-sludge system without pretreatment had final volatile suspended solids values ten times higher (2,225 mg/L) than the effluent from the system fed with pre-hydrolyzed effluent (200 mg/L).

Beyond the technical aspects, economic issues must also be taken into account when considering the use of enzymes in wastewater treatment. In this context, the use of solid-state cultivation to produce hydrolytic enzymes is more economical than the use of submerged cultivation techniques. Using *Penicillium restrictum* and babassu oil cake for the production by solid-state cultivation of a lipolytic "liquid enzyme product" and considering a production scale of 100 m^3 per year, the total capital investment needed is 78% lower than that needed for a submerged liquid cultivation process and the unitary production cost for solid-state cultivation is 47% lower than the market price of an existing liquid lipolytic product. The solid-state cultivation process is very attractive from an economic point of view, with a payback time of 1.5 years, a return on investment of 68% and an internal return rate of 62% for a 5-year-project life (55). These economic advantages of solid-state cultivation for the production of hydrolytic enzymes are mainly due to the low capital investment and to the very cheap raw material used. Considering that products for environmental applications should be extremely cheap, the direct addition of the dried fermented solids to the effluents is a further economic advantage of the process, since no operations concerning enzyme extraction and solid-liquid separation are needed.

A second process that both uses waste residues and produces an environmentally relevant product is the production of cellulase-free xylanase preparations for use in biobleaching of kraft pulp in paper manufacture. The action of the xylanases facilitates the extractability of lignins by conventional bleaching chemicals, resulting in a reduction of the consumption of bleaching chemicals by 20–30%. The use of these chemicals generates toxic byproducts that are mutagenic and persist in the environment, and this reduced consumption leads to a reduction of organic halogen loads in the effluents by 15–20% (56).

2.2.4. An Example of a Class 4 Process

An example of a process for the biotransformation of solids that partially replaces processes that have a more negative impact on the environment is biopulping. Biopulping involves the

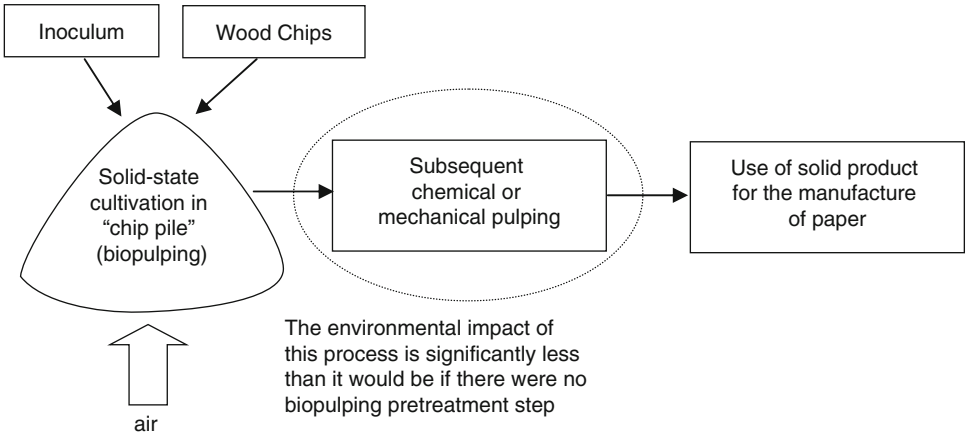

Fig. 7.5. Biopulping of wood chips, an example of a solid-state cultivation process that partially replaces the less environmentally-friendly chemical alternative (57).

pretreatment of wood chips, prior to either mechanical or chemical pulping, with lignin-degrading fungi such as *Ceriporiopsis subvermispora*. This biological pretreatment can lead to energy savings of 40–50% (57). Due to the large volumes that need to be processed, in-vessel treatment would be too expensive; rather the process is carried out in aerated "chip piles" (Fig. 7.5).

2.2.5. Two Examples of Class 5 Processes

Two important examples of solid-state cultivation processes for pollutant removal are biofilters and ex situ bioremediation.

Many industries produce waste gases that contain toxic or odorous substances. Various processes are available to reduce or even eliminate these substances. Biofiltration, in which the gas is passed through a bed of solids on which a mixed microbial population grows and consumes volatile organic compounds from the gas phase (Figure 7.6a), has some advantages over other purification techniques (58). The pollutants are not simply adsorbed and held in another phase, as occurs in adsorption processes, but rather are converted into harmless oxidation products. Moreover, the investments and operational costs of biofilters are cheap when compared to chemical or catalytic oxidation. Recently, the applications of biofiltration have been widely extended due to improvements in filter technology and more severe regulations on the emission of gases.

As an example of ex-situ bioremediation, soil contaminated with petroleum can be removed from the contaminated site, mixed with nutrients and structuring agents, and heaped in piles, up to 3 m high, 5 m wide and 30 m long. These piles are made on the top of a network of air pipes that, in turn, rest on top of an impermeable tarpaulin, in order to prevent the leaching of pollutants into the ground at the treatment site. Treatment times may be as long as 3–12 months, with periodic addition of nutrients. The degree of removal of pollutants depends on

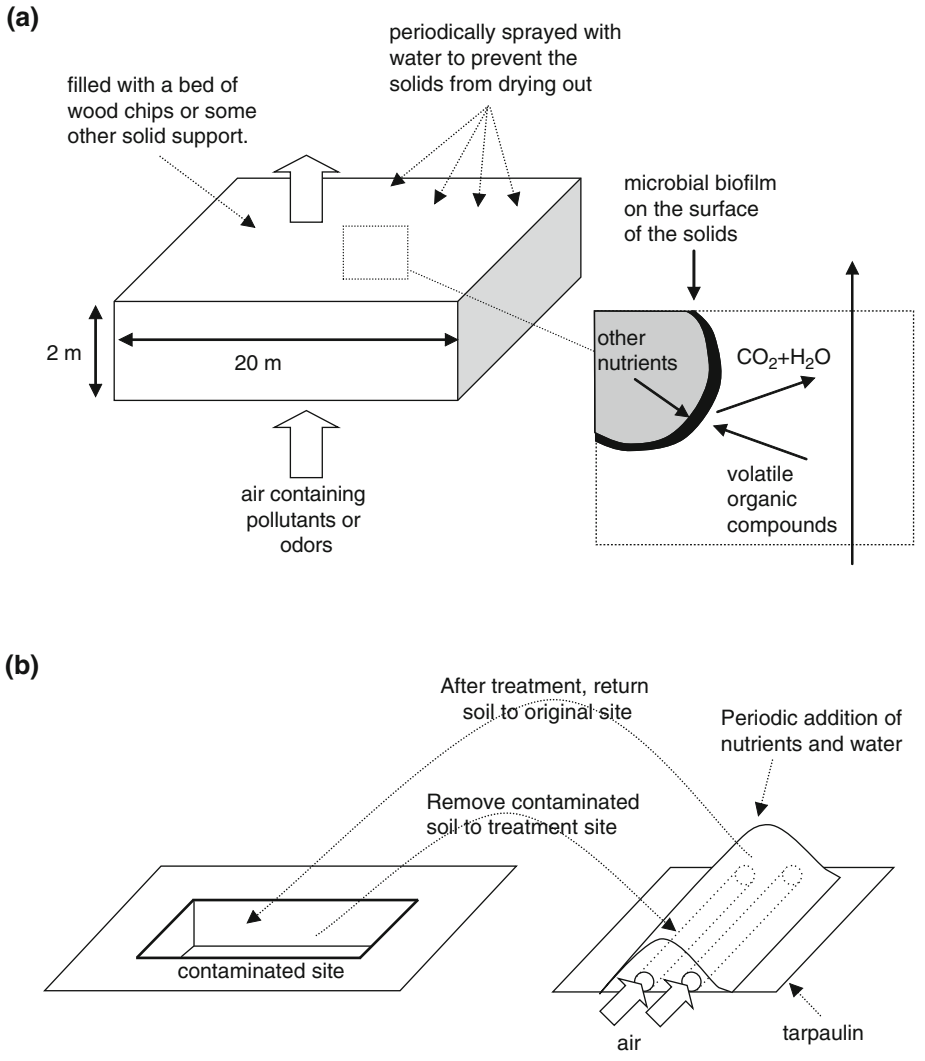

Fig. 7.6. Two examples of solid-state cultivation processes that remove pollutants (58, 59). (**a**) A biofilter used to remove volatile organic compounds that are either pollutants or odors from a waste gas stream. (**b**) Bioremediation of contaminated soil by the "biopile" method.

the efficiency of oxygenation of the soil, and is also affected by temperature, which is not controlled (59).

3. CLASSIFICATION OF PROCESS TYPES

It is possible to analyze the above processes, and other environmentally related applications of solid-state cultivation, according to several different criteria. Each of these criteria has implications for how the bioreactor for the process will be designed and operated. Processes may:

- *Be aerobic or anaerobic*. The majority of solid-state cultivation processes involve the aerobic growth of organisms. However, some environmentally related processes do involve anaerobic growth, such as solid-phase anaerobic digestion and ethanol production from solid residues. The need to supply O_2 to the bioreactor is obviously an important design consideration.
- *Be operated in batch or continuous mode*. The solid nature of the substrate means that the well-mixed continuous culture method that is used in submerged liquid culture is not appropriate, since newly-added substrate particles are not immediately colonized. However, continuous culture of the plug-flow type is feasible, and has been used for an initial composting step in the stabilization of municipal solid waste. Figure 7.7a highlights the differences between batch and continuous operation.
- *Involve the use of pure cultures, defined mixed cultures, or "selected microflora."* For example, the production of the lipolytic enzyme mixture by *Penicillium restrictum* (described in Sect. 2.2.3) involves a pure culture whereas composting processes involve an undefined microflora selected from the original microflora of the material being composted. The use of

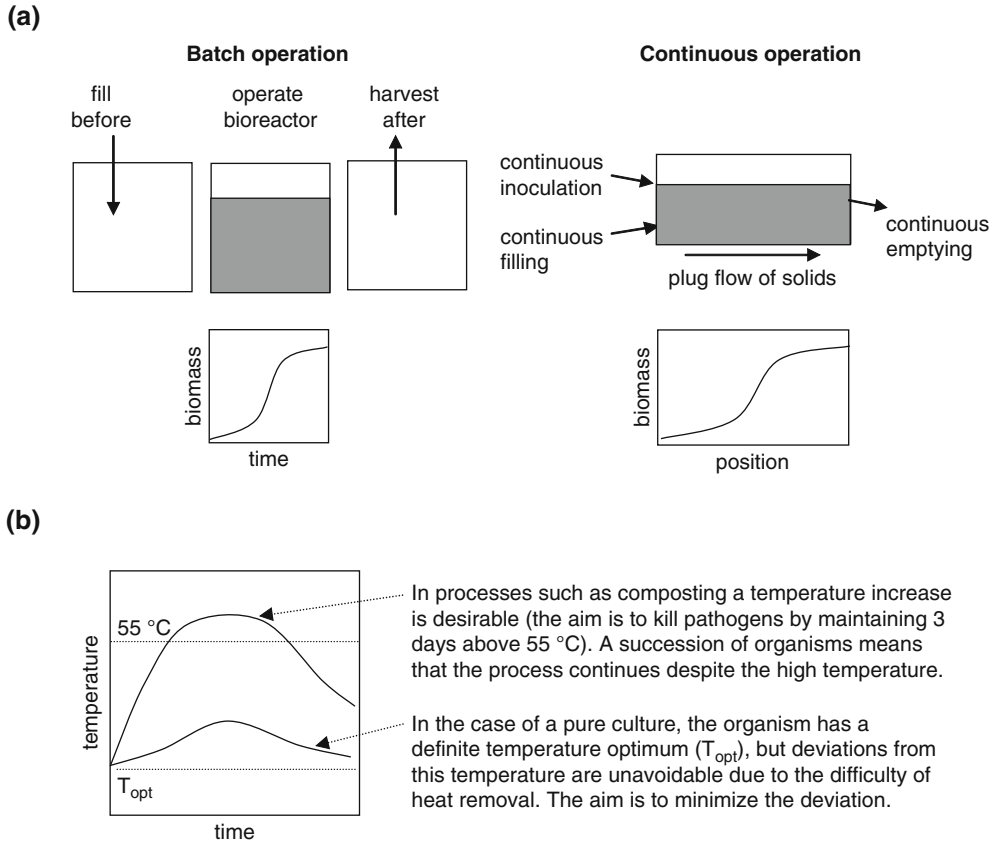

Fig. 7.7. Two of the aspects of bioreactor operation that have implications for process design (60). (**a**) The difference between batch cultivation and continuous operation in the plug-flow mode. (**b**) In some processes, it is highly desirable to control the temperature in the substrate bed, while in others, it is desirable for the temperature to reach high values.

pure cultures is often correlated with a need to design the bioreactor so as to minimize the entry of contaminants during the cultivation. On the other hand, if the culture conditions select for a particular microflora, there may be no particular need to prevent the entry of contaminants.

- *Require good temperature control or either tolerate or need significant variations in the bed temperature during the process*. For many processes, especially those that use pure cultures or defined mixed cultures, there is an optimum temperature for the process (Figure 7.7b) and the bed temperature should be maintained at this optimum value or as close to it as possible. On the other hand, in composting, a desired succession of microbial types accompanies temporal variations in the temperature. In fact, in order to kill pathogens, it is desirable to maintain temperatures above 55°C for several days.
- *Require a high degree of asepsis or not be particularly demanding in terms of asepsis*. Depending on the process conditions and the microorganisms used, solid-state cultivation processes may or may not be resistant to contamination. For example, processes operated at water activities below 0.98 with fungi such as *Penicillium* or *Aspergillus* are typically resistant to contamination by bacteria. If started with a vigorous inoculum, it is often not crucial to go to great lengths to prevent contamination during the process. In some cases, the process organism might be an opportunistic pathogen, produce spores that can trigger allergy in process workers, or present an environmental risk. In these cases, it is desirable to prevent release of the process organism from the bioreactor. These considerations will affect the necessity to include aseptic seals and filters in the bioreactor design.
- *Require to be operated by relatively unskilled workers.* Some processes will be operated at a large-scale central facility wherein relatively skilled labor will be available, allowing the implementation of more complex bioreactor types and more sophisticated technology. However, some processes will be applied in domestic or local industries, requiring more simple technologies, such as cultivation in trays, pots, or bags.

4. THE FUNCTIONS THAT THE SOLID-STATE CULTIVATION BIOREACTOR MUST FULFILL

This section addresses the design and operation of bioreactors for the various processes discussed in Sect. 2. Clearly, the various process classifications presented in Sect. 3 will affect the specific design and operational features of the bioreactor and, as a result, several quite different bioreactor types are used in solid-state cultivation processes. The following sections will outline the various bioreactor types available and the important considerations necessary in order to design and operate them efficiently. The current section will outline in general terms the functions that a bioreactor may have to fulfill. Bioreactors for specific processes may not have all of the features mentioned here, but these specific differences will be made clearer when the various different bioreactors are presented in the later sections.

When cultivating microorganisms in a bioreactor, the aim is to maintain optimal conditions for growth and product formation or for the execution of a desired metabolic activity. The ability to control the conditions in the bed depends on the operating variables that can be manipulated, such as air flow rates and mixing regimes, and on the effectiveness of heat and mass transport phenomena within the bed. Both the available operating variables and the effectiveness of heat and mass transfer depend on the particular bioreactor type used. It is often either impossible or prohibitively expensive to maintain optimal conditions across the

whole bed during the whole cultivation. In this case, the aim becomes to maintain near-optimal conditions over as much of the bed and during as much of the cultivation as possible.

In the majority of solid-state cultivation processes, two key process variables that need to be controlled are the bed temperature and the moisture content. For aerobic processes, it is also necessary to ensure high O_2 concentrations within the void spaces in order to provide an adequate O_2 supply to the particle surface. These variables are important because it is possible to influence them through manipulation of the operating variables. Of course, there are many other variables that affect the growth process. However, "intra-particle variables", such as the dissolved O_2 concentration, the nutrient concentration, and the pH, are typically only affected very indirectly by manipulations of operating variables such as the air flow rate. In fact, our inability to undertake control actions to maintain intra-particle variables at optimum values is a limitation intrinsic to the solid-state cultivation method.

For some processes, there is a single set of conditions that should be maintained throughout the cultivation. For other processes, the optimal conditions change during the process, in which case the aim is to provide the optimal temporal profile. As an example, in producing fungal spores for use as biopesticides, it may be desirable to have higher water activities within the bed during the early stages in order to favor growth, but to have lower water activities in the latter stages in order to favor spore formation. As another example, in composting, it is desirable that the bed temperature should initially increase rapidly, reach and then maintain values of over 55°C for several days before declining again.

In order to allow control of the key process variables, and to fulfill various other functions that a bioreactor has, the bioreactor may need to be designed to (Fig. 7.8):

- *Hold the substrate.* The bioreactor size will be determined on the basis of calculations of the required throughput and the expected average productivity per unit volume of the bioreactor. The size will affect the selection of the material for construction of the bioreactor, based on the required mechanical strength.
- *Enable sterilization*. Sterilization will be most important for those processes in which pure cultures or defined mixed cultures are used. If sterilization is necessary, it must be decided whether the bioreactor and substrate are to be sterilized separately, or the substrate is to be sterilized within the bioreactor. If the substrate is to be sterilized within the bioreactor, the bioreactor must be designed in such a way as to enable as uniform a sterilization of the solid bed as possible and may need to resist high internal pressures. In cases in which the process conditions are highly selective for the process organism, it may be possible simply to cook the substrate rather than to sterilize it.
- *Provide a barrier against contamination or contain the process organism.* These considerations will depend on whether pure or defined-mixed cultures are used, the selectivity of the process conditions for the process organism and the health or environmental risks presented by the process organism.
- *Enable the removal of metabolic heat into cooling water.* Adequate removal of the metabolic heat generated by the process organism is typically the central challenge in the design of solid-state cultivation bioreactors. It may be desirable for the bioreactor to have water jackets or internal heat transfer surfaces to remove heat, although the effectiveness of this method of heat removal is limited by the relatively poor conductivity of solids.
- *Enable air to be supplied to the substrate bed*. Many solid-state cultivation processes are aerobic, and in such processes O_2 must reach the organism growing at the surface of the substrate

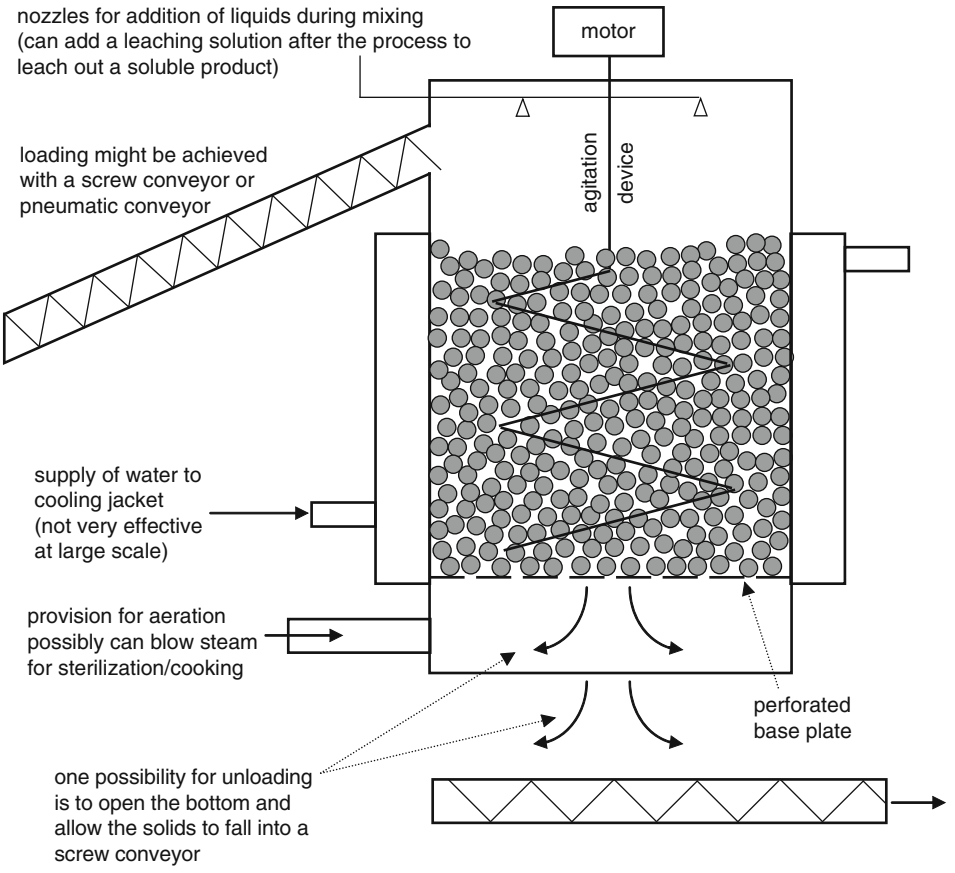

Fig. 7.8. A schematic diagram of a bioreactor for solid-state cultivation, showing various features that it may need (60). Note that some bioreactors appear quite different and not all bioreactors will incorporate all the features shown here.

particles. Beyond this, gas supplied to the substrate bed may play an important role in removal of heat from the bed by convective and evaporative cooling. Two aeration strategies are possible. Air may be blown across the bed surface, without forcing it to pass through the bed, or, alternatively, air may be forcefully blown through the bed. As a general principle, aeration will be more effective as contact increases between the supplied air and the bed. In other words, it is most effective to blow air through the bed; this should be done in such a way as to ensure an even distribution of the air, avoiding preferential flow through cracks in the bed or between the bed and the bioreactor wall.

- *Enable the contents of the bed to be mixed.* As a general principle, mixing promotes uniformity of conditions within the bed, this being desirable in order to obtain uniformity of the microbial processes that occur. However, it may also cause undesirable damage to the process organism, especially in processes in which fungi are used. Three mixing regimes are possible. The substrate bed may be completely static, it may be left static part of the time but suffer intermittent mixing, or it may be continuously mixed. The mixing of a bed of solid particles is a challenging task

that can be greatly affected by the properties of the substrate such as the stickiness, mechanical rigidity, and size and shape of the particles.

- *Facilitate the solids loading and unloading steps*. The design of the bioreactor must take into account the loading and unloading operations. Note that it is not as easy to move solids around as it is to pump liquids into a vessel and drain them out. The difficulty of these solids handling processes increases as the scale of the process increases, that is, as the volume of the solids to be processed increases. The manner in which the bioreactor design provides for the loading and unloading steps will also depend on whether the process is to be operated in batch or continuous mode.
- *Enable the addition of liquids during the cultivation.* In some cases, it might be desirable to add water to the solids during the process to avoid them drying out. Alternatively, it might be necessary to add nutrient solutions or pH-correcting solutions. The uniform distribution of such additions will be important, and, as a general principle, such additions will best be made by misting the solution onto the substrate bed as it is being mixed.
- *Take part in downstream processing operations*. For example, product drying or extraction by leaching of a soluble product could be done in situ within a bioreactor.

5. CLASSIFICATION OF BIOREACTORS USED IN ENVIRONMENTALLY-RELATED SOLID-STATE CULTIVATION PROCESSES

Two key design considerations for bioreactor operations are the aeration and agitation schemes, since these have the greatest effect on the key process variables, namely the bed temperature and water content and the void space O_2 concentration. It is useful to classify bioreactors in groups in terms of how they are aerated and agitated, because of the many similarities in operating variables that can be manipulated to optimize bioreactor performance as well as the design strategies used (60). Four basic groups can be identified:

- Group I – Bioreactors that are neither agitated nor forcefully aerated
- Group II – Bioreactors that are not agitated but are forcefully aerated
- Group III – Bioreactors that are agitated but are not forcefully aerated
- Group IV – Bioreactors that are both agitated and forcefully aerated

The basic features of the bioreactors in these various groups are outlined in the following subsections. Note that the distinction in terms of the agitation regime is not clear-cut. Intermittently mixed bioreactors might either be grouped with unmixed bioreactors or with continuously mixed bioreactors, depending on the frequency of the mixing events. In this context, "frequently mixed" means that the mixing events are sufficiently frequent for the substrate not to become anaerobic for long periods.

5.1. Group I Bioreactors: Not Aerated Forcefully and Not-Mixed

In Group I bioreactors, air is not blown forcefully through the bed but rather is circulated around the bed surfaces. The substrate bed either remains static during the whole process or is mixed only very infrequently, of the order of once or twice per day. Figure 7.9 shows several types of bioreactors that are classified within this group. The classical process is carried out in

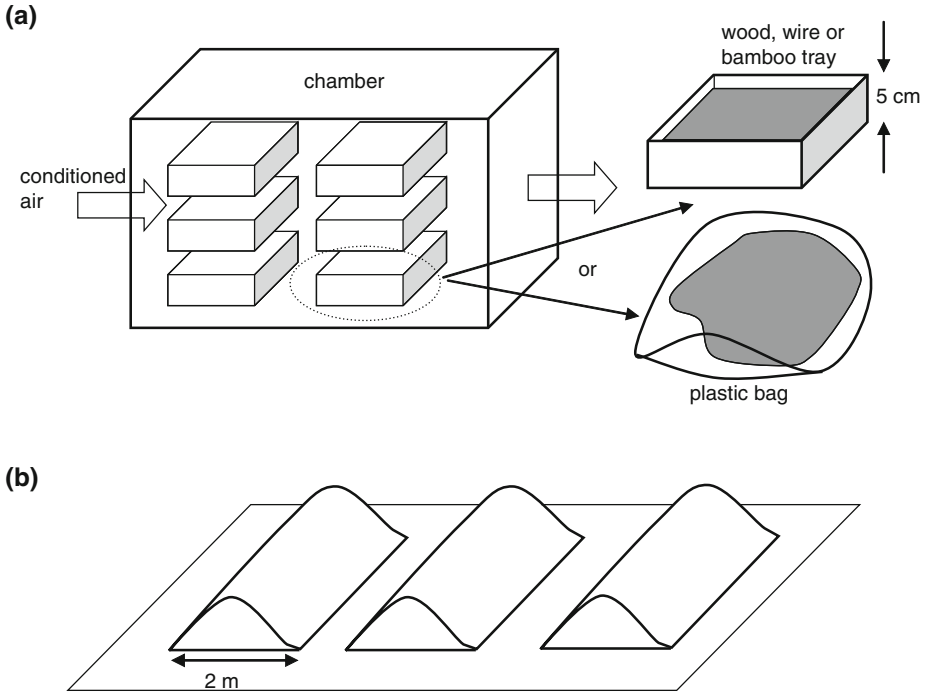

Fig. 7.9. Various bioreactors classified in Group I, that is, bioreactors that are neither forcefully aerated nor mixed (60). (**a**) Tray-type bioreactors. (**b**) Unaerated windrows for composting.

trays, which may be made of wood, bamboo, plastic, or metal. Some processes are carried out in plastic bags. The plastic allows for the exchange of O_2 and CO_2 but minimizes the exchange of water and does not allow the entry of contaminants. These trays or bags are incubated within a chamber, which, depending on the scale of the process, might actually be a room.

Tray bioreactors are appropriate for situations in which relatively small volumes are to be produced with relatively simple technology. For example, tray-type cultivations can be used for the production of biopesticides by local producers. At a very large scale, those composting processes that are based on the use of unaerated, infrequently turned windrows would be classified within this group of bioreactors.

5.2. Group II Bioreactors: Aerated Forcefully but Not-Mixed

The general feature of Group II bioreactors is that the substrate bed is forcefully aerated but remains static; in some processes, it remains static for the whole cultivation, while in others, it may be mixed infrequently, on the order of once or twice per day. The consequence is that the performance of this bioreactor is highly dependent on convective flow phenomena, which, as described later, means that there is a tendency to establish axial gradients (that is, gradients along the direction of air flow).

Typically, in this type of bioreactor, the substrate bed sits on a perforated base and air is forced through the bed. It is more common for the air to enter at the bottom, although it is also

possible to have the air entering at the top (Figure 7.10). A very simple large-scale application of this type of bioreactor is that of forcefully aerated windrows used in composting. The so-called Zymotis bioreactor is an interesting variant of this bioreactor type, in which heat transfer plates are inserted into the bed (61). This bioreactor has been used for the production

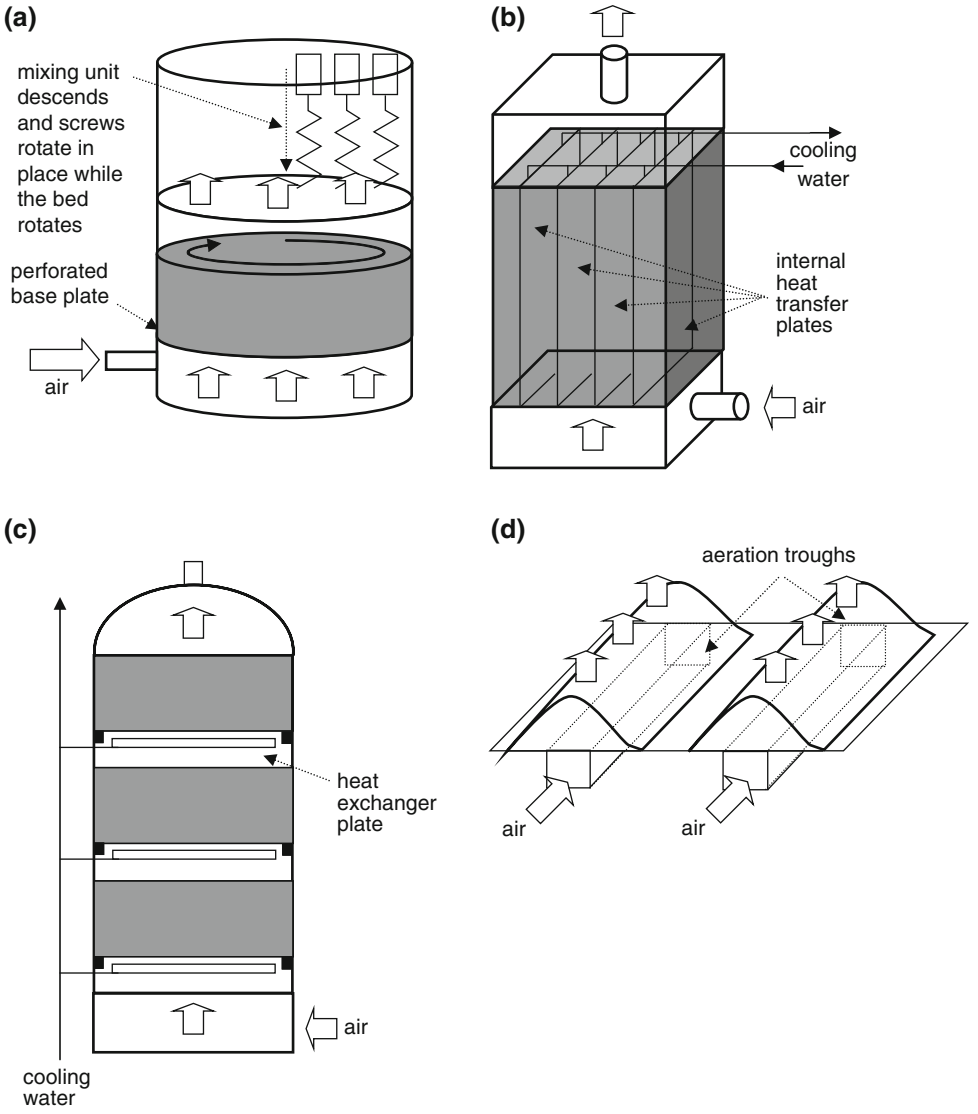

Fig. 7.10. Various bioreactors classified in Group II, that is, bioreactors that are forcefully aerated but not-mixed, or, if mixed, mixed only very infrequently (60–62). (**a**) Traditional design, but with provision for infrequent mixing events. (**b**) The Zymotis bioreactor, which has heat transfer plates within the substrate bed. (**c**) The bioreactor patented by Prophyta, which has multiple beds with a heat transfer plate under each bed. (**d**) Aerated windrows used in composting.

of cellulases using a mixture of two residues (sugar cane bagasse and wheat bran in 80:20 ratio by weight) (61). Another design, recently patented, the so-called Prophyta design, consists of a stack of packed beds with heat exchanger plates at the bottom of each bed and has been used for the production of biopesticides (62). Various designs used for the production of soy sauce *koji*, a non-environmentally related application, could be adapted to environmentally related processes (63).

5.3. Group III Bioreactors: Not Aerated Forcefully but Mixed

The general feature of Group III bioreactors is that the substrate bed is continuously mixed, but air is not blown forcefully through the bed; rather it is blown past the bed surface. Note that, in some cases, the mixing may be intermittent, but occurring at frequent intervals. Most frequently, such bioreactors consist of drums with their central axis being either horizontal or slightly inclined to the horizontal, are partially filled with substrate and have air blown through the headspace. For such bioreactors, there are two mixing options (Figure 7.11). In the "rotating drum" option, the bioreactor body is rotated to provide the mixing action, this possibly being facilitated by internal lifters. In the "stirred drum" option, the bioreactor body remains stationary and the substrate bed is mixed by paddles or scrapers that rotate around a central shaft.

In some cases, attempts have been made to inject air into the substrate, for example, through small holes in the end of each paddle in a stirred bed (64). However, unless provisions are made to distribute this air over a wide area, the effect of this aeration will be limited to a relatively small proportion of the substrate in the bed and such a bioreactor will operate more like a Group III bioreactor than the well-mixed, forcefully aerated bioreactors of Group IV.

Group III bioreactors have been used in various environmentally related applications of solid-state cultivation. Large composters, up to 3.5 m diameter and 45 m in length, have been used for composting of municipal solid-wastes (65). In some cases, compost is produced to be sold as a soil conditioner while in other cases composting is used simply to stabilize the wastes before landfilling, in order to reduce the environmental impact of the landfill.

5.4. Group IV Bioreactors: Aerated Forcefully and Mixed

Group IV bioreactors are both forcefully aerated and continuously mixed, or, if intermittently mixed, they are mixed every hour or two, or more frequently still. Various bioreactors that fall into this group are shown in Figure 7.12. In air-solid fluidized beds, the mixing action is not mechanical (Figure 7.12a). Such a bioreactor of 8,000 L working volume has been used to produce amylases and proteases from wheat bran powder (66). Air-solid fluidized beds have not yet been used for environmentally related applications, but could be expected to have reasonably high operating costs due to the high aeration rates needed to fluidize the bed. In addition, uniform fluidization might be difficult to achieve if heterogeneous waste materials are used as the substrate. In other designs, the bed is mixed mechanically (Figs. 7.12b–d). In some designs, the mixer itself operates continuously, but only mixes a relatively small portion of the bed at any one time and must travel along the bed in order to mix the whole bed, such that any particular region of the bed is mixed only intermittently (Figs. 7.12c, d).

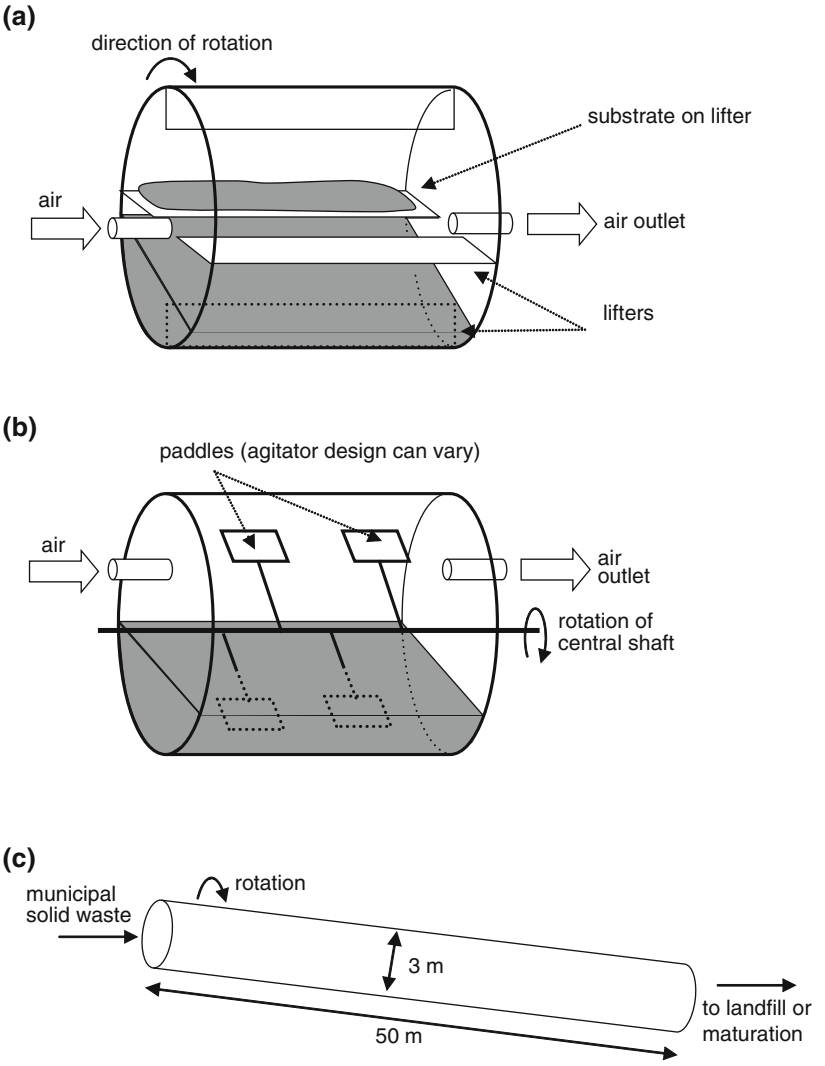

Fig. 7.11. Various bioreactors classified in Group III, that is, bioreactors in which the bed is not forcefully aerated, but are mixed either continuously or frequently (60). (**a**) Rotating drum bioreactor. (**b**) Stirred-drum bioreactor. (**c**) Eweson or Dano-type composters for stabilizing municipal solid waste.

Mechanically mixed Group IV bioreactors have been used at relatively large scales, including various applications of environmental relevance. Various designs have been used in composting processes (65). A bioreactor developed originally at INRA (Platform for Development in Biotechnology), in Dijon, France (67), has been used for the protein enrichment of agro-industrial byproducts for use as animal feed and for the production of enzymes and biopesticides (68). This bioreactor has a bed capacity of 1.6 m^3, enabling it to hold one ton of moist sugar beet pulp (at 75% water content). A larger version 17.6 m in length, 3.6 m in width,

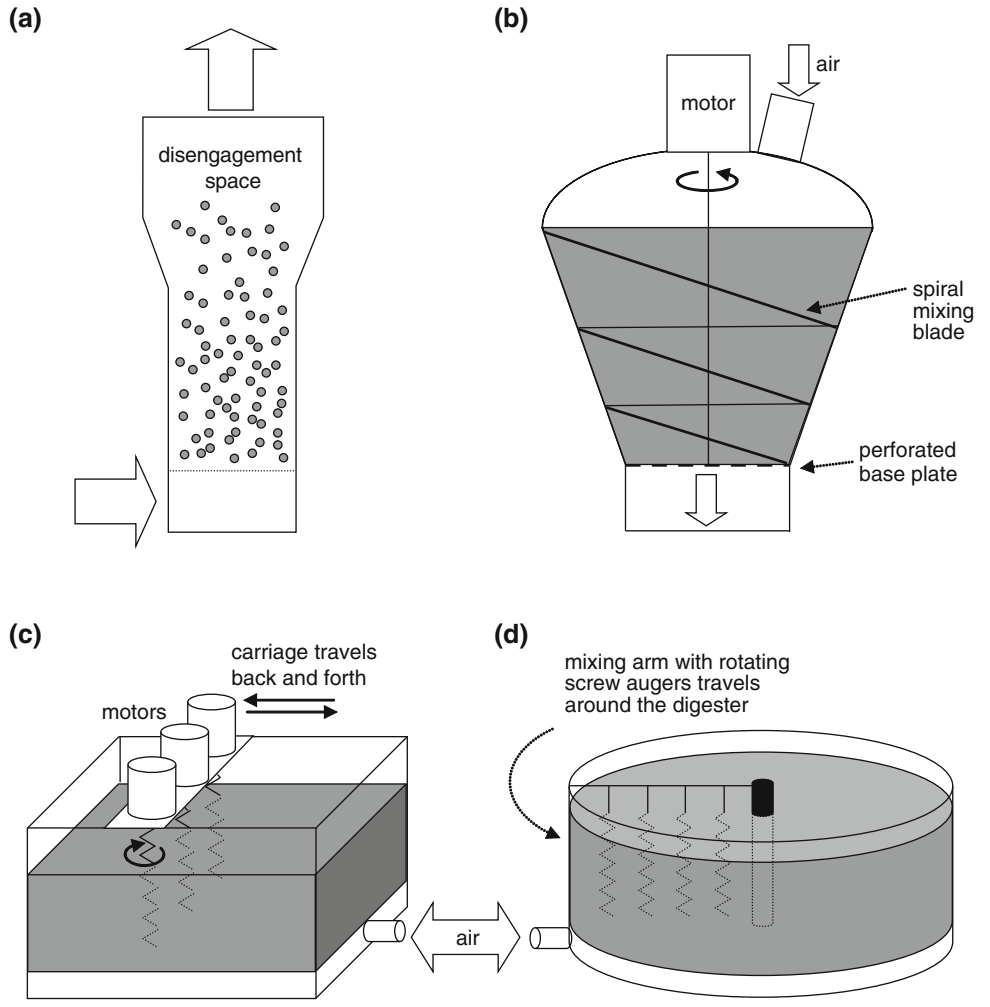

Fig. 7.12. Various bioreactors classified in Group IV, that is, bioreactors in which the bed is both forcefully aerated and mixed either continuously or frequently (60, 65–70). (**a**) An air-solid fluidized bed bioreactor, in which the bed is mixed by the air flow. (**b**) A mechanically-stirred bioreactor without a traveling agitator, an adapted conical solids mixer (70). Note that the bioreactor shown in Fig. 7.8 is another version of this type of stirred-bed bioreactor, and that, in this type of bioreactor, the agitator may be designed differently, for example, planetary mixers may be used. (**c**) A larger scale version of a mechanically-stirred bioreactor with a traveling agitator, as developed Durand and Chereau (67). (**d**) The Fairfield-Hardy digester, which has a traveling agitator and has been used for composting processes (65).

and with a 2.0 m bed height has been used for protein enrichment of 25 ton batches of sugar beet pulp (at 80% water content) (69). Conical commercial solids mixers with helical-blades have also been suggested as bioreactors for solid-state cultivation (Figure 7.12b) (70).

6. DESIGN OF BIOREACTORS FOR ENVIRONMENTALLY-RELATED SOLID-STATE CULTIVATION PROCESSES

Given the various different bioreactor designs (Sect. 5) that can be used and the various different process considerations (Sect. 3), bioreactor design for solid-state cultivation processes is not a simple matter. This section will concentrate on bioreactor design for those environmentally related processes in which it is desirable for the bioreactor to control the temperature at, or as near to as possible, a fixed optimum value throughout the cultivation. Special considerations are required for the design and operation of bioreactors for in-vessel composting, in which temperature variations are desirable and for in-vessel bioremediation and biofilters, in which the main consideration is the removal of pollutants. These will not be discussed here.

6.1. General Considerations for the Selection and Design of Bioreactors

Before addressing methods for the design of specific bioreactor types, it is worthwhile to give a general outline for the bioreactor selection and design process. Various bioreactors that have been developed for environmentally related applications of solid-state cultivation technology in the past have been highly inefficient because they were designed based on a "best-guess" strategy. Our knowledge is currently sufficient to enable the use of quantitative calculations to guide bioreactor design. The process of selecting and designing a solid-state cultivation bioreactor should, therefore, be based on these calculations and will have three steps, each of which can be characterized by a basic question:

- *Understanding the process organism*: What are the key phenomena that, first, affect the process significantly and, second, can be affected in the way that the bioreactor is designed and operated? It is necessary to understand how the organism grows and produces its product and how this growth and production are affected by key process variables. This chapter concentrates on those processes for which the key process variable is the bed temperature. Note that the water content of the bed becomes another key variable in those cases in which evaporation plays a role in temperature control. In other environmentally related processes, such as bioremediation and biofiltering, in which metabolic activities are not so high and, therefore, the generation of waste metabolic heat is lower, high temperatures may not be such a crucial problem; rather, the lowering of the pollutant to acceptable levels may be the crucial design consideration. The tolerance of the process organism to agitation also needs to be understood at this stage.
- *Bioreactor selection*: What is the best bioreactor, taking into account any external constraints? For example, there may be limits on operating costs or on the level of technology to be used. Note that bioreactor selection will be significantly affected firstly by the rate of growth of the organism and the consequences this has for efficient heat removal and secondly by the degree to which the process organism tolerates agitation of the bed.
- *Bioreactor design and optimization*: Having selected the best bioreactor for a particular process, what is the best way to design and operate the selected bioreactor in order to maintain the key process variables at, or as near as possible to, the optimum values for the process? This requires an understanding of the heat and mass transfer phenomena that affect the values of the key process variables, and how the various operating variables influence the efficiency of this heat and mass transfer. As will become clear in the sections addressing the design of the various different bioreactor types, mathematical models that incorporate both the kinetic behavior of the organism and the key heat and mass transfer processes are the powerful tools for guiding the design and optimization of operation of bioreactors.

The design process can be attacked at various levels of sophistication (Figure 7.13). Typically, in the design of an environmentally related process, the interest is in avoiding undue complexity, while making reasonable decisions. The desire to avoid undue complexity means that intra-particle phenomena will typically not be of interest. As described in the following section, this means that relatively simple empirical kinetic equations tend to be used.

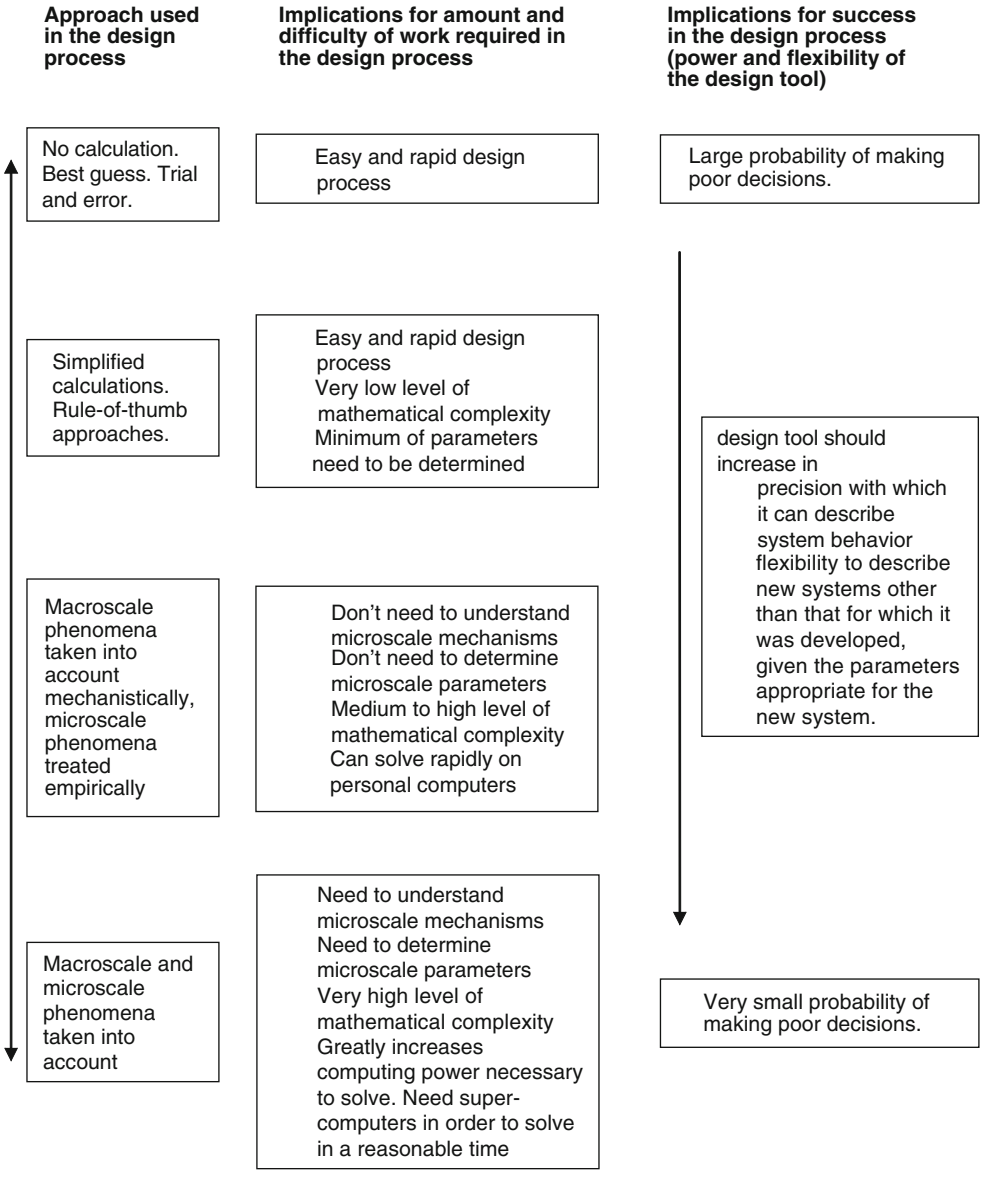

Fig. 7.13. A continuum of approaches to the design task, from the most simple to the most complex (60).

As mentioned earlier, this chapter focuses on those processes in which growth is sufficiently fast for temperature control to be the major challenge in bioreactor design. For these "fast-growth/temperature-problematic" processes, several key questions will guide the design process:

- What is the optimum temperature of the substrate bed?
- What is the degree of aeration that the process requires?
- What is the optimum water activity of the substrate? Note that water becomes an important variable since metabolic heat production promotes evaporation and, furthermore, the bioreactor might be operated in such a manner as to maximize evaporation in order to take advantage of the high heat removal potential of evaporative cooling, but this can potentially decrease the water activity of the substrate to values too low for good growth.
- What are the important limitations on bioreactor operation that might derive from properties of the substrate or organism, such as limitations on the type and frequency of mixing that can be used? Specifically, in processes that involve fungi, the fungal hyphae may be damaged significantly by the mixing action. In addition, certain mixing actions may tend to compact the substrate bed, which would impede O_2 supply to the organism at the particle surface.

With the answers to these questions, the design problem can be further characterized by a more specific set of questions:

- What is the aeration type that should be used, and at what flow rate, temperature, and relative humidity this air should be supplied? Note that it might be advantageous to vary the inlet air properties during the course of the cultivation.
- What is the mixing type that should be used, and what mixing regime should be used, in terms of frequency of mixing, the duration of mixing events if mixing is not continuous, and the intensity of mixing?
- Will there be other significant contributions to heat removal, such as removal through cooling surfaces, and in this case, what will the cooling fluid be (typically air or water) and what should its flow rate and temperature be? Note that it might be advantageous to vary the cooling fluid properties during the course of the cultivation.
- Is it worthwhile to implement monitoring and control schemes to try to maintain the specified key process variables at their desired values by manipulating the regimes for aeration, mixing and cooling through cooling surfaces?

Note that decisions about aeration rates will be most strongly influenced by heat removal considerations, and aeration rates required for adequate heat removal will be adequate to maintain relatively high O_2 concentrations in the void spaces, so considerations of O_2 levels typically do not enter as primary design considerations.

These questions will appear, collocated in a more specific manner, in the individual bioreactor sections, where it will be argued that these questions are best answered on the basis of models of the process in which mass and energy balances over the bioreactor are formulated (Figure 7.14). These balance equations can be formulated in manners that are more complex or simpler to use. These cultivation processes are dynamic processes, that is, the process variables vary significantly over time. In bioreactors in which the bed is not well mixed, there are also often significant spatial variations, which the model should also describe (Figure 7.15). Models that describe both the spatial and temporal variations will then require the solution of partial differential equations, which is more challenging than solving the ordinary differential

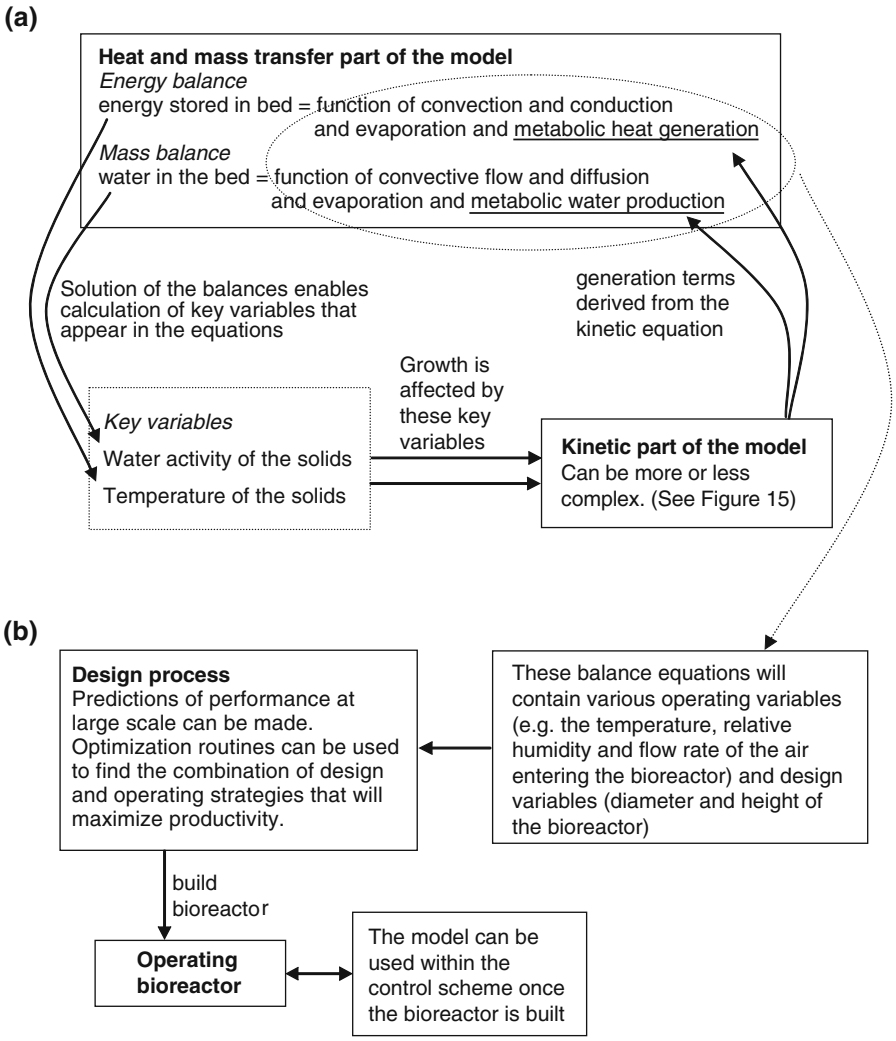

Fig. 7.14. Models as tools for bioreactor design (60) (**a**) The basic structure of models used as tools for guiding the bioreactor design process. (**b**) An indication of how models can be used in the design process.

equations used to describe the temporal profile for a well-mixed system. However, as will become apparent, it is also typically possible to make pseudo steady-state approximations, since the growth processes tend to be slower than the transport processes. This has the effect of transforming a model with partial differential equations into a model with ordinary differential equations in which the independent variable is the spatial position, and of transforming a model with ordinary differential equations into a model with simple algebraic equations.

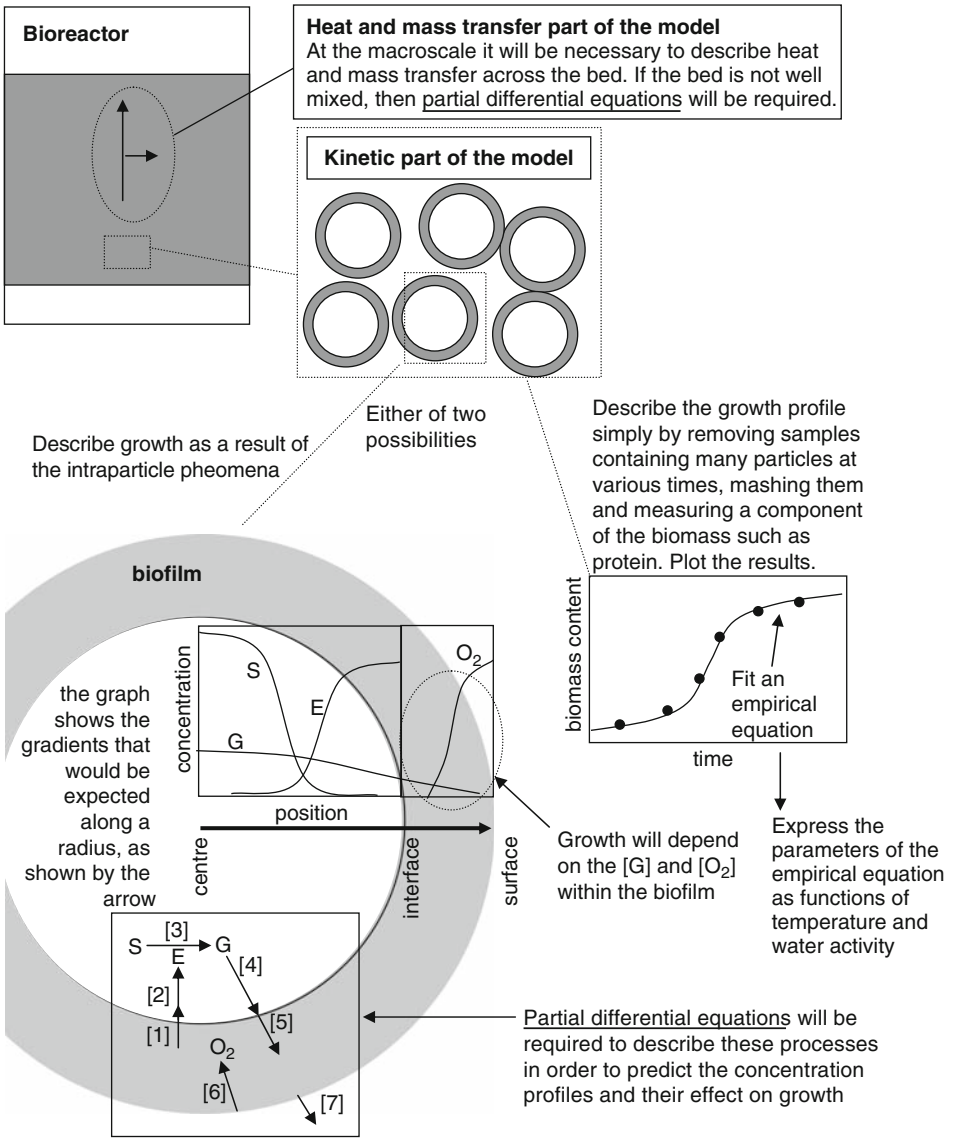

Fig. 7.15. The appropriate level of complexity for the kinetic part of the model (60). As shown by the figure, which represents a process in which a biofilm of a glucoamylase-producing bacterium grows on the surface of a starchy substrate particle, intra-particle variables such as starch, enzyme, and glucose concentrations do affect growth but lead to complex mathematical equations. The empirical approach will lead to much simpler equations. Key: [1] Release of enzyme (E) by the biomass; [2] Diffusion of enzyme into the substrate particle; [3] Action of the enzyme to hydrolyze starch (S) to glucose (G); [4] Diffusion of glucose within the substrate particle; [5] Diffusion and uptake of glucose within the biofilm; [6] Diffusion and uptake of oxygen within the biofilm; [7] Expansion of the biofilm as more biomass is produced.

6.2. The Importance of Characterizing the Growth Kinetics of the Microorganism

As pointed out at the beginning of the previous subsection, in all cases, it is necessary to understand the basic kinetics of growth and product formation by the process organism before making any design decisions. Actually, this is potentially quite a complex task, since ideally the kinetic equation should take into account the key factors that influence the growth of microorganisms in solid-state cultivation systems. Note that several "intra-particle variables" can be important in affecting the growth kinetics, such as local nutrient and dissolved O_2 concentrations and local pH values within the substrate particle (Figure 7.15). However, there are typically significant gradients within the particle, and it is a highly complex matter to characterize these gradients in order to know exactly what "intra-particle conditions" are experienced by the biomass. It is desirable to avoid such complexity in the design process. As a result, a simple empirical approach to characterizing growth kinetics in solid-state cultivation processes is used (Figure 7.15). It involves two steps:

- Empirical characterization of the basic form of the growth profile, describing it with an equation that does not include intra-particle nutrient or O_2 concentrations or the pH;
- Expression of the parameters of the growth equation as functions of the key process variables, these being the temperature and possibly also the water activity of the substrate.

An extensive characterization of growth kinetic profiles in solid-state cultivation systems showed that the logistic equation gives a reasonable approximation of around 75% of growth profiles (71) and, as a result, this equation has been used to describe the growth kinetics in mathematical models for various solid-state cultivation bioreactors. The differential form of the logistic equation, which expresses the growth rate of the organism, can be presented in terms of the volumetric concentration of the biomass (X, kg of dry biomass per m^3 of bed volume):

$$\frac{dX}{dt} = \mu X \left(1 - \frac{X}{X_{max}}\right) \tag{1}$$

where μ is the specific growth rate constant (per hour) and X_{max} is the maximum volumetric biomass concentration reached. Integration of this equation with constant values for the parameters gives a temporal profile of the form shown on the right in Fig. (7.15). For use within a bioreactor model, the specific growth rate constant is expressed as a function of the bed temperature and water activity.

Given that the development of mathematical models of bioreactors requires a large amount of modeling and programming work to write and solve the equations and a large amount of experimental work to determine the parameters, the following bioreactor design sections will show only the equations that are used in simplified design methods. Typically, these rules require only an estimate of the peak heat production rate under optimal growth conditions, avoiding the necessity for extensive characterization of the effect of temperature and water activity on growth. The peak heat production rate occurs at the time of maximum growth rate. Differentiating Eq. (1) with respect to biomass after bringing the term μX inside the

parentheses and then setting the differential to zero gives:

$$\frac{\mathrm{d}\left(\mu X - \frac{\mu X^2}{X_{\max}}\right)}{\mathrm{d}X} = \mu - \frac{2\mu X}{X_{\max}} = 0 \tag{2}$$

which can be solved to show that the maximum growth rate occurs when $X = 0.5X_{\max}$. Substituting this into Eq. (1), the maximum growth rate can be calculated:

$$\left.\frac{\mathrm{d}X}{\mathrm{d}t}\right|_{\max} = \mu 0.5 X_{\max}\left(1 - \frac{0.5X_{\max}}{X_{\max}}\right) = 0.25\mu X_{\max} \tag{3}$$

The maximum volumetric heat production rate (R_{Qmax}, $\mathrm{W/m^3}$) can then be calculated if the stoichiometric relationship between growth and heat production (Y_{QX}, J/kg) is known:

$$R_{\mathrm{Q\,max}} = Y_{\mathrm{QX}}\left.\frac{\mathrm{d}X}{\mathrm{d}t}\right|_{\max} \tag{4}$$

Note that it is not essential to know the growth kinetics in order to use these equations. In fact, the measurement of biomass concentrations in solid-state cultivation systems is often highly problematic, due to the difficulty in separating the microorganism from the solids. An experimental measurement of the O_2 consumption rate will suffice, since the maximum heat generation rate should be directly proportional to the maximum O_2 consumption rate ($R_{\mathrm{O2\,max}}$). The stoichiometric coefficient (Y_{QO}) is 16,562 kJ/kg (72). In this case, the maximum heat production rate would be estimated as:

$$R_{\mathrm{Q\,max}} = Y_{\mathrm{QO}} R_{\mathrm{O2\,max}} \tag{5}$$

The use of this estimate of $R_{\mathrm{Q\,max}}$ will be described in the following subsections.

6.3. Design of Group I Bioreactors

The design and operation decisions that need to be made for Group I (tray-type) bioreactors are:

- If the chamber in which the cultivation to take place is to have conditioned air, how should the inlet and outlet of air in the chamber be designed? What should be the arrangement of trays or bags in the chamber to promote a uniform circulation of air within the chamber itself?
- At what values should the temperature and relative humidity of the air circulated past the trays or bags be controlled?
- How large should each tray or bag be and especially, what depth of the substrate layer should be used in each tray or bag?
- Where infrequent mixing is to be undertaken, what frequency, duration, and intensity of the mixing should be used?

These questions can be answered on the basis of considerations of heat and mass transfer in static, unaerated beds. Note that heat transfer is essentially limited to conduction and mass transfer of O_2, CO_2, and water vapor is essentially limited to diffusion within the inter-particle spaces. There is a tendency to reach high temperatures and low O_2 concentrations within the interior of the bed, with the shape of the profiles depending on whether the bottom of the bed is perforated or not (Figure 7.16).

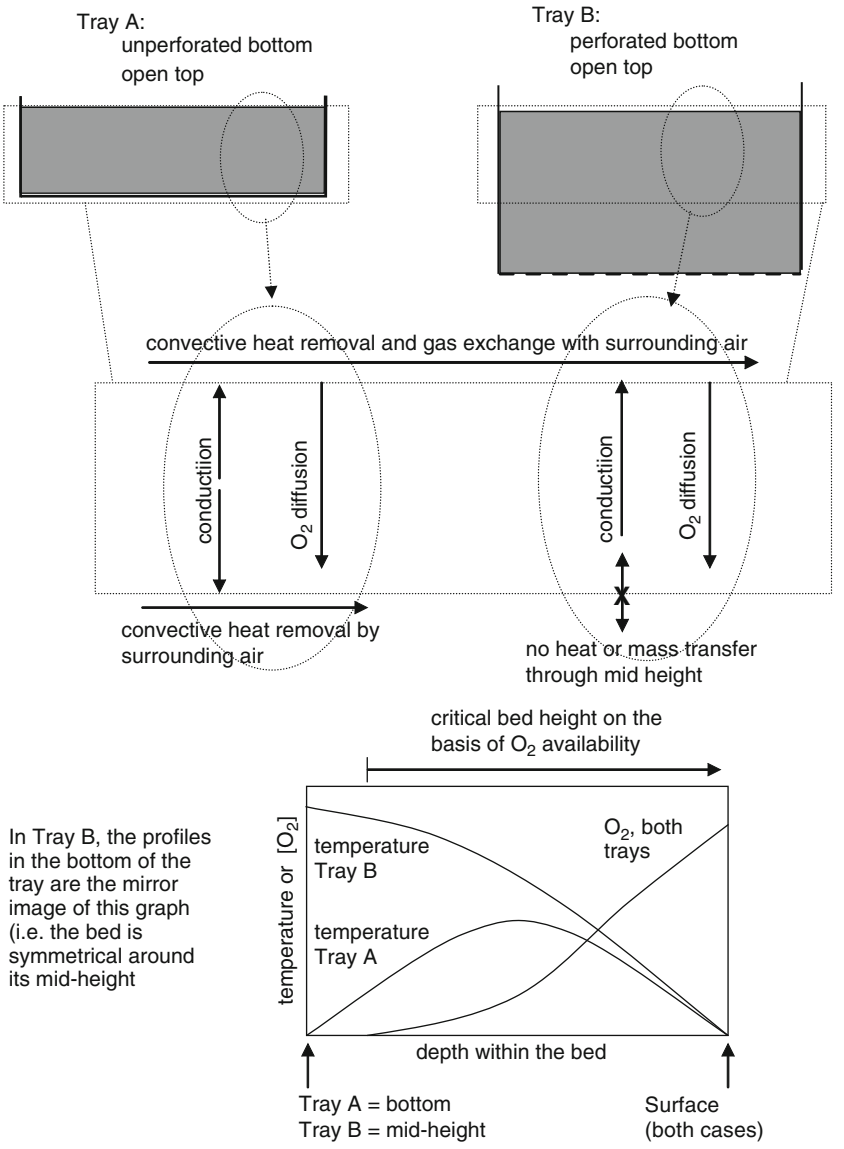

Fig. 7.16. Key heat and mass transfer mechanisms in trays and the resulting effect on temperature and inter-particle O_2 concentration profiles within the bed (73–75). Note that whether or not the bottom of the tray is perforated affects the relative shapes of the O_2 and temperature profiles. Also shown is the critical bed height, defined by Ragheva Rao et al. (73) as being the height at which the O_2 concentration falls to zero at some time during the culture.

6.3.1. Simple Approaches to Making Design Decisions About Group I Bioreactors

A pseudo steady-state approximation for O_2 diffusion and consumption within the substrate bed within a tray gives an equation for the critical bed height (H_c), namely the depth at which O_2 reaches zero concentration at some time during the cultivation (73):

$$H_c = \sqrt{\frac{2D_e C_g Y_{xo}}{R_{O2\,max}}} \tag{6}$$

where D_e is the effective diffusivity of O_2 within the bed, C_g is the O_2 concentration in the gases surrounding the tray, Y_{xo} is the yield coefficient of biomass from O_2, and $R_{O2\,max}$ is the maximum value of the O_2 consumption rate.

Estimating the values of the parameters of this equation is not necessarily an easy task. The effective diffusivity of O_2 within the bed is likely to change during the culture as the microorganism grows into the inter-particle spaces. Ragheva Rao et al. (73) suggested a value of 0.03 cm^2 h. They used a value of 1.07 kg/kg for Y_{xo}. The value of $R_{O2\,max}$ will depend on the particular growth and O_2 consumption kinetics. Ragheva Rao et al. (73) used a value of $R_{O2\,max}$ of 1.62 g/(cm^3 h). With these values, and assuming that the air surrounding the tray was 21% O_2 (v/v), they determined the critical bed height as 4.8 cm. Of course, these calculations will need to be repeated for each new process; however, they do give an order of magnitude estimate of maximum bed heights for Group I bioreactors on the basis of considerations of O_2 consumption.

In terms of heat removal considerations, a pseudo steady-state assumption gives an equation for the temperature profile within the bed as a function of depth below the surface (74):

$$T = -\xi^2\Theta + \frac{N_{Bi}}{N_{Bi}+1}(T_s - T_a + \Theta)\xi + \frac{T_s + \Theta + N_{Bi}T_a}{N_{Bi}+1} \tag{7}$$

where $\Theta = R_Q\delta^2/(2k)$, $N_{Bi} = \alpha\delta/k$ and $\xi = z/\delta$. In these equations, T is the temperature at a particular height z, T_s is the surface temperature of the bed, α is the bed-to-air heat transfer coefficient, R_Q is the volumetric rate of heat generation by the organism, δ is the overall height of the bed, k is the thermal conductivity of the bed, Θ is the temperature difference between the bottom of the solid medium and the upper surface in the case where heat is not transferred across the bottom surface to the air, N_{Bi} is the Biot number and T_a is the temperature of the surrounding air.

To use Eq. (7), it is necessary to have an estimate of the bed-to-air heat transfer coefficient (α), or at the very least, to use a reasonable estimate of the Biot number. A reasonable value may be ten (75). Szewczyk (74) did not actually determine values for the surface to air heat transfer coefficient, but rather used a more complete model to explore the relationship between α and the predicted bed surface temperature, repeating this for various values of the metabolic heat generation rate (R_Q). Such a relationship must actually be provided in order to solve Eq. (7), since it includes both T_s and α and T_s depends on α. In the absence of an explicit equation relating T_s to α, Eq. (7) is not in a form that is easy to apply, however, the analysis undertaken by Szewczyk (74) does give general guidance. For a bed height of only 3 cm, with an air temperature of 30°C and with a low bed-to-air heat transfer coefficient (10 W/(m^2 °C)),

which might be expected if the air is not circulated past the bed at a high rate, bed temperatures can potentially reach values as high as 37°C (if there is reasonable heat transfer through the bottom of the tray) or even 46°C (if there is no heat transfer through the bottom of the tray).

6.3.2. Model-Based Approaches to Making Design Decisions About Group I Bioreactors

Rajagopalan and Modak (75) developed a mathematical model to describe both heat and mass transfer within a tray. The model incorporated the heat and mass transfer phenomena shown for "Tray B" in Figure 7.16. It is therefore capable of predicting both the bed temperature and the O_2 concentration in the inter-particle gas phase within the bed as a function of both bed height and time. Both temperature and O_2 concentration were predicted to be limiting factors, although at different times. During the early stages of growth, the temperature was the dominant factor. At intermediate stages, O_2 limitation was important at the bottom of the bed, while temperature limitation was important in the center of the bed.

The model was used to explore the effects of several design and operating variables. The velocity of the air flow past the surface of the bed had relatively little effect on the temperatures within the bed. The surrounding air temperature and the bed height were more important. Simulations were undertaken to identify the optimum combination of these two variables for an organism with an optimum growth temperature of 38°C. Optimum growth was predicted to occur for a 3 cm bed with surrounding air temperatures of 30–35°C. With larger bed heights, growth was poorer, regardless of what surrounding air temperature was used.

6.3.3. Synthesis of Our Knowledge About How Best to Operate and Design Group I Bioreactors

For a majority of processes, bed heights should probably not exceed 3–5 cm. Given that these bed heights will already have been used in laboratory studies, this means that processes must be scaled up by increasing the total tray area, while maintaining the height of the substrate layer within the tray constant. On a large scale, processes involving Group I bioreactors are likely to be highly labor intensive; however, in processes with batches of the order of 10–100 kg of substrate, such as might occur in small-scale or domestic industries, this type of bioreactor could be appropriate.

6.4. Design of Group II Bioreactors

The design and operation decisions that need to be made for Group II (packed-bed type) bioreactors are:

- What should the height of the bed be?
- What is the required overall capacity? Note that this, combined with the bed height, will determine the necessary horizontal dimensions.
- What should the temperature and flow rate of the process air be?
- Should internal heat transfer surfaces be provided and, if so, what should be the temperature and flow rate of the cooling water?
- Is the bed to be infrequently mixed? In the case that infrequent mixing is to be used, what should be the humidity of the inlet air? Note that unsaturated air can be used in the case of an infrequently mixed bed since water can be added to the bed during the mixing event. For a bed that is to remain static throughout the process, then the inlet air should be saturated in order to minimize

the evaporation rate, otherwise growth may be limited simply because the bed dries out to water activities low enough to inhibit microbial growth.
- If the bed is to be infrequently mixed, what should be the trigger for mixing, what should be the duration and intensity of the mixing events and what additions, if any, should be made to the bed during these events?
- If the bed is to be infrequently mixed, what should the design of the agitator be, and should it be removed from the bed during the periods of static operation?

The decisions that will be made in response to these questions will be affected by the following considerations:

- There is a tendency for bed temperature to increase with bed height for forcefully aerated static beds due to convective cooling, and, therefore, the decision on the bed height to be used will be influenced by the effects of high temperature on the process organism.
- The bed height may also be decided on the basis of expected pressure drops through the bed. Pressure drop can potentially be a problem in static packed beds, especially in processes involving fungi, since growth of fungal hyphae into the void spaces impedes the flow of air through the bed, necessitating a higher pressure at the inlet in order to maintain the flow rate. Note that considerations of pressure drop mean that beds should be of uniform thickness. If a bed is not of uniform thickness, then the air will tend to flow preferentially through those regions of the bed that are less thick (Figure 7.17a).
- Decisions about when to mix might be made on the basis of the need to make intermittent additions, the need to prevent the pressure drop through the bed from becoming too large or the need to destroy any channels that might occur due to the bed either cracking or pulling away from the bioreactor walls. Such channeling is undesirable since it means that the air will flow preferentially through the cracks and not through the bed itself.

6.4.1. Simple Approaches to Making Design Decisions About Group II Bioreactors

The following equation can be used to estimate the maximum outlet temperature as a function of the maximum heat production rate and the air flow rate (76):

$$\mathrm{Da_M} = \frac{R_\mathrm{Q}}{\rho_\mathrm{a}(C_\mathrm{pa} + f\lambda)V_\mathrm{z}(T_\mathrm{out} - T_\mathrm{in})/H} \tag{8}$$

This so-called "modified Damkohler number" ($\mathrm{Da_M}$) is a dimensionless number that represents the ratio of the volumetric heat production rate (R_Q, J/(m^3 h)) to the volumetric heat removal rate. The volumetric heat production rate is estimated by assuming that the air enters the bioreactor saturated at the air inlet temperature and leaves saturated at the outlet air temperature, and takes into account the contributions of the sensible energy of the air and the evaporation of water into the gas phase. The factor f represents a linear approximation to the saturation humidity curve, this approximation being reasonable over short temperature intervals.

Equation (8) is most useful if the modified Damkolher number is set equal to 1, and the equation is rearranged to be explicit in the bed height (H). If a maximum allowable temperature can be identified (T_max), this being a temperature that should not be exceeded in any part of the bed, at any time during the cultivation, then it is possible to calculate the maximum height that the bed can have (H_max). In order to do this, the volumetric metabolic heat production

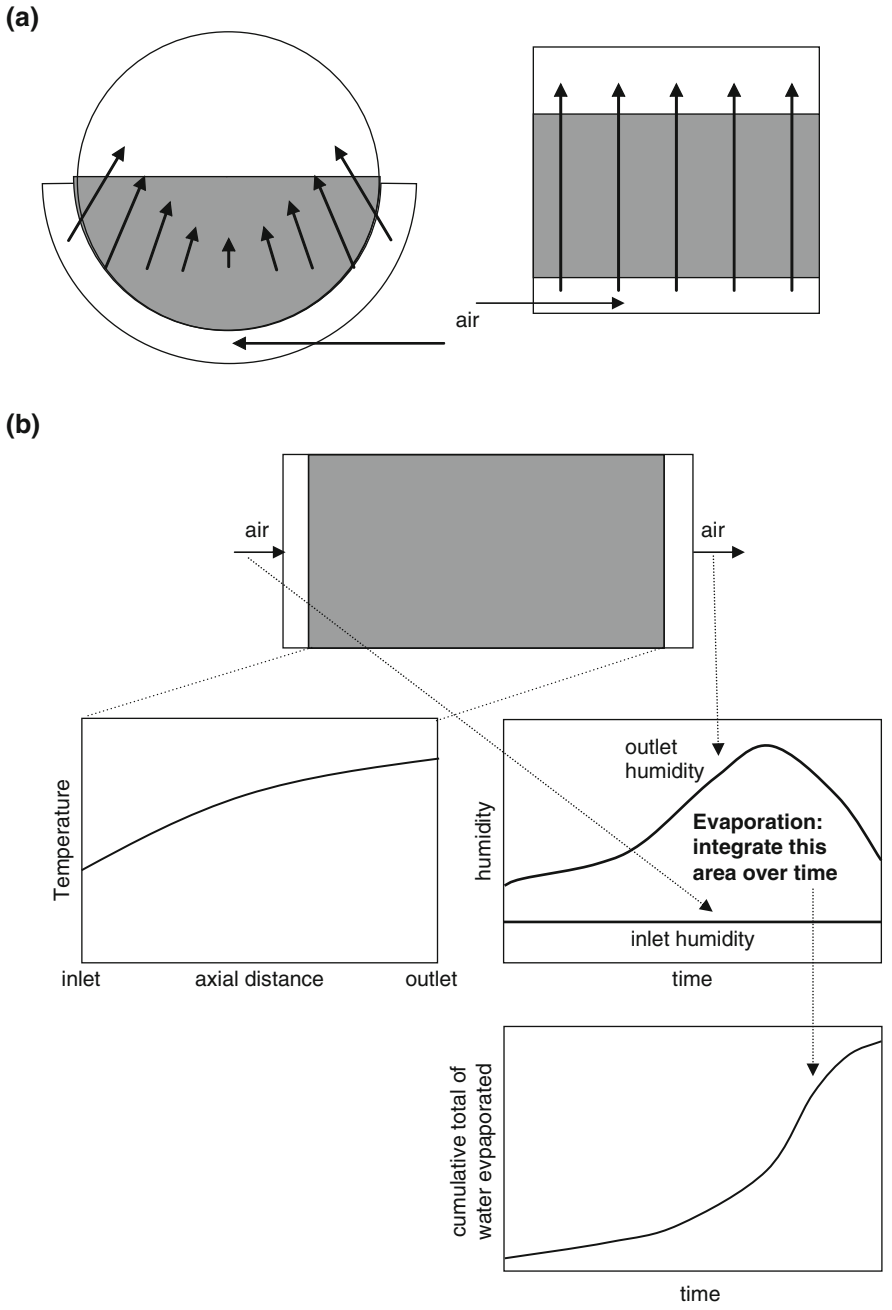

Fig. 7.17. Considerations in the design of packed beds (76). (**a**) The necessity of having a bed of constant thickness in order to obtain uniform aeration. If the bed is not uniformly thick, then the air will preferentially flow through those regions that are less thick because the pressure drop is lower. (**b**) The tendency of packed beds to have axial temperature profiles and the consequence that this has for evaporation. Note that in this case, the packed bed is shown lying on its side.

rate is set to its maximum value ($R_{Q\,max}$) and T_{out} is set to T_{max}, since the highest temperature occurs at the air outlet end of the bed. These manipulations and substitutions give the following equation (76):

$$H_{max} = \frac{\rho_a(C_{pa} + f\lambda)V_z(T_{max} - T_{in})}{R_{Q\,max}} \tag{9}$$

It is then possible to explore the effect of the superficial velocity of the air (V_Z, obtained by dividing the volumetric flow rate of the air by the total cross-sectional area of the bed) and the inlet air temperature (T_{in}) on the maximum allowable height of the bed. In order to do this, the density and heat capacity of air (ρ_a and C_{pa}) can be taken from a reference book, while over the range of 25–45°C, the factor f can be estimated as 0.00246 kg-water/(kg-air°C) (76).

This type of approach can be extended to include considerations of the water balance (77). The temperature profile tends to be steeper at the inlet end of a packed bed, becoming ever less steep with height. This, combined with the exponential nature of the saturation vapor pressure curve, means that the vapor pressure increases approximately linearly with height, suggesting that the bed will dry out relatively uniformly along its length. The drying rate can then be estimated if the expected temporal profile for the outlet air temperature is known, simply by using the outlet air temperature to estimate the difference between the inlet and outlet humidities ($y_{out}-y_{in}$) and then integrating this profile against time (Figure 7.17b). However, this method can only be applied if the profile for outlet temperature is known. Therefore, it will be most useful in a system in which the inlet and outlet temperatures of a large-scale bioreactor are monitored and used to estimate evaporation.

6.4.2. Model-Based Approaches to Making Design Decisions About Group II Bioreactors

Mathematical models have been proposed for several packed-bed bioreactors. The Zymotis bioreactor, with its internal heat transfer plates, might be used in processes in which absolutely no mixing is desired during the cultivation. A model of this bioreactor (78) that takes into account the energy balance, but not the water balance (Figure 7.18a), can be used to explore the effect of various design and operating variables on bioreactor performance:

- Design variables: bed height, spacing between the heat transfer plates.
- Operating variables: air flow rate and temperature, temperature of the cooling water passed through the heat transfer plates.

Simulations with the model show that use of internal heat transfer plates has the potential to decrease, although not remove, the radial and axial temperature gradients (Figs. 7.18b, c). However, careful consideration needs to be undertaken before using this type of bioreactor. Optimal performance requires relatively small spacing between the heat transfer plates, as little as 6 cm for processes involving fast-growing organisms (79). The presence of the heat transfer plates will likely complicate loading operations, it being essential to obtain an even packing of substrate in the compartments in order to prevent channeling. Any changes in bed volume due to bed shrinkage away from the walls during the process will also prevent proper aeration of the bed.

In some solid-state cultivation processes, mixing does have deleterious effects, but infrequent mixing events do not have an unduly negative effect. In such cases, mixing will be used

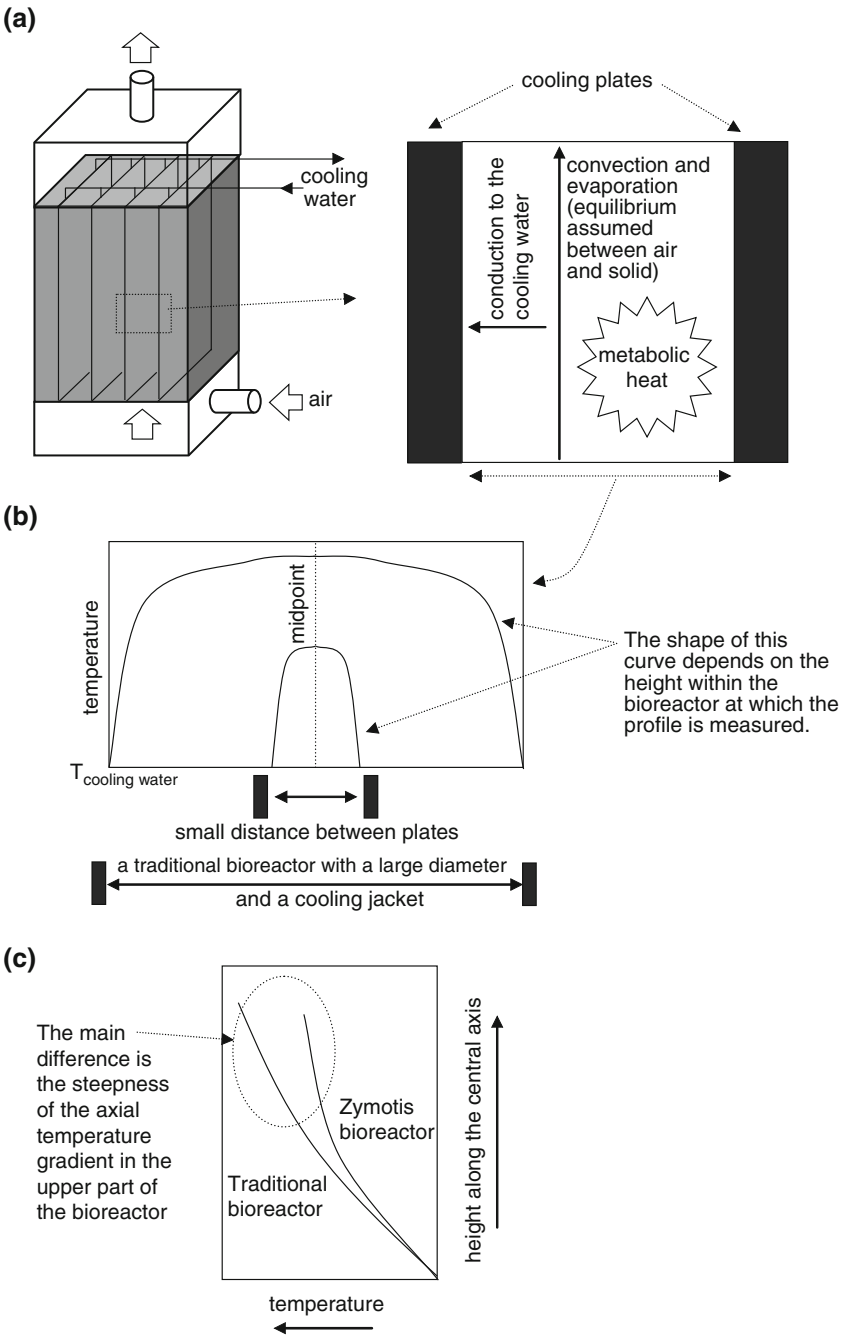

Fig. 7.18. Modeling of the Zymotis bioreactor (78). (**a**) Representation of how the model of Mitchell and von Meien (78) treats the processes occurring. (**b**) and (**c**) Predictions of the model about how the small spacing between the cooling plates reduces (**b**) the radial temperature gradients in the bed and (**c**) the axial temperature gradients in the bed, compared to a traditional packed-bed bioreactor of large diameter with a cooling jacket.

not in an attempt to control temperature, which would require frequent mixing events, but rather in order to allow the replenishment of water to the bed, which opens up the possibility of using unsaturated air at the air inlet in order to promote evaporative cooling. Note that the replenishment of water is only feasible during mixing events, due to the difficulties of uniformly distributing water in a static bed. For example, any attempt to percolate water downwards from the top of the bed would simply flood the top of the bed. von Meien and Mitchell (80) developed a model to describe such a system. It treats the air and solid phases as separate subsystems and, instead of assuming equilibrium between the air and solid phases, describes heat and mass transfer as a result of driving forces (Figure 7.19a).

The predictions of this model allow various insights into how to design and operate infrequently mixed packed beds. An appropriate strategy for initiating mixing events would be to monitor the relative humidity of the outlet air and to initiate a mixing event when this falls below a certain threshold value (Figure 7.19b). During this mixing event, water would be added to the substrate to bring its water activity back to values that are favorable for growth. Such bioreactors should be able to operate with two or three mixing events over a period of 30 h, in the case of a fast-growing process organism. The ability to add water means that the limitation of growth by low water activities in the substrate bed can be avoided (Fig. 7.19b), which leaves temperature control as the most important problem: even though evaporative cooling can be promoted with the use of dry air at the air inlet, axial temperature gradients will still limit the bed height that can be used. Of course, the higher the superficial velocity of the air, the less steep is the axial temperature gradient and the higher the bed can be. The model of von Meien and Mitchell (80) can be used to explore this relationship. There will be limits on acceptable air flow velocities, either due to the operating costs of the aeration system, or due to the fact that at very high velocities the bed will fluidize, although the model is not yet sufficiently sophisticated to take these into account.

6.4.3. Synthesis of Our Knowledge About How Best to Operate and Design Group II Bioreactors

Static packed beds and intermittently stirred packed beds are likely to be suitable for many environmentally related solid-state cultivation processes, especially for less shear-tolerant organisms, as they minimize shear damage to the organism and also minimize operating costs associated with the mixing of the substrate bed. On the basis of the results of the quantitative design approaches described earlier, plus the experience of the soy sauce *koji* industry (63), it is likely that maximum bed heights will be of the order of 0.5–1.0 m, necessitating large areas if large volumes are to be processed.

Order of magnitude design decisions can be made using the simplified strategies outlined in Sect. 6.4.1. However, these methods do make some assumptions that are questionable. For example, Weber et al. (81) showed experimentally that the outlet gas from packed-beds is not necessarily saturated, falling to below 90% relative humidity during periods of peak heat production. Models that allow for this situation, such as that developed by von Meien and Mitchell (80), will be more accurate design tools. However, use of such models does require estimates of the solid-to-gas heat and mass transfer coefficients, which can potentially vary significantly between processes and are not simple to measure experimentally.

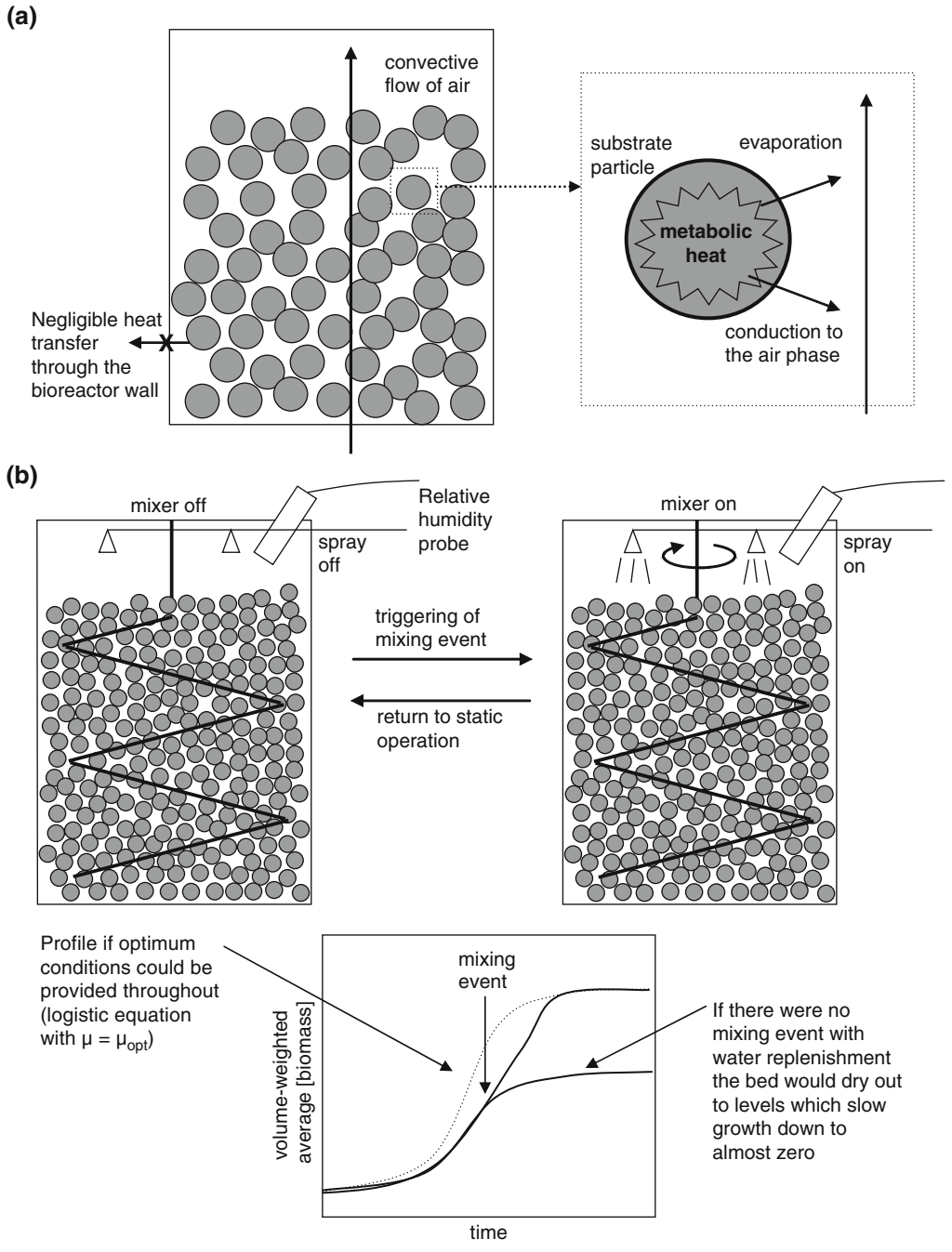

Fig. 7.19. Modeling of intermittently-agitated packed-bed bioreactors (70). (**a**) How the model of von Meien and Mitchell (80) treats the processes occurring. (**b**) Details of the operating strategy and the predicted performance of the bioreactor with this mode of operation.

6.5. Design of Group III Bioreactors

The design and operation decisions that need to be made for Group III (rotating-drum and stirred-drum type) bioreactors are:

- How large should the bioreactor be, and what should be the length to diameter ratio?
- How much of the total bioreactor volume should the substrate bed occupy?
- At what values should the headspace air temperature and relative humidity and flow rate be controlled?
- In rotating drums, should lifters be incorporated and, if so, how many and of what size and shape? In stirred drums, what is the best design for the agitation system?
- Should the bioreactor axis be horizontal or inclined?
- What rotation rate should be used, for the drum body for rotating drum bioreactors and for the agitator for stirred-drum bioreactors?
- Should the outer surface be cooled and, if so, in what manner? Note that it is possible to blow air past the bioreactor, to spray cooling water onto the outer surface or to incorporate a cooling jacket into the design.

The decisions that will be made in response to these questions will be affected by how the various design and operating variables influence the heat and mass transfer phenomena within the bioreactor. As shown by Fig. 7.20, these include:

- *Flow patterns within the solid substrate bed.* In rotating drums in the absence of lifters, axial and radial mixing of the bed can be quite inefficient, especially at the relatively low rotational rates that are typically used. In rotating drums with lifters, the lifters will promote radial mixing, and can be designed in such a manner as to promote axial mixing by pushing substrate back and forth within the drum. In any case, mixing in horizontal drums tends to be inefficient when the bed occupies more than 30% of the total drum volume, so this can be taken as an upper limit as to how full the drum should be.
- *Flow patterns of gas within the headspace*. These flow patterns affect the efficiency of bed-to-headspace heat and mass transfer.
- *Bed-to-headspace heat and mass transfer*. This transfer will be improved by those factors that increase the overall area of contact.

These considerations mean that lifters should typically be used in rotating drums, since lifters prevent the slumping flow regime, even at very low rotation rates. This improves radial mixing patterns within the bed, exposing the substrate more uniformly at the surface. In addition, as the particles are lifted and then fall back as a curtain of particles, there is intimate contact between these particles and the headspace, promoting bed-to-headspace heat and mass transfer. In order to achieve good radial mixing and bed-headspace contact in rotating drums without lifters, it would be necessary to use high rotation rates to obtain the cataracting flow regime. Note that lifters can also be used to promote axial mixing, in which case it is probably best to have the bioreactor at an angle such that the movement imparted by the lifters to one end of the drum is counterbalanced by return of the substrate due to gravity (82).

6.5.1. Simple Approaches to Making Design Decisions About Group III Bioreactors

Hardin et al. (83) developed what they called a "dimensionless design factor" (DDF) for rotating drum bioreactors. It assumes that both the headspace and substrate are well mixed, and represents the ratio of the estimated maximum heat production rate (R_Q) to the heat

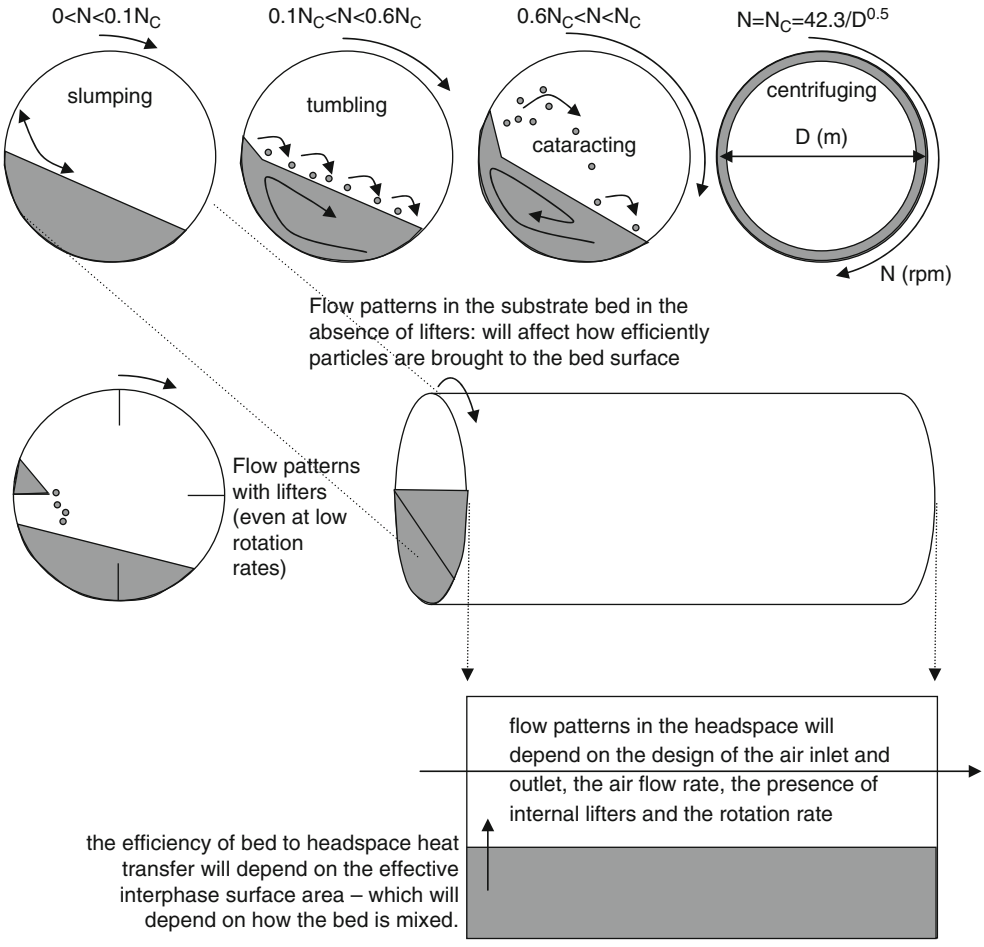

Fig. 7.20. Various design and operating variables that influence the heat and mass transfer phenomena within rotating drum bioreactors (60). Note that in the slumping regime, there is minimal mixing within the bed; it simply rises and falls as a single mass. N_C is the critical drum speed at which the solids are centrifuged against the sides of the drum.

removal rate.

$$\mathrm{DDF} = \frac{R_Q V_{bed}}{F_a C_{pa}(T_{out} - T_{in}) + F_a(y_{out} - y_{in})\lambda + h_{con}\pi D(D/2 + L)(T_{out} - T_a)} \tag{10}$$

where V_{bed} is the volume of the bed, F_a is the air mass flow rate, T_{in}, T_{out}, and T_a are the inlet air, outlet air, and surrounding air temperatures, respectively, y_{in} and y_{out} are the inlet and outlet humidities, respectively, D and L are the drum diameter and length, respectively, C_{pa} is the heat capacity of the air, λ is the heat of evaporation of water and h_{con} is the overall coefficient for heat transfer from the bed to the surrounding air by conduction through the drum wall.

Equation (10) can be used to provide an estimate of the air flow rates required by setting the DDF to 1 (that is, putting the rate of heat removal equal to the rate of heat production), R_Q to its maximum expected value ($R_{Q\,max}$), and the outlet air temperature to its maximum allowable value (T_{max}):

$$F_a = \frac{R_Q V_{bed} - h_{con}\pi D(D/2 + L)(T_{max} - T_a)}{C_{pa}(T_{max} - T_{in}) + (y_{out} - y_{in})\lambda} \tag{11}$$

Various parameter values need to be known in order to use this equation. As with Eq. (9), C_{pa} and λ can be obtained from reference books. The humidities y_{in} and y_{out} can be calculated from the Antoine equation, assuming that the air enters the bioreactor saturated at T_{in} and leaves saturated at T_{out}. The challenge is then to find a suitable estimate for the overall heat transfer coefficient of the bioreactor (h_{con}), before actually building the bioreactor, since the equation is being used for design purposes. Stuart et al. (84) used an equation developed by Kays and Bjorklund (85), which takes into account the effect of the drum diameter and rotational speed on convective heat removal from the drum surface. However, these have relatively little effect, so Stuart et al. (84) recommended the use of a value of h_{con} of 18,000 J/(h m^2 °C). Once these values are substituted, Eq. (11) gives an estimate of the necessary air flow rate for a given drum size and geometry, that is, for given values for the drum length and diameter. This air flow rate will depend on the values used for the operating variables T_{in} and T_a.

Hardin et al. (83) rearranged Eq. (11) to be explicit in T_{max} and used this rearranged equation to predict maximum temperatures expected for various experimental systems from the literature in which the operating conditions of the rotating drums were reported. This simplified approach overestimated the maximum temperature achieved in the bed by 2–5°C, meaning that it could be quite useful as a conservative design tool.

6.5.2. Model-based Approaches to Making Design Decisions about Group III Bioreactors

The DDF approach of Hardin et al. (83) assumes that the bed and headspace are in thermal and moisture equilibrium, with the headspace gases being saturated at the temperature of the solids, which is probably not a good assumption in practice. Two mathematical models have been proposed for rotating drum bioreactors that do not assume equilibrium but rather take into account bed-to-headspace heat and mass transfer. One model assumes that the substrate bed and headspace gas phases are each well-mixed in both in the axial and radial directions (84). This could be a reasonable approximation if the length to diameter ratio of the bioreactor is small, or even if the bioreactor is longer, but only if provisions are made for end-to-end movement of the solids, through adequate design of the lifters, and if multiple air inlets and outlets are provided. The other model assumes that both the substrate bed and the headspace gases are well mixed in the radial direction but that there is no mixing of either phase in the axial direction (86).

The model of the well-mixed rotating-drum bioreactor not only recognizes the substrate and headspace phases, but also formally treats the bioreactor body as a separate phase (Fig. 7.21a). Heat transfer between the various phases is described, as well as evaporation from the substrate bed to the headspace. The model agreed well with experimental data obtained at a laboratory scale (84). Using correlations that enable the estimation of heat

transfer coefficients for different scales (87), the model can be used to explore the design of large-scale bioreactors. If geometric similarity is maintained (that is, a constant L:D ratio), then heat removal through the bioreactor wall to the surrounding air makes an ever decreasing contribution to overall heat removal as the scale increases. This suggests that, if laboratory-scale studies are undertaken, the outer surface of the bioreactor should be insulated, in order to simulate the poor contribution of this heat removal mechanism at large scale. This strategy will enable better estimation of the air flow rate and relative humidity that should be used at a large scale.

The model's predictions give insights regarding the order of magnitude of air flow rates that would be necessary in order to enable adequate temperature control at small and large scale. For adequate temperature control, this being defined as bed temperatures never exceeding 38°C when a fast-growing organism with an optimum temperature of 30°C is cultivated, aeration rates as high as 0.3 vvm (volumes of 15% relative humidity air per total volume of bioreactor per minute) are necessary for a process involving 2 kg of cooked wheat bran in a 20 L bioreactor. With 250 kg of substrate, the aeration rate must be of the order of 3 vvm whereas with 2,000 kg of substrate, the aeration rate must be as high as 8 vvm.

Exploration of the predictions of the model that describes a rotating drum bioreactor in which there is no axial mixing suggests that for this type of operation, depending on the combination of operating conditions, significant axial temperature profiles can occur or the whole bed may reach high temperatures (Fig. 7.21b) (86). For example, in a simulation of the growth of *Aspergillus oryzae* in a 20 L rotating drum bioreactor with 2 kg of cooked wheat bran, the axial temperature profile in the bed was as large as 5°C. The model was used to predict the order of magnitude of the aeration rates that will be necessary to achieve adequate temperature control in larger-scale bioreactors. If the inlet air were maintained at 90% relative humidity throughout the cultivation, then normalized superficial velocities (that is values of V_Z/L, where V_Z is the volumetric flow rate divided by the cross sectional area of the headspace) of around 0.2/s would be required for a 204 L bioreactor and of around 1.2/s would be required for a 2,400 L bioreactor. However, if a control system were incorporated that switched the inlet air from 90% to 15% relative humidity whenever the outlet air temperature exceeded a set-point, then adequate temperature control could be achieved in the 2,400 L bioreactor with a normalized superficial velocity of only 0.5/s. Note that the use of dry air increases the evaporation rate and, therefore, necessitates the addition of water to the substrate during the cultivation. However, this is not a problem for rotating drums, as water can be distributed evenly simply spraying it onto the surface of the revolving bed.

6.5.3. Recent Directions in Characterizing the Phenomena in Group III Bioreactors

Recent studies have shed some light on the appropriateness of the idealized mixing regimes assumed by Stuart et al. (84) and Mitchell et al. (86). Hardin et al. (88) used CO as a tracer gas and analyzed the residence time distribution patterns in order to infer the flow pattern within the headspace. The data were consistent with the flow pattern shown in Fig. 7.21c. However, it is difficult to apply these results generally to rotating drum bioreactors, since headspace flow patterns are significantly affected by the design of the air inlet and outlet, the air flow rate, and the presence of lifters. Once such flow patterns are determined, it is possible to use them

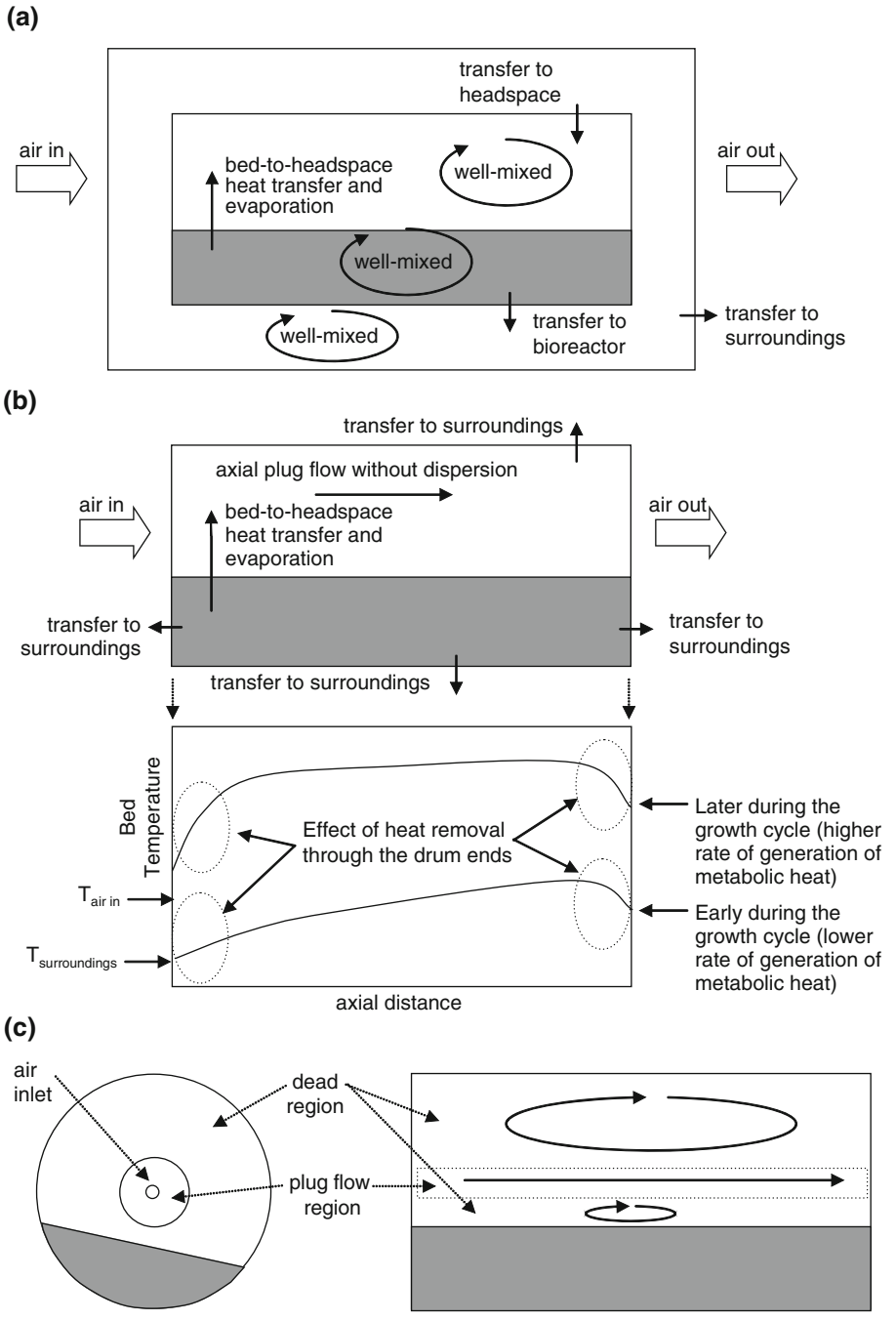

Fig. 7.21. Modeling of rotating drum bioreactors (84, 86, 88). (**a**) How the model of Stuart et al. (84) describes a well-mixed rotating drum bioreactor. (**b**) How the model of Mitchell et al. (86) treats a rotating drum bioreactor in which axial mixing is insignificant, and typical predictions that this model makes about axial temperature profiles in the substrate bed. (**c**) More realistic headspace flow patterns as determined by Hardin et al. (88).

to obtain estimates for the overall coefficients that characterize the bed-to-headspace heat and mass transfer and to express them as functions of the air flow rate and the rotation rate (89). However, as yet there is insufficient knowledge for the development of general relationships; it will be necessary to undertake experimental studies with a wider range of geometries.

Studies have also been undertaken regarding the degree of axial mixing within the substrate bed in rotating drums, using colored substrate particles (90, 91) and nuclear magnetic resonance (92). More recently, Schutyser et al. (82) introduced the powerful tool of discrete particle modeling into the analysis and prediction of mixing in solid-state cultivation bioreactors. They applied this technique to investigate baffles designs in rotating drums and showed that the use of curved baffles in an inclined drum would lead to the most effective radial and axial mixing.

6.5.4. Synthesis of Our Knowledge about How Best to Operate and Design Group III Bioreactors

It is possible to draw some general conclusions about the design and operation of rotating drum bioreactors in batch mode:

- Lifters are highly desirable in order to promote bed-to-headspace heat and mass transfer and radial mixing, and can also be designed so as to promote axial mixing.
- In order to avoid significant axial temperature gradients, it is best not to have a very high length to diameter ratio, in which case, curved baffles can give adequate axial mixing of the substrate bed. If high length to diameter ratios are used, then multiple air inlets and outlets should be provided.
- At large scale, air flow rates of as much as 10 volumes of air per bioreactor volume per minute will be required for adequate temperature control.
- Although discontinuous rotation has been suggested as a possible operating strategy (93), it is unlikely to enable adequate temperature control at large scale.

More work is required to characterize flow patterns with the headspace and beds and, based on these studies, to arrive at correlations that allow bed-to-headspace heat and mass transfer coefficients to be estimated as functions of key design and operating variables.

Although rotating drums are well suited to continuous processes, little attention has been given to developing quantitative design rules for this mode of operation. Further, relatively little attention has been given to the operation of stirred drums. They will have many similarities with rotating drums, however, the design of the agitator and the resultant mixing patterns in the bed have received little attention.

6.6. Design of Group IV Bioreactors

The design and operation decisions that need to be made for Group IV bioreactors are:

- How large should the bed be, and what should be its geometric proportions?
- What should the flow rate, temperature, and humidity of the process air be? Note that since water can easily be added uniformly to a mixed bed, unsaturated air can be used at the air inlet in order to promote evaporative cooling.
- When should additions (e.g., replenishment of water) be made to the bed?
- Should any cooling surfaces be provided, and, if so, what should be the cooling water flow rate and temperature?

- What type of mixing action should be provided and, for mechanically-mixed beds, how should the agitator be designed?
- What should be the intensity of mixing? Should mixing be intermittent but frequent? If so, what should be the frequency and duration of mixing events? Note that, for frequently-mixed beds, mixing plays a role in temperature control, unlike infrequently-mixed beds wherein its role is to allow water replenishment or prevent high pressure drops (Sect. 6.4).

The decisions that will be made in response to these questions will be affected by the following considerations:

- The rate of heat generation by the process organism.
- The degree to which evaporative cooling is used as a cooling mechanism.
- The effect of the various options for mixing method and mixer design on the organism and substrate.

6.6.1. Simple Approaches to Making Design Decisions about Group IV Bioreactors

The modified Damkohler number of Mitchell et al. (76) (Eq. (8)) can be modified to be applied to well-mixed beds, assuming that heat removal through the bioreactor wall makes a contribution that is sufficiently small to be ignored:

$$\mathrm{Da_M} = \frac{R_\mathrm{Q} V_\mathrm{bed}}{F_\mathrm{a}(C_\mathrm{pa} + f\lambda)(T_\mathrm{out} - T_\mathrm{in})} \tag{12}$$

where the symbols are as previously defined. By setting this modified Damkolher number to 1, Eq. (12) can be used for to estimate the aeration rate (F_a) that will be necessary in order to maintain the bed temperature below a certain upper limit (T_max):

$$F_\mathrm{a} = \frac{R_\mathrm{Q\,max} V_\mathrm{bed}}{(C_\mathrm{pa} + f\lambda)(T_\mathrm{max} - T_\mathrm{in})} \tag{13}$$

6.6.2. Model-Based Approaches to Making Design Decisions about Group IV Bioreactors

If it is reasonable to assume that the substrate bed is well-mixed, then the water and energy balance equations will be ordinary differential equations, which are reasonably easy to solve with standard numerical integration programs. Consequently, researchers who have developed models for Group IV bioreactors have typically paid more attention to the kinetics of growth and product formation. Such models can then be used to explore the effect of operating variables on predicted bioreactor performance. This has been done in several studies:

- Nagel et al. (64) proposed a model that divides the water in the substrate particle into intracellular and extracellular water, and used the model to explore on-line control of moisture content and temperature.
- dos Santos et al. (94) used a model to describe the effect of operating conditions on the bed temperatures reached within a simple well-mixed bioreactor, and the effect of these temperatures on the denaturation of enzymes produced by the process organism.

More recently, Schutyser et al. (70) applied discrete particle modeling to investigate mixing patterns in the conical helical-blade mixer, which they proposed as a potentially useful design for well-mixed solid-state cultivation bioreactors.

Models for fluidized bed bioreactors have not considered the energy balance because the high aeration rates provide sufficient cooling. The main design challenges are related to adequate fluidization of the substrate particles, a subject that will not be addressed here.

To refine these models, it is probably interesting to incorporate the effects of mixing on growth into the model, especially for the case in which the process organism is a fungus. Although the deleterious effect of agitation on fungal hyphae is well known, as yet there is insufficient quantitative information to describe this effect mathematically.

7. ASSOCIATED ISSUES THAT MUST BE CONSIDERED IN BIOREACTOR DESIGN

7.1. A Challenge in all Bioreactor Types: Design of the Air Preparation System

For almost all bioreactors, it is necessary to design an air preparation system. The only exceptions are those tray-type bioreactors that are incubated in a chamber without conditioned air. The basic task is to provide air at the required flow rate, temperature, and humidity. The required conditions for the inlet air are deduced from quantitative bioreactor design strategies, such as those outlined in Sect. 6. Note that it is not necessarily the case that the design and optimization of operation of the bioreactor is decided and only then the air preparation system is designed. Limitations on what it is feasible or economical to do with the air preparation system can affect decisions about how to operate the bioreactor.

Typically, it will be advantageous to change the conditions of the inlet air during the process, this being because the aeration system has a key role to play in temperature control and the need for heat removal typically varies significantly through the growth cycle of the organism. Note that the simple design approaches represented by Eqs. (9), (11), and (13) can be used to calculate the aeration rates necessary at the time of peak heat production. These rates should be sufficient to cool the bioreactor during the entire growth cycle. However, use of an aeration rate based on the peak heat production rate means that the aeration rate will be unnecessarily high during the great majority of the growth cycle. Therefore the model-based approach, which incorporates growth kinetic equations, has the advantage of being able to be used to determine how the need for heat removal changes during the process and consequently, how the required aeration conditions change during the process.

In order to vary the cooling effect provided by the aeration system, any of various variables can be manipulated, namely the inlet air temperature, humidity, and flow rate. It is possible to design an air preparation system to control humidity and temperature simultaneously, however, it would be economically unfeasible due to the sophistication that would be necessary since air humidity has a non-linear dependence on the temperature. In general, solid-state cultivation processes operate at low profit margins, especially those in environmentally related applications. As a result, the air preparation system must have low operating and maintenance costs. A good compromise can be obtained using a humidification column that provides saturated air at a particular temperature with a by-pass if evaporative cooling with dry air is required. In the system suggested in Fig. 7.22, which is similar to that of Agosin et al. (95), the humidifying column assures air saturation and the water temperature can be used to control the temperature of the outlet air.

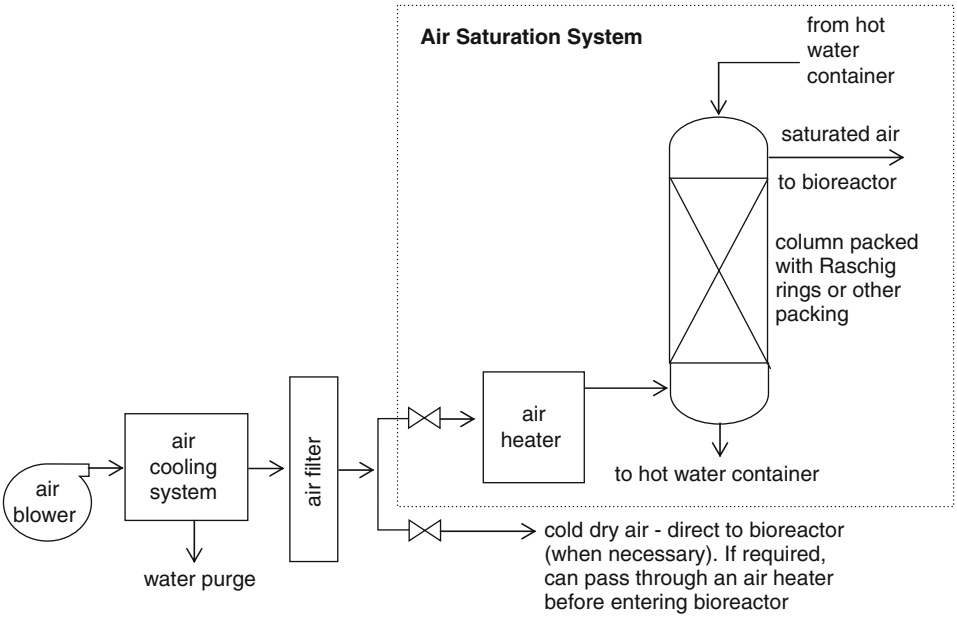

Fig. 7.22. Basic features of an air preparation system that can be used to provide saturated or dry air to a bioreactor, as required (95). A control system will be needed to direct the air flow in the desired direction (i.e., to provide saturated or dry air to the bioreactor).

7.2. Monitoring and Control Systems for Bioreactors

Given that the required operating conditions change over time with changes in the microbial activity, it is essential to have a monitoring (data acquisition) system and a control system for the bioreactor that makes on-line measurements of key process variables and makes changes in selected operating variables (manipulated variables), in the most effective manner possible, in response to these measured values.

7.2.1. Equipment for On-Line Monitoring

Key process variables for which it would be desirable to have on-line measurements include:

- *The bed temperature, possibly at different locations within the bed.* Bed temperature is important because it has significant effects on microbial growth and when it deviates from the optimum value it will be necessary to undertake control actions.
- *The pressure drop over the bed*. Pressure drop is important in those bioreactors that are forcefully aerated, since it represents a resistance to air flow and, if the pressure drop gets too high, it may be desirable to undertake a control action, such as agitating the bed to increase the bed porosity.
- *The rate of growth of the organism*. This is a key variable that can be used to evaluate process performance. Note that direct measurement of growth rates is highly problematic in solid-state cultivation systems. However, an indirect estimate can be obtained from respiration rates.
- *The water activity of the bed*. The water activity of the bed is important because it has significant effects on microbial growth. If it is likely to deviate significantly during the process, which

depends on the sorption isotherm of the substrate and how the bioreactor is operated, then it will be necessary to undertake control actions. Note that if the sorption isotherm of the substrate is known, it may be sufficient to determine the water content, although the presence of the microorganism and changes in the substrate due to growth-related processes may mean that the sorption isotherm changes during the process, a phenomenon that has received little attention.

- *The flow rate, temperature and humidity of the air supplied to and leaving the process*. As discussed in Sect. 1, it is important to control the inlet air conditions at the values required by the process. However, it is not a simple matter to do this, and it is necessary to monitor the inlet air variables to ensure that they are in fact at their desired values and, if they are not, to activate the system responsible for controlling the inlet air. The outlet air conditions can give valuable information about water losses from the bed.

Note that other key variables, such as average biomass and average nutrient concentrations, can only be measured off-line and do not give results quickly enough to be used in control systems. This section focuses on equipment and methods for on-line measurements. However, the necessity to remove samples for off-line measurements does raise the issue of the design and operation of the sampling systems. Note that it will typically be desirable to remove samples from the substrate bed during the process. If the bed remains static, it will probably be necessary to remove samples from several locations since the conditions in the bed and the resulting growth and product formation will be heterogeneous. This may need to be done without disturbing bioreactor operation, but this will be difficult. For example, the removal of samples from the interior of a packed-bed will typically leave holes that will allow preferential flow of the air, diminishing the effectiveness of the aeration of the solids. Note also that for processes that are not resistant to contamination, it will be necessary to design the sampling system so as to operate aseptically.

In designing the bioreactor, it is necessary to consider what on-line monitoring equipment should be incorporated into the design. Various possibilities, some of which are illustrated in Fig. 7.23, are listed below.

- *Temperature measurement*. Bed temperatures can be measured with thermocouples or thermistors inserted into the substrate bed. Although thermistors are more expensive and more fragile than thermocouples, they are more stable and precise. The temperature probes may need to be withdrawn from the bed before the bed is mixed. The measurement of temperature at different positions gives information about the efficiency of heat removal and will allow the implementation of a more adequate and robust control strategy.
- *Measurement of the pressure drop across the bed*. The pressure drop across the bed can be measured using differential pressure meters that work on the piezoelectric principle. These meters have short response times and are not affected by the gas temperature.
- *Measurement of bed water content*. Although various probes could potentially be used to measure bed water contents on-line, such as capacitance or conductivity-based devices, these will be affected by the probe-solid contact, which can vary significantly during the process. Possibly the most promising devices are those based on the emission of microwaves and analysis of the reflected signal by Time Domain Reflectometry (96). Potentially, this method can be used to map moisture contents in three dimensions, although the methods for doing this are still under development (96).
- *Measurement of gas phase temperature and relative humidity*. Gas phase relative humidity in the inlet and outlet gas can be measured with various probes in which the capacitance or resistance

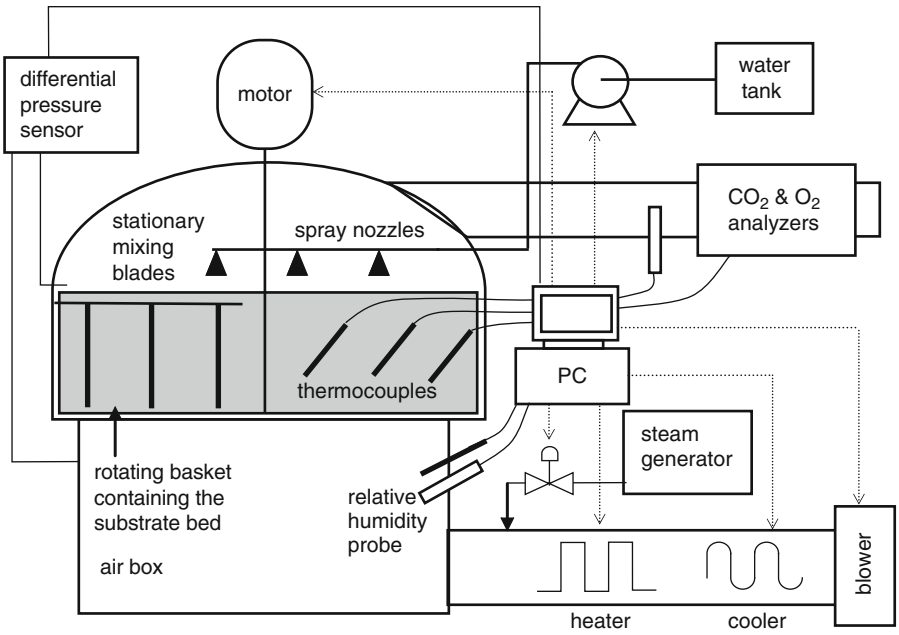

Fig. 7.23. A possible system for monitoring and control of a solid-state cultivation bioreactor, based on the system used by Agosin et al. (95).

of a sensor varies with the relative humidity of the gas phase with which it is in equilibrium. However, these tend to have relatively long response times. Relative humidity meters that operate on the dew point principle may be preferable as they have rapid response times (96). The temperature measurement can be done using thermocouples or thermistors.

- *Measurement of gas phase O_2 and CO_2 concentrations*. The respiration rate of the process organism, calculated through monitoring O_2 and CO_2 concentrations in the off-gases from the bioreactor, can give valuable information about the growth rate and metabolic state. Although on-line gas chromatography can be used to monitor not only these gases but also various volatile metabolites, gas analyzers, such as paramagnetic analyzers for O_2 and infrared analyzers for CO_2, tend to be more precise and have faster response times. Although they are relatively expensive, they are durable, precise, and suffer relatively little from interference with other gases if the gas is dried before analysis.
- *Measurement of gas flow rates*. There are many methods available to measure gas flow rates. The volumetric flow rate of the gas can be measured with Pitot tubes, Venturi tubes, rotameters, anemometers, and turbines, to name a few. The mass flow rate of the gas can be determined with thermal mass meters, which are more expensive.

Note that this survey of available measuring devices is only very brief. In order to choose the appropriate equipment when more than one device is available for a given measurement, it is necessary to understand the abilities and limitations of the various devices and to weigh this against its capital and maintenance costs.

In some cases, equipment for a desired measurement is not available or is prohibitively expensive. In these cases, it may be possible to develop inference models (so-called "soft-sensors"), which use other measurable variables to estimate the desired variable. For example,

Penha y Lillo et al. (97) used a mass balance, based on the relative humidity of the inlet and outlet gases and the consumption and production of water in growth-related processes, to estimate the water content of the substrate bed. This brings up the need for data filtering. All measuring devices suffer from random or systematic measurement errors, and data filtering will be necessary to smooth out the readings in order to identify the underlying "real" trends.

7.2.2. Control Strategies for Solid-State Cultivation Bioreactors

When measured process variables such as those discussed in the previous section deviate from values that are optimal for the process, it is necessary to manipulate the operating variables in a manner such as to counteract the deviation and, as far as is possible, to bring the process variables back to their optimal values. For example, when the bed temperature rises above the optimum, the flow rate of the inlet air might be increased or its temperature decreased, or both. The design of solid-state cultivation bioreactors involves not only decisions about the physical appearance of the bioreactor, but also decisions about the operating strategy, in other words part of the design task is to devise the control strategy.

A discussion of process control strategies is beyond the scope of this chapter. However, experience in solid-state cultivation systems has shown that simplistic control strategies, in which a single operating variable is manipulated in response to a single measured variable, are inadequate for controlling solid-state cultivation bioreactors (98). Given that evaporative cooling is often involved in temperature control, simultaneous control of the temperature and moisture levels can only be achieved with a multiple-input, multiple-output scheme.

Data filtering, mentioned in the previous section, is an essential part of any control scheme, as it obviously makes no sense to undertake control actions in response to random noise or even systematic deviations of the data acquisition equipment. Therefore, before being used in the control algorithm, the data is treated with mathematical filtering procedures such as Kalman filtering or Butterworth filtering to eliminate this measurement noise.

Given the complex nature of solid-state cultivation systems, and that much of the behavior of the system is driven by the growth kinetics of the process organism, which follow defined rules, it is appropriate to use predictive control algorithms, such as Dynamic Matrix Control, that take into account the predicted future behavior of the system based on a model of the process, rather than simpler algorithms, such as PID, which is simply reactive in nature.

Control of solid-state cultivation systems in large-scale bioreactors has proved to be a very difficult task, and is still far from a satisfactory solution (99, 100). Indeed, there are only few examples of rational use of modern control strategies in the optimization of large-scale bioreactors. Figure 7.23 shows an example of the monitoring and control system for a pilot-scale bioreactor built in Chile (95, 101).

8. FUTURE PERSPECTIVES

As discussed in this chapter, quantitative design rules for bioreactors for solid-state cultivation processes are already available, including simplified methods that can give answers of the right order of magnitude to design questions. Mathematical models are potentially more powerful design tools. Various of these models are discussed in greater detail in Mitchell et al. (102). However, the models that have been developed to date have not yet been converted

into fully flexible and user-friendly tools. In other words, for an engineer to adapt one of the current models to their own system, which might simply have a different isotherm, a different kinetic equation for microbial growth or a different geometry, it would be necessary to change the original source code, which requires the engineer to have modeling and programming skills. An ideal design tool would allow such equations to be entered directly into a specific dialog box on the computer screen. Of course, incorporating such flexibility into a program is a challenging task. Furthermore, there is much scope for improvement of the models themselves, including the following:

- Development of general relationships to express key model parameters (e.g., heat transfer coefficients) as functions of design and operating variables;
- Improvement in the modeling of growth kinetics, especially in the face of conditions that vary during the growth cycle;
- Extension to describe systems more realistically. For example, in various models, it will be appropriate to replace assumptions of equilibrium between the solid and gas with equations that describe heat and mass transfer.

The discrete particle modeling approach that has been used to simulate mixing patterns in rotating drum bioreactors can be extended to other bioreactors and, with the incorporation of water transfer and heat transfer into the model, it is potentially a powerful tool for use in the bioreactor design process (103). However, despite the promise of this approach, it will probably be some time before discrete particle modeling is used widely in the design of solid-state cultivation bioreactors, since the method requires advanced mathematical skills and the models make very heavy demands on computing power. Such models may take days or even weeks to run, during which time several cultivations could be undertaken experimentally.

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NOMENCLATURE

α = bed to air heat transfer coefficient, $J/(h\,m^2\,^\circ C)$
ξ = dimensionless height within the bed, dimensionless
δ = overall bed height within a tray, m
Θ = temperature difference between the bottom of the solid medium and the upper surface in a tray in the case where heat is not transferred across the bottom surface to the air, $^\circ C$
λ = enthalpy of evaporation of water, J/kg
μ_{opt} = specific growth rate constant under optimal conditions, per hour

μ = specific growth rate constant, per hour
ρ_a = density of air, kg/m^3
BOD = Biological Oxygen Demand, mg/L
COD = Chemical Oxygen Demand, mg/L
$C_g = O_2$ concentration in the gas phase surrounding the tray, kg/m^3
C_{pa} = heat capacity of air, J/(kg °C)
D = diameter, m
Da_M = modified Damkohler number, dimensionless
D_e = effective O_2 diffusivity within the substrate bed within a tray, m^2/h
f = rate at which the saturation humidity of air increases with temperature, kg/(kg °C)
F_a = air flow rate, kg/h
h_{con} = coefficient for heat transfer through the bioreactor wall, J/(h m^2 °C)
H_c = critical bed height for a tray, m
k = thermal conductivity of the bed, J/(h m °C)
L = length, m
N_{Bi} = Biot number, dimensionless
N_C = Critical drum rotational speed, rpm
$R_{O2\,max}$ = maximum volumetric rate of O_2 consumption kg/(h m^3)
R_Q = volumetric rate of heat generation, J/(h m^3)
$R_{Q\,max,}$ = maximum volumetric heat production rate, J/(h m^3)
t = time, h
T = bed temperature, °C
T_a = surrounding air temperature, °C
T_{in} = Inlet air temperature, °C
T_{out} = Outlet air temperature, °C
T_s = temperature at the surface of the bed, °C
V = volume of the substrate bed, m^3
V_Z = superficial velocity of the air, m/h
Y_{QO} = stoichiometric coefficient relating heat yield with oxygen consumption, J/kg
X = biomass content, kg/m^3
X_{max} = maximum possible biomass content, kg/m^3
y_{out} = humidity of the outlet air, kg/kg
y_{in} = inlet air humidity, kg/kg
Y_{QX} = stoichiometric heat yield from growth, J/kg
Y_{xo} = Yield of biomass from O_2, kg/kg
z = height within the bed, m

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8

Value-Added Biotechnological Products from Organic Wastes

Olena Stabnikova, Jing-Yuan Wang, and Volodymyr Ivanov

CONTENTS

Abstract Different organic wastes may be used as raw material for value-added products. The chapter represents organic wastes as a raw material for biotechnological transformation and gives brief descriptions of biotechnologies for transformation of organic wastes into such value-added products as enzymes, organic acids, flavours, polysaccharides, mushrooms, biodegradable plastics, animal feed, biomass for bioremediation, dietary fibers, pharmaceuticals, gibberellic acid, chemicals (acetone and butanol, glycerol) and fuel (ethanol and hydrogen). It describes microorganisms, which can be used for biosynthesis of value-added products, and highlights most essential factors affecting the process of their biosynthesis. Examples of biotechnologies for biotransformation of organic wastes into value-added products are presented. Such environmental biotechnologies as bioconversion of organic waste into compost or fertilizer, recovery of metals from solid and liquid wastes, recovery of phosphate and ammonium from liquid wastes by application of iron-reducing and iron-oxidizing bacteria are described in the chapter.

Key Words organic waste • biotransformation • biotechnological products • environmental biotechnology.

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1. ORGANIC WASTES AS A RAW MATERIAL FOR BIOTECHNOLOGICAL TRANSFORMATION

Many organic wastes may be used in biotechnological transformation of value-added products, such as enzymes, single-cell protein, fuels, chemicals, and medicines. For instance, the inedible parts of plants, agricultural residues from the harvesting, and food processing waste can be used as raw materials for biotransformation, amounting to more than 13×10^9 tons/year (1). These organic wastes contain valuable components, such as starch in potato and cassava pulp, pectin in apple pomace, sucrose in molasses, cellulose and hemicellulose in wood, garden waste, and rice hull. The European Union alone produces 1,000 million tons of agricultural waste, 500 million tons of garden and forestry waste, and 250 million tons of organic waste from food processing industry. A significant part of these wastes can be transferred into value-added products. Table 8.1 illustrates the scale of organic waste production in different countries. The elemental composition of selected food processing waste is shown in Table 8.2.

2. BIOTECHNOLOGICAL PRODUCTS OF ORGANIC WASTE TRANSFORMATION

Vegetable and fruit processing wastes contain mainly starch, cellulose, and organic acids. These substances can be used for biotechnology as raw material. Table 8.3 lists various biotechnological products that can be produced from different food processing wastes.

Table 8.1
Food processing waste production in different countries

Waste	Quantity, tons/year	Country	Reference
Organic waste from potato, vegetable and fruit processing	380,000	Germany	(2)
Apple pomace	250,000	Germany	(3)
Grape pomace	9,000,000	World production	(4)
Grape pomace	300,000	USA (California)	(5)
Tomato pomace	14,000	Portugal	(6)
Pineapple peel	400,000	Australia	(7)
Olive pomace	36,000	Jordan	(8)
Olive pomace	100,000–120,000	Turkey	(9)
Olive pomace	250,000	Spain	(10)
Cranberry pomace	9,525	USA	(11)
Orange peel	3,300,000	USA (Florida only)	(12)
Sugar beet pulp (dry matter)	14,000,000	All countries of European Union	(13)
Cassava pulp	1,000,000	Thailand	(14)

Table 8.2
Elemental composition of selected food processing waste

Food processing waste from the treatment of	Elemental composition of food processing wastes, element mg/kg of dry matter			
	C	N	P	K
Cabbage	438,300	20,117	4, 114	24, 245
Cucumber	436,600	27,300	5, 823	41, 011
Eggs shell	146,600	3,867	0, 862	0, 522
Green vegetables	373,300	55,300	6, 167	50, 553
Long bean	446,700	48,700	3, 290	21, 615
Mixed vegetables	444,700	55,200	4, 401	36, 563
Noodle	442,600	30,033	0, 826	1, 246
Potato	483,333	15,430	2, 240	13, 678
Rice	445,000	22,600	1, 248	0, 390
Green bean	409,900	40,000	3, 021	7, 775
Red bean	410,100	44,133	3, 149	7, 370
Soya bean	481,300	69,900	4, 254	11, 902
Fish	494,300	73,700	7, 200	8, 287
Pork	435,400	88,100	6, 920	7, 756
Chicken	549,100	67,100	6, 311	7, 699
Beef	587,700	70,300	5, 497	6, 801
Duck	492,500	68,200	11, 195	5, 752

2.1. Solid-State Fermentation for Bioconversion of Agricultural and Food Processing Waste into Value-Added Products

Solid-state fermentation (SSF), a general method for food processing waste bioconversion, is a process in which microorganisms grow on or within solid substrates in the absence of free water. However, substrates must possess enough moisture to support the growth and metabolism of microorganisms (56). The solid material in this process acts both as physical support and source of nutrients. To simplify product isolation from the medium, for example, polyurethane foam may be used instead of natural raw material such as wheat bran. SSF has been conventionally more applicable for filamentous fungi, but yeast and even bacteria are successfully used for biotechnological production by solid-state fermentation. SSF is a low-level technology in comparison with industrial submerged fermentation, but it appears to be a promising technology for the utilization of solid wastes. SSF is of special interest to countries with an abundance of agro-industrial residues that can be used as inexpensive raw materials. SSF has many advantages in processing agro-industrial residues as compared with submerged fermentation: lower energy requirements, process simplicity, cheaper aeration, absence of rigorous control of fermentation parameters and production of smaller quantity of

Table 8.3
Bioconversion of food processing waste in products

Waste	Value-added product	Microorganism	Reference
Apple pomace, cranberry pomace and strawberry pomace	Enzyme Polygalacturonase	*Lentinus edodes*	(11)
Wheat bran, sunflower flour, coffee husk, soybean meal, rice bran, corn bran, rice hull, aspen wood, sweet potato residue, waste hair	Enzymes Proteases (acidic, neutral and alkaline)	*Aspergillus* sp., *Penicillium* sp., *Rhizopus* sp., *Bacillus* sp., *Trichoderma* sp.	(15)
The mixture of sugar cane bagasse with wheat bran or orange bagasse	Enzyme Pectin lyase and polygalacturonase	*Thermoascus aurantiacus*	(16)
Lemon pulp	Enzyme Pectinase	*Trichoderma viride*	(17)
Wheat bran, rice bran, apple pomace	Enzyme Pectinase	*Bacillus* sp.	(18)
Wheat bran, rice bran, coconut oil cake and corn flour	Enzyme Inulinase	*Staphylococcus* sp., *Kluyveromyces marxianus*	(19)
Lignocellulosic wastes, sugarcane bagasse	Enzymes Cellulase β-glucosidase	*Aspergillus ellipticus*, *Aspergillus fumigatus*	(20)
Banana waste	Enzymes α-amylase Cellulases	*Bacillus subtilis*	(21, 22)
Tea waste	Enzyme Glucoamylase	*Aspergillus niger*	(23)
Wheat bran, rice straw and minerals	Enzymes Cellulases	*Aspergillus ustus*, *Botrytis* sp., *Trichoderma* sp., *Sporotrichum pulverulentum*	(24)
Copra paste, and spent coffee	Enzyme β-mannanase	*Aspergillus oryzae* *Aspergillus niger*	(25)
Carrot-processing waste	Organic acid Lactic acid	*Rhizopus oryzae*	(26)
Hemicellulosic hydrolysate from *Pinus taeda* chips	Organic acid Lactic acid	*Rhizopus oryzae*	(27)
Kumara, a starch-containing root crop	Organic acid Citric acid	*Aspergillus niger*	(28)

(Continued)

Table 8.3
(Continued)

Pineapple waste	Organic acid Citric acid	*Aspergillus foetidus* *Aspergillus niger*	(29)
Sugarcane bagasse	Amino acid L-glutamic acid	*Brevibacterium* sp.	(30)
Sweet potato residue	Antibiotic Tetracycline Chlorotetracycline	*S. viridifaciens*	(31)
Soybean curd residue, okara	Antibiotic Iturin A	*Bacillus subtilis*	(32)
Wheat bran	Plant growth hormone Gibberellic acid	*Gibberella fujikuroi*	(33)
Cassava flour, sugar cane bagasse	Plant growth hormone Gibberellic acid	*Gibberella fujikuroi*	(34)
Hydrolyzed tomato pomace	Vitamin B_{12}	*Propionibacterium shermanii*	(35)
Prawn-shell waste	Single cell protein	*Candida* spp. *Rhodotorula* spp.	(36)
Olive pomace after delignification and saccharification	Poultry feed enriched by protein	*Candida utilis* or *Saccharomyces cerevisiae*	(8)
Apple pomace	Animal feed enriched by protein	*Aspergillus niger* and *Candida utilis*	(37)
Hydrolyzed potato starch waste	Exopolysaccharide Pullulan	*Aureobasidium pullulans*	(38)
Grape skin pulp extract, starch waste, olive oil waste effluents, molasses	Exopolysaccharide Pullulan	*Aureobasidium pullulans*	(39)
Sent malt grains, apple pomace, grape pomace, and citrus peels	Exopolysaccharide Xanthan	*Xanthomonas campestris*	(40)
Olive mill wastewaters	Exopolysaccharide Xanthan	*Xanthomonas campestris*	(41)
Coconut waste	Bacterial endotoxins Insecticide	*Bacillus thuringiensis*	(31)

(Continued)

Table 8.3
(Continued)

Sugar beet pulp	Flavor Vanillin	*Pycnoporus cinnabarinus*.	(42)
Cassava bagasse and giant palm bran	Fruity aroma compounds	*Kluyveromyces marxianus*	(43)
Waste material of the pineapple juice production	Ethanol	*Zymomonas mobilis*	(44)
Pineapple cannery waste	Ethanol	*Saccharomyces cerevisiae*	(45)
Corn fiber (after acidification)	Butyrate	*Clostridium tyrobutyricum*	(46)
Sugar refinery wastewater	Hydrogen	*Rhodobacter sphaeroides*	(47)
Starch-manufacturing wastes (sweet potato starch residue)	Hydrogen	*Clostridium butyricum* *Enterobacter aerogenes*	(48)
Waste water of a starch factory	Hydrogen	Anaerobic microflora	(49)
Organic waste (wheat grains)	Hydrogen	*Bacillus licheniformis*	(50)
Organic waste (whey)	Hydrogen	*Rhodopseudomonas*	(51)
Olive pomace	Biogas	Anaerobic microflora	(9)
Molasses	Biosurfactant	*Bacillus subtilis*	(52)
Potato wastes	Biosurfactant	*Bacillus subtilis*	(53)
Potato processing waste	Poly-β-hydroxybutyrate	*Alcaligenes eutrophus*	(54)
Organic waste	Compost	Consortium of microorganisms	(55)

wastewater. SSF was studied for the production of value-added products such as antibiotics, alkaloids, plant growth factors, enzymes, organic acids, biopesticides, biosurfactants, biofuel, and aroma compounds and for the enhancement of crop residues using protein and vitamins (2, 15, 31, 57). SSF is used commercially in Japan and India (Biocon Company) to produce industrial enzymes (58).

The most important parameters for solid state fermentation are water content, temperature, pH, and aeration. Water content for solid state fermentation varies from 10 to 80%. The minimum water content for molds and yeast growth is 10–20% (1). Bacteria usually grow at higher water content than needed for molds and yeasts. Optimal water content is specific for different microorganisms and for different physiological processes such as growth, sporulation, and production of primary or secondary metabolites. Microbial cultures, which are usually used for SSF, represent mesophilic organisms with optimal temperatures from 25 to 32°C. Heat is generated during the growth of microorganisms; therefore, to maintain

the necessary temperature in a SSF bioreactor, different methods of cooling, such as forced intensive aeration, can be used. Molds can grow at a pH ranging from 5.0 to 8.0, while yeasts prefer slightly acid pH in the range from 4.5 to 6.0. The optimal pH for most bacterial cultures is near neutral. Usually, pH is adjusted at the beginning of process and is not monitored and controlled during SSF. Aeration is needed to maintain aerobic conditions, to control temperature, and to regulate water content in the matter. Mixing of the fermented substrate is done to ensure homogeneity of matter, distribution of inoculum, better aeration, and the same conditions for microbial growth in different parts of reactor. The basic steps of the waste biotransformation by SSF are shown in Fig. 8.1. Some steps, such as pretreatment, and sterilization, product isolation, are optional. Pre-treatment may include shredding, and soft acid, or alkaline hydrolysis of waste. Centrifugation, filtration, or extraction of product by chemical solvents can be used.

The design of a SSF bioreactor should take into account mass transfer, cooling, diffusion, and extraction of metabolites (59, 60). The basic principle of SSF is the "solid substrate bed," which contains moist solids and an interparticle voids phase. Mixing and aeration regimes vary for different types of SSF bioreactors. Traditionally, tray and drum type bioreactors have been used. A tray bioreactor can have unmixed beds without forced aeration (oldest and simplest design). Packed beds, on the other hand, consist of unmixed beds with forced aeration, and

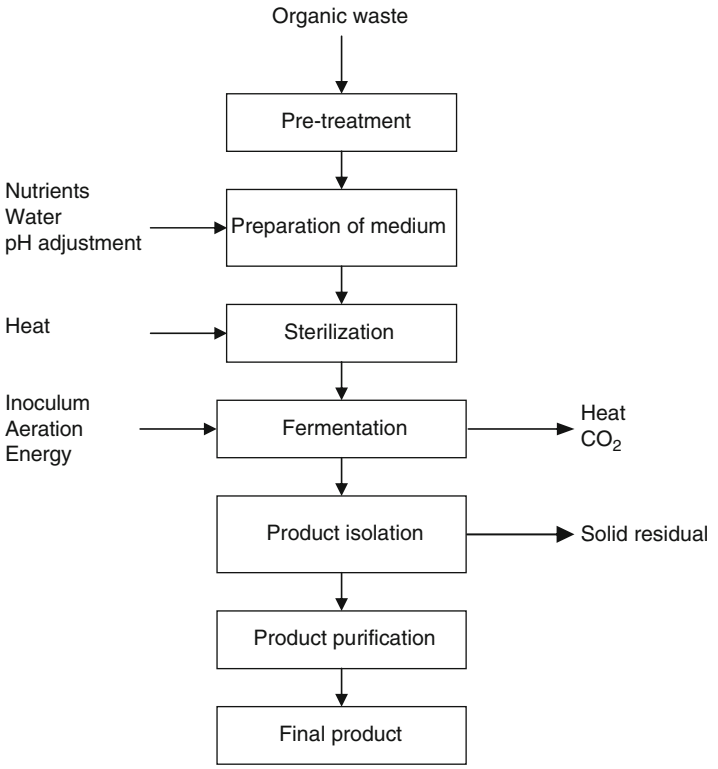

Fig. 8.1. Flowchart of waste biotransformation into value-added products by solid state fermentation.

rotating drums have intermittent agitation without forced aeration, operating on continuous or semi-continuous mode. Intermittently, mixed bed bioreactors with forced aeration can be described as packed beds in which conditioned air passes through the bed. There are also SSF bioreactors with both continuous mixing and forced aeration such as the gas–solid fluidized bed, the continuously stirred aerated bed, and the rocking drum bioreactor.

2.2. *Production of Enzymes*

An enzyme is a biological catalyst that increases the rate of specific biochemical reactions by many times at room temperature. The classification of enzymes by the International Union of Biochemists includes six functional classes: (a) oxidoreductases act on different chemical groups to add or remove hydrogen atoms; (b) transferases transfer functional groups between donor and acceptor molecules; (c) hydrolases add water across a bond, hydrolyzing it; (d) lyases add water, ammonia, or carbon dioxide across double bonds or remove these molecules to produce double bonds; (e) isomerases carry out all kinds of isomerization; (f) ligases catalyze reactions in which two chemical groups are joined with the use of energy from ATP.

Enzymes are widely used in food industry, textile industry, as components of detergents, in medicine, and cosmetics. The industrial production of many enzymes includes cultivation of microorganisms (fungi or bacteria) on a specific nutrient medium, which must be completely aseptic (do not contain other viable microbes than cultivated strain). Microorganisms can produce intracellular enzymes (inside cell) and extracellular enzymes (enzymes, which are excreted from cells to medium). Isolation and purification processes, known as downstream processing, follow microbial cultivation. Intracellular and extracellular enzymes must be extracted from biomass or medium after cultivation and then purified. The composition of the medium greatly affects the enzyme biosynthesis process. To enhance productivity, some ingredients, namely inductors, can be added to the media.

Food processing waste can be used as a substrate for the low-cost microbial production of amylolitic enzymes such as α-amylase, which is widely used in the food, textile, and paper industries. Extracellular enzymes are used to split 1,3-α-D-glucosidic linkages between adjacent glucose units into the linear amylase chains. Different wastes such as potato, soybean meal, and fruit processing waste can be used for α-amylase production. For example, the production of α-amylase by *Bacillus subtilis* was proposed on banana fruit stalk through solid sate fermentation (21). The technology included the following steps: sterilization of banana waste at 121°C for 60 min; addition of nutrients (ammonium sulfate/sodium nitrate, 1.0%, beef extract/peptone, 0.5%, glucose/sucrose/starch/maltose, 0.1%, and potassium chloride/sodium chloride, 1.0%) to the sterilized banana waste with a moisture content of 70% and particle size 400 μm; adjustment of pH to 7.0; addition of inoculum with the ratio 10% (v/w) to substrate; and cultivation at 35°C for 24 h. A yield of enzyme activity of 5,345,000 U/mg min was recorded. The findings showed that tea waste, supplemented with minerals and malt extract, was a suitable substrate for the synthesis of glucoamylase by *Aspergillus niger*. Initial moisture content of the solid medium was 60%. Cultivation was provided at a pH of 4.5 and a temperature of 30°C for 96 h. The maximum activity of glucoamylase was 226 IU/g dry substrate (23).

Pectinolytic enzymes are widely used to increase yields, improve liquefaction, clarification and filterability of fruit juices for better maceration and extractability of plant tissues in the fruit processing industry, and for clarification of wine. Pectic substances are acid polysaccharides with a backbone of galacturonic acid residues linked by (1–4) linkages, with varying degrees of methyl esterification. D-galacturonic acid is a major component of pectin molecule; some neutral sugars, such as ramnose, arabinose, galactose, and xylose, are also commonly present in pectin. Pectin contains from a few hundred to a thousand saccharide units in a chain-like configuration with average molecular weights from about 50,000–150,000. Pectin is present in all higher plants, but is mainly extracted industrially from apple promace and citrus peels. Pectic substances are classified into four main types: protopectin, pectic acid, pectinic acid, and pectin. Protopectin is a water-insoluble substance, while the other three are either totally or partially soluble in water.

Pectinases are represented by three types of pectic enzymes: pectinesterases with de-esterifying function; depolymericizing enzymes, which include hydrolases and lyases pectinases; and protopectinases, which solubililized protopectin. Pectinesterases catalyze the de-esterification of the methoxyl group of pectin forming pectic acid (galacturonans contained a small amount of methoxyl group). The depolymerases split the α-(1, 4)-glycosidic bonds between galacturonic monomers in pectic substances either by hydrolis (hydrolyses) or trans-elimination (lyases). Protopectinases divide the water-soluble part from protopectin. The main aim of their application is the degradation of the long and complex molecules of pectin. There are known applications of alkaline pectinases in the textile industry for the retting and degumming of fiber crops in production of good quality paper, in fermentation of coffee and tea, oil extractions, and treatment of pectic waste water (18). The estimated value of sales of all industrial enzymes in 1995 was $1 billion, of which about $75 million was assessed for pectinases (61). In recent years, there has been increased interest in the production of the pectinases from food processing wastes. Fungi, mainly, *Aspergillus niger* and the yeast *Kluyveromyces marxianus* are used for the production of commercial pectinases, but it was shown that the edible mushroom *Lentinus edodes* (Shiitake) and bacteria *Bacillus* sp. can be also applied in the bioconversion of apple pomace and other lignocellulosic wastes into enzymes. Food processing waste such as orange peels, orange finished pulp, wheat bran, sugar beet pulping waste, apple pomace, cranberry pomace and strawberry pomace can be used for the production of microbial polygalacturonase. Solid state fermentation is probably the most effective and low-cost process for pectinase production (Table 8.4).

For example, strawberry pomace was used as a substrate for solid state fermentation to produce polygalacturonase (11). The pressed strawberry pomace was dried to a moisture content of 10%, ground to a particle size of 1 mm, amended with water in a ratio 1:2 (w/v) and sterilized at 121°C for 15 min. Mycelium of *Lentinus edodes* was used as inoculum. These fungi have the advantage of possessing GRAS (generally regarded as safe) status, which permits the use of their metabolites in the food processing industry. Enzyme biosynthesis was conducted at 25°C for 50 days. The highest polygalacturonase activity was obtained after 40 days of cultivation and the enzyme yield was 29.4 U/g of pomace. A crude enzyme extraction was provided by following procedure: distilled water was added to the material in volume

Table 8.4
Production of pectinases from food processing wastes

Waste	Microorganism	Enzyme	Method	Activity	Reference
Apple pomace	*Aspergillus niger*	Pectinese terase and polygalacturonase	SSF	Polygalacturonase, 15 mg/kg SM; pectinesterase, 200 mg/kg SM with activity of enzyme mixture 900 AJDA U/mL	(62)
Apple pomace	*Aspergillus niger*	Polygalacturonase	SSF	25,000 U/kg of apple pomace fermented	(63)
Raw orange peel	*Aureobasidium pullulans*	Polygalacturonase	Slurry fermentation	130–140 VU/cm^3	(64)
Orange bagasse and sugar cane bagasse	*Thermoascus aurantiacus*	Polygalacturonase and pectin lyase	SSF	Polugalacturonase, 32 U/g, pectin lyase, 40,189 U/g	(16)
Strawberry pomace	*Lentinus edodes*	Polygalacturonase	SSF	29.4 U/g of pomace	(11)
Wheat bran	*Bacillus* sp.	Pectinase	SSF	8,050 U/g dry substrate	(18)
Wheat bran	Aspergillus foetidus	Pectinase	SSF	2,535 VU/g of wet substrate	(65)
Citrus waste	*Aspergillus foetidus*	Pectinase	SSF	1,600–1,700 VU/g of wet substrate	(66)
Wheat bran	*Bacillus* sp.	Pectinase	SSF	8,050 U/g dry substrate	(18)

SM solid medium; *AJDA* apple juice depectinizing assay; *U* units. One U releases 1 μmol of D-galacturonic acid from polygalacturonic acid per minute at 37°C, pH 5.0; *VU* viscosimetric units. One VU was defined as the amount of enzyme that decreased the initial viscosity of the pectin solution by 50% in 1 min.

ratio 3:10, the mixture was homogenized and centrifuged. To obtain a crude enzyme, solution supernatant was filtered and dialyzed.

Thermostable polygalacturonase was produced by *Thermoascus aurantiacus* by SSF using food processing wastes (16). Orange bagasse (pressed mixture of pulp and peel) and sugar cane bagasse washed in tap water were ground and dried at 80°C. The mixture of 90% orange bagasse, and 10% (w/w) sugar cane bagasse, was sterilized at 120°C for 40 min and inoculated with *Thermoascus aurantiacus* in a 1:100 ratio of dry mycelium to dry substrate. A nutrient solution containing NH_4NO_3, 0.1%; $NH_4H_2PO_4$, 0.1% and $MgSO_4 \cdot 7H_2O$, 0.1% was added in a ratio of 1:2 (w/v) to a final moisture content of approximately 67%. SSF was carried out at 50°C for 14 days. The maximum production of polygalacturonase (32 U/g) occurred between the 2nd and 4th day and maximum value of pectin lyase (40,180 U/g) was observed between 8th and 10th days of cultivation.

Cellulases are a group of enzymes which catalyze the bioconversion of cellulose to soluble sugar, glucose. Microorganisms produce mainly three types of cellulases either separately or in the form of a complex: endo-1,4-β-D-glucanase, exo-1,4-β-D-glucanase and β-glucosidase. Cellulase is an inducible enzyme; therefore, to obtain the high level of cellulase production, the cultivation medium must contain cellulose. It was proposed that such waste as coir pith, wheat brain, rice straw, and banana residue be used as substrates for cellulases production. For example, cellulases production by the bacterial strain *Bacillus subtilis* on the banana fruit stalk by SSF used a medium containing sterilized waste (autoclaved at 121°C for 60 min, particles of 400 μm size) with a 70% moisture content, added by $(NH_4)_2SO_4$ or $NaNO_3$ or glucose, 1.0% (w/w) and an inoculum-to-substrate ratio of 15% (v/w) with a pH of 7.0 (22). Enzyme biosynthesis was provided at 35°C for 72 h. The final product had a cellulase activity of 4.5 IU/g of dry solids.

2.3. Production of Organic Acids

Organic acids are used in the food and pharmaceutical industries as preservatives or chemical intermediates. Such acids, including propionic, lactic, pyruvic, succinic, fumaric, maleic, malic, itaconic, tartaric, citric, and isocitric acids, are usually obtained by the batch or continuous aerobic submerged fermentation in liquid medium using suspended microbial culture or immobilized biomass. After biomass separation, organic acids can be isolated and purified by liquid extraction, chromatography, evaporation, ultrafiltration, reverse osmosis, dialysis, crystallization, precipitation, and drying. Due to the biodegradability of organic acids, there is a growing demand for these compounds in the production of biodegradable polymers such as polylactic acid. Conventional processes of extraction and purification of acids generate a large amount of polluted effluent. An alternative technology for organic acids separation may be electrodyalisis with bipolar membrane (67).

Citric acid, which is a tricarboxylic acid, (a natural component of many citrus fruits) is the major organic acid produced by conventional submerged fermentation with *Aspergillus niger*. It is used in the food, beverage, chemical, pharmaceutical, and other industries. Worldwide demand for citric acid is approximately 600,000 tons/year. The main substance used in its production is molasses. Potassium ferrocyanide ($K_4Fe(CN)_6$) is added to the media in quantity about 0.06% (w/v) to enhance citric acid biosynthesis. Citric acid is a hydrophilic substance

and is thus poorly extractable by common organic solvents; however, it can be recovered from aqueous solutions with high molecular weight aliphatic amines. Apple pomace is a promising substrate for citric acid production by solid state fermentation (68).

L(+)-lactic acid (2–2-hydropropionic acid), $CH_3CHOHCOOH$, is an important chemical used as acidulant, flavor, and preservative in the food, cosmetics, pharmaceuticals, leather, and textile production. The biggest potential application of lactic acid is the production of polylactic acid, which can be used in the manufacturing of biodegradable plastics. The worldwide biotechnological production of L-lactic acid is 80,000 tons (69). Biotechnological production of lactic acid consists of 90% of total volume, and the rest is produced by the chemical synthesis. The advantage of biological synthesis is that it produces an optical pure product, while a mixture of D- and L-isomers of lactic acid is produced by chemical synthesis. Lactic acid bacteria are used for the biological production of lactic acids. Originally, lactic acid bacteria are inhabitants of biosurfaces and leaves of plants. They have no ability to synthesize some vitamins and amino acids, and therefore, demand those factors for their growth. The most important producers of lactic acid are the strains of *Lactobacillus helveticus*, *Lactobacterium plantarum*, *L. lactis*, *L. delbrueckii*, *L. bulgaricus*. The majority of lactic acid producers are mesophilic bacteria with optimal temperature for growth of $30^{\circ}C \pm 2^{\circ}C$, but there are some thermophilic strains such as *L. bulgaricum*, *L. delbrueckii*, *Streptococcus thermophilus*, which grow at temperatures higher than $40^{\circ}C$. Some lactic acid bacteria produce only lactic acid (homofermentation), and others can convert sugars into lactic acid, carbon dioxide, and ethanol (heterofermentation). Different wastes have been proposed to be used for lactic acid production. The raw material is whey permeate, which can be used for the large-scale production of lactic acid. It is an inexpensive and abundant by-product of the dairy industry, especially ultrafiltration-based cheese manufacturing. About 12.7 million tons of liquid whey from cheese manufacturing, which can be used for lactic acid production, are being wasted in the U.S. Cheese whey is the liquid effluent containing approximately 5% lactose, and 0.7% protein, generated at a rate of about 9 kg for every 1 kg of produced cheese. Whey has a high level of biochemical oxygen demand (35,000–40,000 ppm) and its discharge creates a serious environmental problem. It also includes lactose as a carbon source, nitrogenous compounds, trace minerals, and vitamins and can be used as a substrate for biotechnological processes. Lactose of whey is hydrolyzed into glucose and galactose. To ensure the growth of lactic acid bacteria, whey ultrafiltrate must be supplemented with nitrogen, vitamins, amino acids, and trace elements. Yeast extract was commonly recommended as the best source for growth stimulation and lactic acid production by lactic acid bacteria. Lactic acid can be produced on whey permeate by batch cultivation, continuous process, or by immobilized cell process (Table 8.5).

The batch process is the most studied and most commonly used method for lactic acid production on whey permeate. To prevent biosynthesis inhibition by the accumulated product, pH must be maintained at the optimum level of 6.0. The free lactic acid concentration in that case will be approximately 0.3 g/L, which is below the inhibitory level. pH can be maintained by adding sodium hydroxide, ammonium hydroxide, or calcium ions. For example, *Lactobacillus helveticus* was cultivated batchwise on whey permeate supplemented with 30 g of yeast extract (71). Whey permeate powder was used in a concentration of 57 g/L, which corresponded to a

Table 8.5
Lactic acid production from whey permeate by different methods

Type of process	Microorganism	Lactic acid, g/L	Yield, g lactic acid/g substrate	Productivity, g/L h	Reference
Batch	*Lactobacillus bulgaricus*	44	NA	3–5	(70)
Batch	*Lactobacillus helveticus*	36	0.75	NA	(71)
Batch	*Lactobacillus casei*	44	0.90	NA	(72)
Batch	*Lactobacillus bulgaricus*	44	0.95	NA	(73)
Batch with electrodialysis	*Lactobacillus helveticus*	47	NA	NA	(74)
Batch	*Lactobacillus casei* and *Lactococcus lactis*	22.5	0.48	0.93	(75)
Fed-batch	*Lactobacillus casei* and *Lactococcus lactis*	46.0	0.77	1.91	(75)
Continuous	*Lactobacillus bulgaricus*	13	0.28	NA	(73)
Continuous with immobilized cells	*Lactobacillus helveticus*	8.0	0.95	7.0	(76)
Continuous	*Lactobacillus casei subsp. casei*		NA	5.5	(77)
Continuous with immobilized cells	*Lactobacillus casi subsp. casei*		NA	13.5	(77)
Continuous	*Lactobacillus bulgaricas*		NA	6.2	(78)
Continuous with electrodialysis	*Lactobacillus helveticus*	125	NA	NA	(74)
Fed-batch process with co-immobilized cells in Ca-alginate beads	*Lactobacillus casei* and *Lactococcus lactis*	47	NA	NA	(75)
Process in membrane bioreactor with in the cell recycling	*Lactobacillus bulgaricus*	43	NA	85	(70)

NA not applicable.

Table 8.6
Comparison of batch and fed- batch culture for lactic acid production from deproteinized whey by mixed cultures of *L. casei* and *L. lactis* (modified from ref.(75))

Parameters	Batch culture	Fed-batch culture
Lactic acid concentration, g lactic acid /L	22.5	46.0
Lactic acid productivity, g lactic acid /L h	0.93	1.91
Lactic acid yield, g lactic acid/g sugar utilized	0.48	0.77
Specific lactic acid production rate, g lactic acid/cfu h	2.7×10^{-11}	7.0×10^{-11}
Specific sugar uptake rate, g sugar/cfu h	5.7×10^{-11}	5.5×10^{-10}
Fermentation efficiency, g sugar utilized/100 g initial sugar	93.0	80.0

lactose concentration of 48 g/L. Clarified whey was supplemented by yeast extract and used for lactic acid bacteria cultivation at 42°C and pH 5.9 for 20 h to the final concentration of lactic acid 40 g/L.

To improve the process of lactic acid fermentation, an application of fed-batch culture was proposed (75). The production of lactic acid by a mixed culture of suspended *Lactobacillus casei* and *Lactococcus lactis* cells in batch cultivation was compared with a fed-batch culture grown on deproteinized whey. The base medium for lactic acid bacteria cultivation contained 50 g/L lactose, the volume of medium for the batch cultivation in the fermentor was 6 L, and the duration of the process was 24 h. The fed-batch process included two phases: in the first 12 h, it was similar to the batch cultivation, but initial volume of medium was 3 L; in the next 12 h, the medium containing 100 g/L lactose was continuously added at a constant feeding rate 250 mL/L to a total volume of 6 L. The fermentations were provided at 32°C with a pH maintained at 6.0 by addition of 5 N NaOH. The quantity of inoculum was the same for both fermentations. Table 8.6 shows the results of the comparison between the fed-batch process and batch cultivation.

The disadvantages of batch process include a long start-up period (lag phase), long fermentation time, large volume of fermentor, and high operational costs. The advantages of continuous cultivation for lactic acid production are higher productivity and stability of the process. Continuous cultivation can be provided at a dilution rate ranging from 0.03 to 0.35 h^{-1} (79). To enhance the bioconversion of substrate into lactic acid, retention, or recycling of bacterial cells in a bioreactor can be applied. It is possible to increase cell concentration by their recycling during continuous fermentation, by immobilization of cells in gels (more often in Ca-alginate beads), and by cultivation of lactic acid bacteria in membrane reactors or reactors with electrodialysis. The procedure of immobilizing bacterial cells is well known (75). A suspension of bacterial cells with the concentration about $1.2–1.4 \times 10^{10}$ cells/mL was mixed with 5% sterile sodium alginate solution in a 1:2 ratio (v/v). The mixture was extruded drop by drop with a peristaltic pump into a sterile 2% $CaCl_2$ solution at room temperature under continuous stirring. The beads (2–3 mm diameter) were hardened in $CaCl_2$ solution for 2 h. The particles were then washed with sterile 0.9% sodium chloride to remove excess calcium ions and free cells. Continuous fermentation of immobilized cells of *Lactobacillus helveticus* at pH 5.5 and 42°C resulted in a productivity of 7 g/(L h), which was approximately ten times higher than

obtained in batch cultivations with free cells (76). The comparison of continuous fermentation with free and immobilized cells of bacterium *Lactobacillus casei subsp. casei* showed that in a stirred reactor a volumetric productivity was 5.5 g/(L h) at a 100% substrate conversion and dilution rate of $0.22\,h^{-1}$. A fluidized bed reactor with an immobilized biomass of 105 g/kg support had a productivity of 10 g/(L h) at a dilution rate of $0.4\,h^{-1}$ and substrate conversion of 93%. The productivity of the reactor was 13.5 g/(L h) at dilution rate $1.0\,h^{-1}$ and a substrate conversion of 50% (77).

The current method for lactic acid production consists of cultivating lactic acid bacteria in the membrane bioreactors. Cultivation of *Lactobacillus bulgaricus* on whey permeate obtained by ultrafiltration of cottage cheese whey and supplemented with yeast extract in a high-performance membrane bioreactor configured in the cell recycle mode yielded an optimum productivity of lactic acid 35 g/(L h) at a cell concentration 10 g/L. Higher cell concentration 60 g/L resulted in productivity over 80 g/(L h) with complete substrate utilization (70). Lactic acid is continuously removed from fermenter during electrodialysis fermentation.

The inexpensive substrate for lactic acid production may be an agricultural waste contained cellulose and hemicellulose, which can be converted into soluble sugars by chemical or enzymatic hydrolysis for further use for microbial synthesis of L(+)-lactic acid, for example, wheat straw (80). Europe's wheat production in 2000 alone was 184 million tons and the average yield of straw was 1.3–1.4 kg/kg of grain. Wheat straw consists of cellulose (35–40%), hemicellulose (30–35%), and a hardly biodegradable polymer lignin. To use lignocellulosic material as a substrate for biotechnological processes, it is necessary to separate cellulose and hemicellulose from lignin, and then to produce free sugars from cellulose and hemicellulose through their depolymerization. Glucose is produced from cellulose, and a mixture of monosaccharides including hexoses (mannose, galactose, glucose) and pentoses (arabinose and xylose) can be obtained from hemicellulose. The presence of xylose, the dominated monosaccharide released from hemicellulose, which is not used by the majority of microorganisms, complicates the usage of the hemicellulose as a raw material for biotechnology.

To improve lactic acid production from lignocellulosic substrate, the use of *Lactobacillus pentosus*, which is able to utilize pentoses as well of hexoses, was proposed (81). The biotechnological production of lactic acid from agricultural lignocellulosic waste includes some major steps such as: pretreatment of substrate to convert cellulose and hemicellulose into sugars, bioconversion of sugars into microbial biomass and lactic acid, separation of the solution of lactic acid from bacterial cells and solid particles, and isolation and purification of lactic acid. Hemicellulose hydrolysate from wheat straw was used for production of lactic acid by the lactic acid bacteria *Lactobacillus pentosus* (80). The chemical hydrolysis was carried out by adding 4% (w/w) sulfuric acid to the waste followed by treatment at 100°C for 2 h in a closed container. After cooling to ambient temperature, the pH was adjusted to 6.5 by adding 10 M NaOH. The solution was supplied with 10 g/L casein peptone, 10 g/L meat extract, 5 g/L yeast extract, 1 g/L Tween 80, 2 g/L K_2HPO_4, 5 g/L sodium acetate, 2 g/L diammonium citrate, 0.2 g/L $MgSO_4 \cdot 7H_2O$, and 0.05 g/L $MnSO_4 \cdot H_2O$ and was autoclaved at 121°C for 20 min. 10% (v/v) of inoculum was added and the beginning of batch fermentation performed under strictly anaerobic conditions at 33.5°C for 72 h. The final lactic acid concentration was 7.5 g/L. Molasses, a by-product of the sugar manufacturing, is another large-scale waste substrate

suitable for lactic acid production. The main source of carbon in molasses is sucrose. Species *Lactobacterium delbrueckii* usually is used for the lactic acid production on molasses.

Fungi can be also used as an L(+)-lactic acid producer, as demonstrated on corncobs (82). Shredded corncobs were pretreated by 0.1 N NaOH (1:20, w/v) for 2 h under stirring, pH was adjusted to 5.0 with concentrated HCl and sterilized at 121°C for 15 min. After cooling, the medium was inoculated with 10^6 viable conidia of *Rhizopus oryzae* per l00 mL. A commercial apple juice processing enzyme preparation Rapidase Pomaliq, derived from *Aspergillus niger* and *Trichoderma reesei*, contained 2,600 U/mL carboxylmethyl cellulase and 490 U/mL xylanase, 0.5 mL/100 mL was added to cultural media. $CaCO_3$, 0.2 g/100 mL, was added after 1 day of fermentation as buffering agent. The fermentation was provided at 30°C under aeration (shaking, 200 rpm) for 48 h. The yield of L(+)-lactic acid was 299 g/kg dry matter of corncobs.

Most lactic acid bacteria require a medium rich in amino acids, vitamins, purines, and pyrimidines for their growth and biological activity. Yeast extract is a commonly used supplement that provides lactic acid bacteria with necessary growth stimulators. However, yeast extract contributes over 30% to the total production cost (83). Organic waste was proposed as an inexpensive alternative for the industrial lactic acid production; these include beet and cane molasses, hydrolyzed whey protein, mustard oilseed cake, grass extract, with high content of amino acids and vitamins. The use of hydrolysate from ram horn, a byproduct of the meat industry, was proposed as a substrate for lactic acid production using Lactobacillus casei (84). Ram horns contain a high percentage of protein keratin; therefore, its hydrolysate is rich in amino acids, and can be used as a supplement for lactic acid bacteria cultivation. According to the proposed technology, horns were washed and dried at 100°C, cut into smaller pieces and ground to the particles with size 5 mm. Hydrolyses was provided with 6N HCl or 6N H_2SO_4 first for 24 h at 70°C and then for 4 h at 130°C, neutralized to pH of 7.0 and filtrated. Hydrolyzate (60 mL) was added to 1 L of the synthetic medium containing: yeast extract (5 g/L); glucose (50 g/L); sodium acetate (5 g/L); sodium citrate (2 g/L); K_2HPO_4, (2). Tween 80 (1 mL) was added to 1 L of the medium; pH was adjusted to 6 with 1N HCl. Medium was sterilized at 121°C for 15 min. Batch aerobic cultivation of *Lactobacillus casei* was conducted at 38°C for 26 h. The final concentration of lactic acid in the culture broth was 44 g/L. Malt combing nuts, a low value by-product from the malting industry, was also proposed as an inexpensive source of nitrogen and vitamins instead of yeast extract in lactic acid production by *Lactobacillus casei* (85). Addition of 5% (w/v) of malt combing nuts to whey permeate with 55 g/L of lactose had the similar effect as supplementation of the medium with 0.3% (w/v) of yeast extract.

2.4. Production of Flavors

The world market of aroma chemicals, fragrances, and flavors has an annual growth rate of 4–5% (2). Their total production in 1995 was estimated at US $\$9.6 \times 10^9$ (86). Most of the flavoring compounds are presently produced by chemical synthesis or extraction from natural materials. The cost of many natural flavors exceeded the cost of synthetic ones. For example, the prices per kg (in US$) for synthetic and natural flavors are as follow: vanillin, $31 and $345; raspberry ketone, $58 and $3,000; γ-Deca lactone, $75 and $1,400;

Table 8.7
Selected flavors produced by microbial bioconversion of food wastes

Food waste	Microorganism	Aroma compounds	Reference
Cassava bagasse and giant palm bran	*Kluyveromyces marxianus*	Fruity aroma compounds	(13)
Wheat bran, cassava bagasse and sugar cane bagasse	*Ceratocystis fimbriata*	Fruity aroma compounds	(89)
Cassava bagasse plus soybean meal	*Rhizopus oryzae*	Acetaldehyde and 3-methyl butanol	(90)
Coffee husk	*Ceratocystis fimbriata*	Fruity flavor	(91)
Sugar-beet pulp	*Aspergillus niger* and *P. cinnabarinus*	Vanillin	(88)
Wheat bran			

δ-Deca lactone, $130 and $5,500; isoamyl butyrate, $31 and $345; phenethyl alcohol, $58 and $3,000; respectively (87). However, natural flavors are the preferred choice for food and beverage production. Both European and American legislations consider flavors/aromas produced from the feedstock of plants or animals by microbiological processes to be natural (88). Microbiological bioconversion of food waste can be the most cost-effective alternative method for natural aroma compounds production. It has been shown that different microorganisms including bacteria, yeast, and fungi are able to produce different volatile components, which can be used as aroma compounds. Agroindustrial residues and food processing waste are suitable substances for the microbial production of flavors. Some examples of aroma compounds production from agricultural residues and food processing waste are shown in Table 8.7.

The composition of a medium greatly influences the production of fruity aromas. The fungi *Ceratocystis fimbriata* produces a fruity aroma on sugar cane bagasse supplemented with a synthetic medium containing glucose, whereas the addition of leucine or valine results in a strong banana aroma (89). *Ceratocystis fimbriata* growing on steam-treated coffee husk supplemented with glucose develops a strong pineapple aroma. Meanwhile, the addition of leucine gives a strong banana odor (91). The yeasts *Klyyveromyces marxianus* belonging to the GRAS group of microorganisms have been shown to produce fruity aromas via state fermentation on cassava bagasse and palm bran (13). In this process, agricultural wastes were dried at 60°C for 24 h. The dried substrates were then milled and sieved to obtain particles of 0.4–0.8 mm size, moistened using distilled water to their saturation level and sterilized at 121°C for 15 min. Inoculum was added to create the concentration of yeast cells 10^7 cells/g of dry matter and solid state fermentation was conducted at 28°C for 72 h. The predominant aroma compounds determined by gas chromatography were ethyl acetate, ethanol, and acetaldehyde. Ethyl acetate was the major compound produced with cassava bagasse (highest

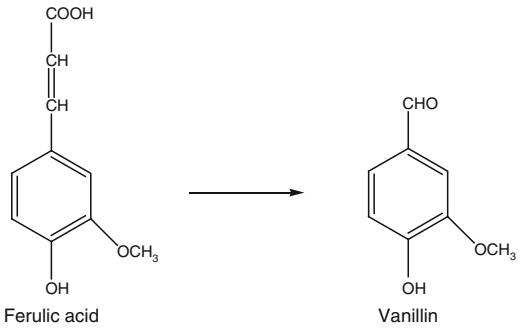

Fig. 8.2. Chemical structure of ferulic acid and vanillin.

concentration was 1, 395 μmol/g L), but ethanol was the predominant compound when palm bran was used. The fruity aroma was attributed due to the production of esters. The fermented substrates were then proposed to be used as feed supplements for cattle.

Vanillin (3-methoxy-4-hydroxybenzaldehyde) is presently the most important flavor, having a total production exceeding 12,000 tons annually. Approximately 50 tons are produced from natural vanillin extracted from vanilla pods (*Vanilla planifolia*), a tropical orchid, which grows as a vine around trees. Synthetic vanillin provides the remainder (92). Vanillin is chemically synthesized from guaiacol (catechol monomethylether), or by the oxidative breakdown of lignin, both of which are byproducts of paper pulp manufacture. Bioconversion of ferulic acid obtained from vegetable and fruit agro-industrial wastes into vanillin by liquid cultures of fungi may be an alternative way of vanillin production. Structure of ferulic acid and vanillin is shown in Fig. 8.2.

Ferulic acid is ester-linked to pectic sidechains in beet and ether-linked to lignin in cereals. It was found that ferulic acid can be recovered from wheat bran by a ferulic acid esterase of *Aspergillus niger* (93), from sugar beet pulp by commercial enzyme preparations (94), and from barley spent grain and wheat bran by esterase- and xylanase-rich extracts of barley (95). A two-step process of bioconversion was proposed to convert ferulic acid from sugar-beet pulp and from wheat bran into vanillin by filamentous fungi *Aspergillus niger* and *Pycnoporus cinnabarinus* (88). Sugar beet pulp contains 0.8 g of ferulic acid per 100 g of dry matter (94) and wheat bran has 0.5 g of ferulic acid per 100 g of dry matter (88). Fungal ferulic acid esterases and xylanases were used for enzymatic degradation of raw material. Almost 95% of the total ferulic acid was released from wheat bran using ferulic acid esterases and endoxylanase from fungi *Trichoderma viride*. To extract ferulic acid from the mixture of neutral and acidic sugars, such solvents as methanol or acetic acid are used. Adsorbents such as resins and activated carbon showed high specificity for ferulic acid and can be used for recovery. Ethanol can be used as eluant for desorption of ferulic acid. Ferulic acid was obtained from sugar-beet pulp with a purity of approximately 50%. The first step of vanillin production was the biotransformation of ferulic acid to vanillic acid by ascomycetes *Aspergillus niger*, and the second step was biotransformation of vanillic acid to vanillin by basidiomycetes *Pycnoporus*

cinnabarinus. The final concentrations of 90 and 300 mg/L of vanillin were obtained from ferulic acid enzymically released from wheat bran and sugar-beet pulp, respectively.

2.5. Production of Polysaccharides

Polysaccharides production includes three main steps: medium preparation, fermentation, isolation, and purification of the final product. Pullulan is a water-soluble exocellular homopolysaccharide composed of maltotriose units linked through α-1,6-glycosidic bonds. It can be produced by fungi *Aureobasidium pullulans*. Pullulan forms solutions with a high viscosity at a relatively low concentration and can be applied for the production of oxygen-impermeable films and fibers, which are biodegradable, transparent, oil resistant, and impermeable to oxygen. Therefore, pullulan may be used as food coating and packaging material. Different organic wastes were proposed for the production of pullulan, such as grape skin pulp extract, starch waste, olive oil waste effluents, molasses, and potato processing waste. Figure 8.3 shows a flowchart of pullulan production from potato processing waste (according to the results of ref. (38)).

Potato processing waste was treated with α-amylase followed by further hydrolysis with pullulanase and amyloglucosidase. Enzymatic hydrolysis converted potato starch into substrate suitable for growth and exopolysaccharide production by *Aureobasidium pullulans*. The hydrolyzate was enriched by the mineral sources of nitrogen and phosphorus, pH of the medium was adjusted to 6.0, and inoculum was added. Fermentation was conducted for 7 days. Pullulan was then recovered from the fermented material by ethanol precipitation after the removal of cells.

Xanthan gum is another widely used water-soluble heteropolysaccharide and is the most important microbial polysaccharide from a commercial point of view, with a worldwide annual production of 30,000 tons. It is applied in food processing for emulsification, stabilization, temperature stability, improval of rheological properties, and is an important ingredient in dietary products for stouts. It is also used in manufacturing of cosmetic and pharmaceutics products. Because of its water solubility and a high viscosity of its solutions, xanthan is used in the petroleum industry for preparation of drilling fluids and enhanced oil recovery. Xanthan gum is produced industrially from sucrose or glucose by fermentation using gram-negative bacterium *Xanthomonas campestris*. It has been proposed for use in production of xanthan food processing waste such as spent malt grains, apple pomace, grape pomace, citrus peels, olive mill wastewaters, and waste sugar beet pulp. For example, xanthan production by *Xanthomonas campestris* on apple pomace and spent malt grains was demonstrated (40). Dried apple pomace was moisturized by soaking in KOH solution for 1 h to neutralize organic acids. Then, solution containing sodium glutamate (4 g/L); K_2HPO_4 (1 g/L); and $MgSO_4 \cdot 7H_2O$ (0.2 g/L), was added to create a final moisture content higher than 70% w/w. Dry spent malt grains as a porous inert support was added to the moisturized apple pomace at a 3:2 ratio. Spent malt grains, a by-product of the brewery industry, had a high water sorption capacity of over 80%, an average particle size of 1–4 mm, and prevent packing of the fermented matter. Solid media was sterilized at 121°C for 15 min, and inoculated with 5% (v/v) of *Xanthomonas campestris*. Solid state fermentation was provided at 30°C in a humidified atmosphere for 6 days. The yield of xanthan was 37.2 g/kg of wet substrate.

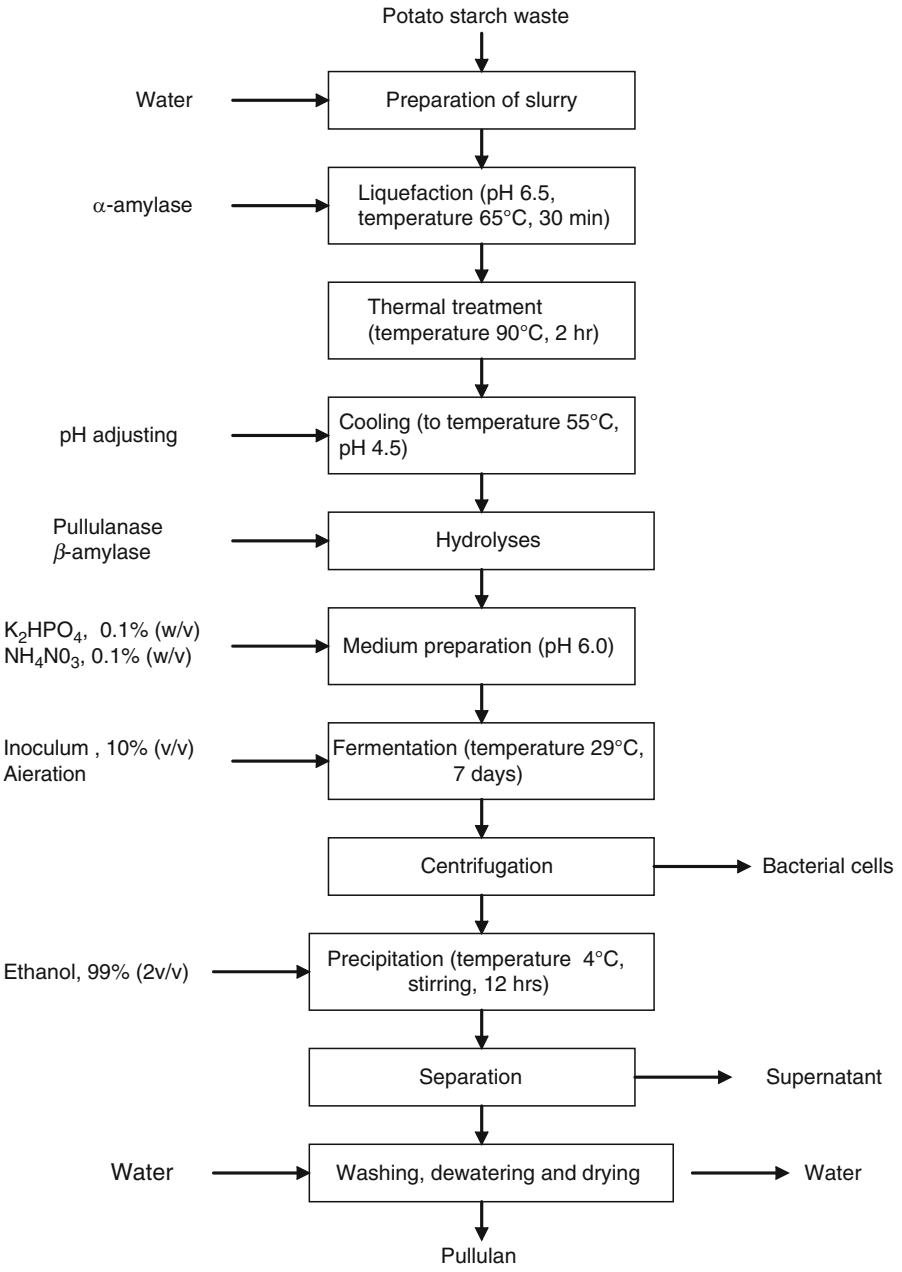

Fig. 8.3. Flowchart of potato waste bioconversion into pullulan by *Aureobasidium pullulans*.

2.6. Mushroom Production

World commercial mushroom production consists of approximately 5×10^6 tons of fresh weight annually (96). Different organic and lignocellulosic wastes can be used as substrates for cultivation of edible mushrooms. *Pleurotus ostreatus* (oyster mushrooms), *Agaricus campestris*, and *Agaricus bisporus* (field mushrooms) are cultivated all over the world, while *Lentinus edoides* (shiitaki), *Volvariella volvacea* (edible straw mushroom), and *Ganoderma lucidum* (red reishi) are cultivated mainly in Asia. In total, at least 20 mushrooms species are cultivated for commercial purposes such as food consumption as well as medicine to prevent such diseases as hypertensions and hypercholesterolemia due to the presence of dietary fibers, mainly chitin (97), and for antitumor, antiviral, and immunomodulating treatments due to special polysaccharides (98). At present, pharmaceutical and nutraceutical products from mushrooms may be worth more than USD 1.2 billion. It is considered that 100 g of mushrooms supply from 9 to 40% of the daily recommended allowance (DRA) of dietary fiber (99).

The cultivation of mushrooms includes two major steps: preparation of the compost or solid medium and mycelium growth until fructification. For production of compost or medium for mushrooms cultivation, a great number of different materials can be used, such as wood chips, sawdust, hay, maize waste, paddy straw, cassava bagasse, waste paper, cotton seed hulls, water hyacinth, apple pomace, oil palm bunch, husk rice, banana leaves cheese whey, horse manure, chicken manure, and others. It was shown, for example, that it is possible to produce *Pleurotus ostreatus* on a non-composted or shortly composted mixture of grass, 70%, coffee pulp, 30%, with the addition of 2% $Ca(OH)_2$ to an initial pH of 8.7 (100). *Agaricus bisporus* is the most cultivated mushroom in the world, and it is the dominant species cultivated in Europe. The *Agaricus* species are cultivated on a medium consisting of horse manure, wheat straw, corn cobs, chicken manure, and others. The materials are mixed and composted for 8–9 days, then compost is packed in boxes and pasteurized. After inoculation and mycelium growth, compost is covered with a mixture of soil, peat, and chalk. The optimal temperature for mycelium growth is 24°C, and the optimal temperature for fruiting body production is from 14 to 18°C. The yield of mushrooms is estimated as 0.5–1 kg from 1 kg of compost dry mater. Figure 8.4 shows the flowchart for mushroom production.

Some technologies of mushroom cultivation include pasteurization or sterilization of substrate used for mushrooms growth, but some mushrooms can be grown on nonpasterized substrate. The preferred pH for mushrooms growth is 7.5 or higher. The majority of species are developed at 23–25°C. Every species of mushroom has its own preference for growth. For example, to obtain a harvest of red reishi, the wood logs of Japanese oak with the introduced mycelium are covered with wood crumbs and kept at 25–30°C and a humidity of 70–90%. A synthetic medium of nutrient supplemented sawdust packed in polypropylene bags or bottles is the most common method used to produce shiitaki or oyster mushrooms in Japan and Singapore (Figs. 8.5 and 8.6). The residual compost may be used as a soil fertilizer and as a prospective agent for bioremediation of contaminated soil including polycyclic aromatic hydrocarbons (101).

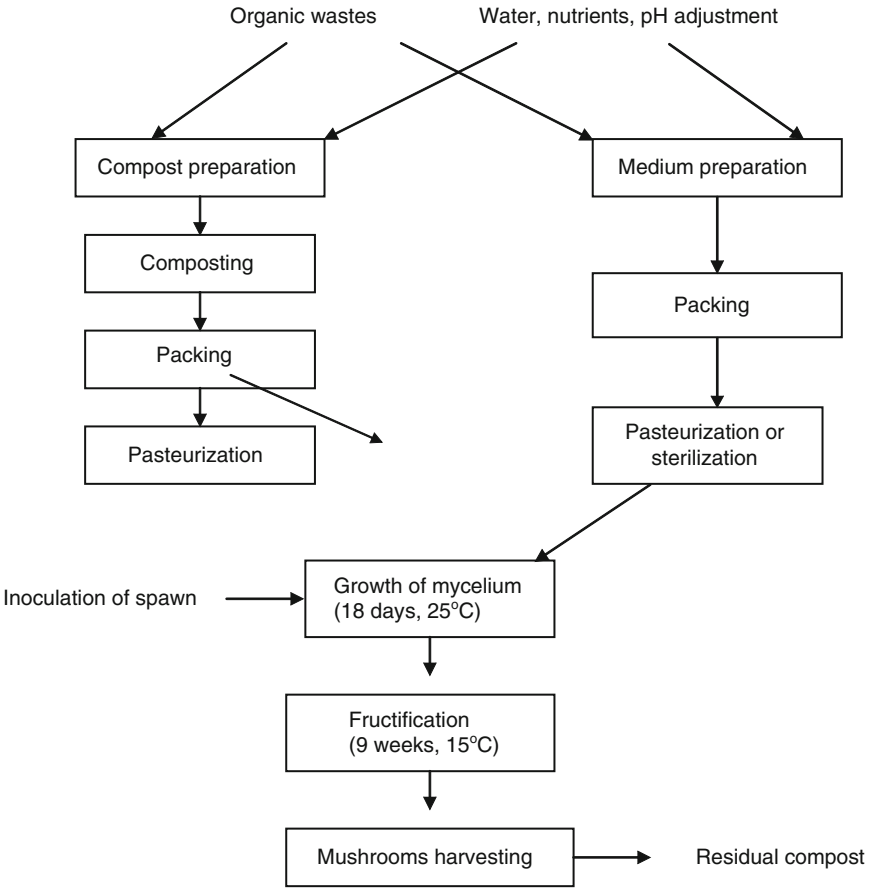

Fig. 8.4. Biotransformation of organic waste into mushrooms.

2.7. Production of Biodegradable Plastics

About 100 million tons of plastics are produced in the world annually. Disposed plastic materials are a significant source of the environmental pollution. The use of biodegradable plastic can help to solve this problem, but its production is expensive and is now used for manufacturing of high-value items used in medicine, mainly in surgery. The search of an inexpensive substrate for the microbial production of inexpensive biodegradable material is part of the ongoing biotechnological direction. Potato processing waste for production of biopolymer poly-β-hydroxybutyrate was proposed as a biodegradable substitute for conventional thermoplastics (54). Potato starch was enzymatically converted into a concentrated glucose solution with a glucose concentration of 208 mg/mL. Barley malt was used as a source of amylase with the ratio of 10:90 g/g of potato waste. The medium for the batch cultivation of the bacterial strain *Alcaligenes eutrophus* was obtained by dilution of hydrolyzate and addition of mineral salts. The final concentration of poly-β-hydroxybutyrate was 5.0 g/L.

Fig. 8.5. Shiitake cultivation in plastic bags.

Lactic acid can be used as a raw material for synthesis of polylactic acid for manufacturing of biodegradable plastic. The properties of the poly-lactate plastic are greatly improved if the lactic acid is only in the L-form. For this case, microbiological synthesis of lactic acid is preferable. The inexpensive substrate for lactic acid production may be an agricultural waste containing starch, cellulose, and hemicellulose, which can be enzymatically converted into

Fig. 8.6. Cultivation of oysters in plastic bags.

soluble sugars for further use for microbial synthesis of L(+)-lactic acid. The technology for lactic acid production was described earlier.

2.8. Production of Animal Feed

2.8.1. Enrichment of Lignocellulosic Material by Single Cell Protein

Agricultural lignocellulosic waste production is at least 123×10^6 tons annually (102). The content of protein in most of the agro-industrial wastes is low, but the content of fibers with low digestibility is high. Therefore, such materials cannot be used as a feed for non-ruminant animals. To increase the protein level and decrease the content of indigestible cellulose, the microbial bioconversion of lignocellulosic wastes into feed may be provided. The chemical or enzymatic pretreatment of wastes serves to improve the microbial bioconversion. Chemical pretreatment usually consists of the soaking of wastes in diluted solutions of acid or alkaline to hydrolyze cellulose and hemicellulose to the substances with lower molecular mass. The temperature of chemical hydrolysis is maintained at 50–70°C. Some essential nutrients, mainly nitrogen and phosphorus, can be added to hydrolyzed wastes to enhance the microbiological process.

Enzymatic hydrolyses is a more expensive procedure than chemical pretreatment. Therefore, the best biotreatment method is the application of fungi, which can produce cellulases to hydrolyze cellulose into more convenient products for microbial consumption. Fungi can simultaneously hydrolyze cellulose, consume the products of hydrolysis, and synthesize the

biomass, thus enriching the wastes by proteins and vitamins. It is possible to apply yeast together with the fungi. Yeast can consume the products of hydrolysis, which were produced by fungi, and synthesize biomass with a high protein content (50% of dry weight) and vitamins. For example, to enhance apple pomace by protein, cellulolytic filamentous fungi *Thrichoderma viride* and *Aspergillus niger* and yeasts *Saccharomyces cerevisiae*, *Candida utilis*, and *C. tropicalis* in different combinations were used. The filamentous fungi degraded cellulose and hemicellulose at the beginning of the process, and yeasts subsequently utilized the sugars produced. The best combination was the yeast strain *Candida utilis* and fungi strain *Aspergillus niger*, resulting in a 200% increase in protein content after only 7 days of solid-state fermentation (37).

A study in Jordan proposed the bioconversion of solid waste (50% moisture) from olive oil processing into animal feed (8). This procedure included alkaline pretreatment of waste, delignification of pretreated wastes by fungi *Phanerochaete chrysosporium*. The fermented material was then saccharified by fungi *Trichoderma reesei* to provide a substrate for the yeasts, *Saccharomyces cerevisiae*. The level of crude protein increased from 5.9% in the raw pomace to 40.3% in the fermented material. The final product was recommended as animal feed for the poultry industry in Jordan. It is important to note that the final product of microbial bioconversion of waste may be approved as animal feed only after thorough toxicological evaluation (103) since many species of fungi produce toxins.

2.8.2. Use of Organic Waste as Substance for Microbial Cells Production

The process of producing single-cell proteins includes medium preparation, fermentation, separation of biomass from liquid fraction, and downstream processing steps such as washing, cell disruption, protein extraction, purification, and drying. The cultivation is usually performed via suspended fermentation. Yeasts are widely applied for production of biomass as feed for animals. Algae, fungi, and bacteria can also be used for this purpose. However, the use of fungi is limited by the relatively low growth rate and possible presence of mycotoxins in biomass. Bacteria usually have the highest growth rate and content of protein in biomass as compared with other microorganisms, but the procedure of bacterial biomass concentration is complicated due to the smaller size of cells. The bacterial cells may be flocculated to simplify centrifugation. Cellulose, which is present in the algae cell wall, decreases the nutrient value of algae biomass. The production of algae requires much space and depends on climate conditions.

Many liquid wastes can be used for the production of single-cell proteins. Vegetable and fruit processing wastes contain starch, cellulose, organic acids and are suitable substrates for yeasts utilizing organic acids. Waste brine generated from kimchi production was proposed for cultivation of osmotolerant yeast *Pichia guilliermondii* (104). Yeasts *Candida utilis*, *Pichia stipitis*, *Kluyveromyces marxianus*, and *Saccharomyces cerevisiae* grew well in Chinese cabbage juice (105). Plant original liquid waste was a good medium for the growth of *Saccharomyces cerevisiae*, *Torula utilis*, and *Candida lipolytica* (106). Corn silage juice was suitable for *Kluyveromyces marxianus* (107). Water extracts from vegetable and fruit processing waste can be used by *Saccharomyces cerevisiae* (108). Acid hydrolysate of shrimp-shell wastes also can be used for yeast biomass production by cultivation of *Saccharomyces cerevisiae*

(109). Yeast biomass is rich in protein (50% of dry weight), which contains all essential amino acids and many vitamins, such as thiamine, riboflavin, biotin, niacin, pantothenic acid, pyridoxine, choline, folic acid, and *p*-aminobenzoic acid. Amino acid composition of protein from biomass of mixed culture consisted of fungi *Trichoderma reesei* and yeast *Kluyveromyces marxianus* grown on beet pulp was richer than those of the FAO guideline and soy bean oil meal (110). The content of amino acid lysine, deficient in cereal diet, is high in yeast protein. The protein from fungi is rich in essential sulfur-containing amino acids (111).

2.9. Use of Organic Waste for Production of Fungi Biomass for Bioremediation

Lignin-degrading white-rot fungi have the unique ability to degrade/mineralize a great number of different toxic environmental pollutants including munitions waste, pesticides, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, bleach plant effluent, synthetic dyes, synthetic polymers, and wood preservatives (112, 113). White-rot fungi are basidiomycetes producing extracellular enzymes including manganese peroxidases, lignin peroxidases, and laccases which are involved in the degradation of lignin in lignocellulose-containing substrates and can also be used for the degradation of xenobiotic compounds. One white rot fungi application is the bioremediation of soil polluted with polycyclic aromatic hydrocarbons (PAHs). PAHs are released into the environment during oil spills, incomplete burning of coal, oil, gas, wood, garbage, or from petroleum and coal treatment processes. Several PAHs are toxic, mutagenic, and carcinogenic; therefore, soil contamination by PAHs is a serious environmental problem. Several genera of basidiomysetes, including *Phanerochaete chrysosporium*, *Pleurotus ostreatus*, *Coriolopsis polyzona*, and *Trametes versicolor*, showed a high ability for PAH mineralization. The process of soil bioremediation with white-rot fungi can be provided by in situ treatment when the biomass of white rot fungi is added to treated soil. The biomass of such fungi can be obtained by their growth on lignocellulosic waste, such as corn cobs, alfalfa straw (112), or sugarcane bagasse (113). White rot fungi, added to soil for its bioremediation, can co-exist with indigenous microflora and mineralize PAH. An additional source of lignocellulose-containing substrate, for example, wheat straw (115), must be added to ensure the fungi activity in soil. It was reported that up to 49% of added benzo[*a*]pyrene was removed from soil by *Pleurotus ostreatus* after 3 months incubation (116). Fungi *Pleurotus* sp. removed 29–42% of 5–7-ringed PAH from artificially contaminated soil (117). The mycelium remaining after gathering of the fruit bodies of edible mushrooms is the most cost-effective and safe biological agent for soil bioremediation.

2.10. Dietary Fiber Production from Organic Waste

Fiber (non-starch polysaccharides) is an important component of the human diet. Soluble dietary fibers such as pectic substances and hydrocolloids are present in fruits, vegetables, legumes, and oat brain, while insoluble fibers (cellulose and hemicellulose) are found in cereal grains. It was shown that in human populations with low average intake of dietary fiber, an approximate doubling of total fiber intake from foods could reduce the risk of colorectal cancer by 40% (118). Different fruit and vegetable processing wastes have a high content of fiber (Table 8.8) and in recent years have found their application as a food ingredient.

Table 8.8
Content and composition of selected dietary fiber of some fruit and vegetable waste (modified from ref. (2))

Waste	Fiber			Pectin
	Total	Insoluble	Soluble	
Apple pomace	62.5	48.3	14.2	15.7
Carrot pomace	29.6	18.9	10.7	22.0–25.0
Kiwi pomace	25.8	18.7	7.1	7.3
Lemon peel	50.9	28.2	22.7	25.2
Lemon pulp	45.8	26.0	19.8	12.0
Peach pomace	43.9	36.3	7.6	7.1
Potato pulp	15.8	9.4	6.4	15.0
White wine pomace	58.6	56.3	2.3	3.9–5.5

Fiber supplementation has been used to increase their content in different products including cereal food, meat food, imitation cheeses, and sauces. Different food processing residues contain a lot of fiber and are potential sources of dietary fiber supplements. Carrot pomace was proposed for use in the production of bread, bakery goods, cereals, and dairy meals and beverages. Biotechnological processing of the vegetable residues to a dietary fiber food additive can be accomplished by lactic acid solid state fermentation to inhibit enterobacteria and fungi, which can be present in pomace (2). Carrot and grape pomace remaining after lactic acid fermentation were converted into product with high content of fiber, which can be used for crude fiber enrichment of bakery products.

2.11. Production of Pharmaceuticals from Organic Waste

Submerged fermentation is typically used for the commercial biotechnological production of pharmaceuticals, but at recent years, much research has been done on the pharmaceutical preparations production using different agricultural waste by solid state fermentation (Table 8.9). Some examples of production of antibiotics and other medicines from agricultural wastes by SSF are described in the following lines.

Antibiotics are the low-molecular-weight organic natural products of some microorganisms, which are active against other microorganisms at low concentrations (123). They can be produced either by microbial synthesis or by semi-synthetic or synthetic processes. For the microbial production of antibiotics, suspended cultures are usually used. Some results indicate that solid state fermentation can compete with liquid submerged fermentation. Thus, penicillin production by *Penicillium chrysogenum* increased by 500–600% when SSF was used instead of submerged fermentation (124). Production of iturin, an antifungal peptide effective at suppressing phytopathogens, by *Bacillus subtilis* using soy bean curd residue was six to eight times more efficient in solid state fermentation than submerged fermentation on the basis of unit wet weight (125). The maximum neomycin production by *Streptomyces marinensis* on synthetic medium by SSF was $5,813\ \mu g/mL$, but it was increased by 1.85

Table 8.9
Agricultural and food processing waste for production of pharmaceutical preparations by SSF

Substrate	Pharmaceutical preparation	Microorganism	Productivity, μg or mg/g substrate	Reference
Wheat rawa with raspberry seed powder	Antibiotic Neomycin	*Streptomyces marinensis*	10, 755 μg/kg	(119)
Wheat rawa	Antibiotic Cephalosporin C	*Acremonium chrysogenum*	22, 281 μg/kg	(20)
Corncob	Antibiotic Oxytetracycline	*Streptomyces rimosus*	10,000–11,000 mg/kg	(121)
Wheat brain	Immunesuppressive agent Cyclosporin-A	*Tolypocladium inflatum*	5,043 mg/kg	(122)

times and consisted 10, 855 μg/g substrate when wheat rawa with raspberry seed powder was used in a SSF process (119).

Cephalosporins, which are less toxic broad-spectrum antibiotics comparable in action to ampicillin, are β-lactam antibiotics derived from a species of fungi of the genus *Cephalosporium*. They are effective against Gram-positive and Gram-negative bacteria by inhibiting bacterial cell wall synthesis. *Acremonium chrysogenum* was used for cephalosporin C production on wheat rawa (120). The optimum productivity of cephalosporin C was achieved when wheat rawa was added with soluble starch, 1% (w/w), yeast extract, 1% (w/w), and mineral solution in ratio 1.5:10 (v/w of wheat rawa) with the following composition: K_2HPO_4 (0.5 g/L), $MgSO_4 \cdot 7H_2O$ (0.5 g/L), $FeSO_4 \cdot 7H_2O$ (0.01 g/L), and NaCl (0.5 g/L). Initial moisture content was 80% and a pH of 6.5. The sterilized and cooled medium was inoculated with spores of *Acremonium chrysogenum*. Inoculum with concentration 10^8 spores/mL was added in quantity 10% (v/w) to the medium and cultivation was conducted at 30°C for 5 days. Yield of cephalosporin C was 22, 281 μg/g of substrate.

Oxytetracycline was produced by Streptomyces rimosus TM-55 in solid state fermentation on a corncob supplemented with 20% (w/w) rice bran or 1.5–2.5% $(NH_4)_2SO_4$ as the sole nitrogen source, 1% $CaCO_3$, 2% $MgSO_4 \cdot 7H_2O$, 0.5% KH_2PO_4, and 0.6–0.8% aspartic acid or lysine, with incubation for 8 days at 25–30°C (121). Optimal antibiotic production was at initial pH from 5.2 to 6.3, and at initial moisture content from 64 to 67%. Yield was 10–11 mg of oxytetracycline per gram of substrate.

Cyclosporin A, a potent immunesuppressive agent and an anti-hypertensive agent, is widely used in tissue and organ transplantation to prevent tissue rejection. It is a cyclic oligopeptide produced by the fungi *Tolypocladium inflatum*, *Cylindrocarpon lucidum*, *Fusarium solani*, and *Neocosmospora varinfecta* (126). The major steps of cyclosporin production are: fermentation, downstream processing, and formulation of the final product. The mutant of

Tolypocladium inflatum produced 5,043 mg cyclosporin-A from kg of wheat bran under optimum fermentation conditions in 10 days. It was grown on a sterile wheat bran medium containing 20% millet flour, 10% jowar flour, 0.15% zinc sulfate, 0.25% ferric chloride, and 0.05% cobaltous chloride (122). The optimal parameters for cyclosporin production were found as follows: addition of inoculum to bran in a 6:10 ratio (v/w), initial moisture content of 70%, initial pH of bran 2.0 and incubation temperature of 25°C. Cyclosporin-A was purified by solvent extraction with ethyl acetate, followed by column chromatography. The isolated product complies with the United States Pharmacopoeia specifications.

Lovastin, an antihypercholesteremic agent, is a competitive inhibitor for enzyme 3-hydroxy-3-methylglutaryl coenzyme reductase. Its application decreases the concentration of cholesterol in the plasma of humans and animals. The Biocon India company manufactures lovastatin by solid state fermentation on wheat brain media (58). Extracted sweet sorghum pulp supplemented with cheese whey and minerals was also suitable for lovastatin production with fungi *Aspergillus terreus* by SSF (127).

Notwithstanding the successful applications, there is no real economic reason to use wastes for the production of medicines because the price of product is high as compared with the price of raw material.

An additional disadvantage is that the content and quality of wastes as raw material is variable.

2.12. Production of Gibberellic Acid

Gibberellic acid ($C_{19}H_{22}O_6$), a plant hormone stimulating plant growth, can be produced by fungi *Gibberella fujikuroi* or *Fusarium moniforme*. It is widely used in agriculture to regulate plant development, promote flowering, or accelerate germination of barley in the brewery industry. It was shown that higher concentrations of gibberellic acid can be obtained in the SSF process comparable with SmF with lower production and extraction costs (34). Production of gibberellic acid by *Gibberella fujikoroï* was carried out by fed-batch solid state fermentation on a wheat bran supplemented medium (33). Wheat bran was autoclaved for 3 h at 121°C, mixed with soluble starch in ratio 24:1, sterilized for 2 h at 121°C, and wetted with sterile nutrient solution to increase moisture content to 50%. The biomass of *Gibberella fujikoroï* was aseptically added to the medium. The cultivation was carried out at 28°C and constant moisture content for 300 h. Sterile corn starch was mixed with sterile distilled water and this suspension was added in a 1:6 ratio to wheat brain (w/w) three times at 72, 96, and 120 h of cultivation, respectively. The medium was automatically mixed for 10 s every 2 h. The maximum yield of gibberellic acid, 3 g/kg dry matter, was obtained after 10 days of cultivation.

2.13. Production of Chemicals

According to the estimation of McKinsey and Company, the consulting firm, 2% of today's $1.25 trillion chemical market is produced using biotechnology processes. McKinsey predicts that by 2010 this biotechnological production of chemicals will grow to 30% because biotechnologies have demonstrated such benefits as a lower capital costs, lower break-even utilization rates, less waste, lower energy use, and fewer processing steps (www.bcintlcorp.com).

2.13.1. Production of Acetone and Butanol

Production of the solvents acetone and butanol by bacteria *Clostridium acetobytiricus* is one of the largest known biotechnological processes. During World War I, Chaim Weizmann developed this method for production of acetone, which was needed for the manufacturing of smokeless gunpowder. In 1945, 66% of the total butanol and 10% of the total acetone were obtained by the biotechnological process, but in 1960s, it was replaced by chemical synthesis of alcohols and other solvents. However, in some countries, fermentation plays an essential role for acetone production. For example, about 50% of the acetone requirements in China are still covered by microbial production (128). To make the biotechnological production of solvents economically competitive, it is necessary to overcome three major problems: the high cost of substrate (molasses, which is usually used for industrial fermentation), the low product concentration (about 2% because of solvent toxicity), and the high product recovery costs (extraction of the solvents and distillation has been used in past) (128). Increasing interest in microbial production of chemicals, based on novel fermentation and product recovery technologies has been observed in recent years. A combination of new genetically modified strains of *Clostridium* spp. with high productivity, using inexpensive agricultural wastes as substrates in continuous cultivation, and new product recovery technologies by gas stripping, liquid/liquid extraction or membrane-based systems can revive industrial acetone/butanol fermentation. Substrates for biotechnological production of the solvents are renewable resources such as starch containing wastes and cellulose from agricultural waste (Table 8.10).

An attempt to reconstitute an industrial process for acetone and butanol fermentation by new achievements was described for a pilot plant in Austria (128). *Clostridium beijerickii* NRRL B-592 was used for solvent formation (134). This strain produced neutral solvents (acetone, *n*-butanol, and ethanol) at an overall dilution rate of 0.13 h^{-1} with solvent concentration 9.27 g/L and solvent productivity of 1.24 g/(L h) in a two-stage continuous cultivation. The volumes of two-stage pilot plate reactors were 50 L and 300 L. Substrates were agricultural starch containing material. Potatoes were steam-exploded, treated with α-amylase and sterilized. No growth additives were required. Product separation was planned by gas stripping with heating of the effluent to 70°C and condensation of the solvent/water vapors.

2.13.2. Production of Glycerol

The microbial process for glycerol ($CH_2OH–CHOH–CH_2OH$) production by *Saccharomyces cerevisiae* (via sulfite steered fermentation) was developed in 1914 in Germany because glycerol was needed for manufacturing of explosive nitroglycerol. Microbial production of glycerol was then replaced by a less expensive chemical technology of glycerol synthesis from petroleum by-products. At present, glycerol is widely used in pharmaceutical, cosmetic, food, and chemical industries. Industrial microbial production of glycerol is limited by the difficulties of glycerol recovery from cultural medium. An increasing interest in microbiological glycerol production has been observed in recent years based on the use of inexpensive renewable substances and development of new effective separation techniques, such as ultrafiltration, reverse osmosis, and ion exchange. The most popular waste proposed for biotechnological glycerol production is whey (Table 8.11).

Table 8.10
Agricultural and food processing waste for production of solvents(acetone, butanol and ethanol)

Substrate	Microorganism	Process	Reference
Apple pomace	*Clostridium beijerinckii*	Batch	(129)
Potato waste	*Clostridium acetobutylicum*	Batch with integrated membrane extraction	(130)
Palm oil mill effluent	*Clostridium aurantibutyricum*	Batch	(131)
Starch-based packing peanuts	*Clostridium beijerinckii*	Batch	(132)
Agricultural wastes			
Concentrated hydrolysate of domestic organic waste	Clostridium acetobutylicum	Batch	(133)
Potato waste	*Clostridium beijerinckii*	Two-stage continuous	(134)
Whey permeate	*Clostridium* sp.	Continuous with cells immobilized in calcium alginate beads	(135)

Glycerol is synthesized by the reduction of dihydroxyacetone phosphate to glycerol 3-phosphate by 3-phosphate dehydrogenase, followed by dephosphorylation of glycerol 3-phosphate to glycerol. Glycerol can be obtained during the yeast fermentation of sugars if sodium bisulfite is added to the medium to react with acetaldehyde before it can be converted to ethanol. Fermentation can be provided in batch or continuous mode. The use of immobilized microbial cells allows the process to be conducted at high cell density, to prevent washout of cells from the reactor, to operate the process with low retention time, and to receive a high yield of glycerol. The osmophilic yeast strains accumulate glycerol as a compatible solute to counterbalance high osmotic pressure (140). Representatives from genera *Saccharomyces cerevisiae* and *Kluyveromyces fragilis* are more often used for glycerol biosynthesis. The aerobic or anaerobic bioconversions are applied for yeast glycerol production.

An example of glycerol production by yeast *Kluyveromyces fragilis* on whey permeate in bath process with immobilized cells is given in the following lines (136, 138). Salted whey containing 7.5% NaCl supplemented with peptone, malt extract, and 1% of Na_2SO_3 was sterilized and used as a substrate. The immobilization of *Kluyveromyces fragilis* cells in agar gel was accomplished as follows: the cells were grown to the stationary phase at 37°C and the cell suspension was mixed with an equal volume of sterilized warm aqueous solution of 3% (w/v) agar. The mixture was then poured into sterile Petri dishes and incubated overnight

Table 8.11
Agricultural and food processing waste for production of glycerol

Substrate	Microorganism	Concentration, g/L	Process	Reference
Whey	*Kluyveromyces fragilis*	13.2	Batch with immobilized cells in agar gel	(136)
Whey	*Kluyveromyces fragilis*	18.7	Continuous in membrane cell recycle bioreactor	(136)
Whey permeate	*Kluyveromyces fragilis*	11.6	Batch	(137)
Sugar molasses	*Saccharomyces cerevisiae*	53.0	Continuous with cells immobilized in agar gel	(138)
Hydrolized wheat milling residues	*Saccharomyces cerevisiae*	19.8	Continuous with cells immobilized in agar gel	(138)
Ram horn hydrolizate as a source of nitrogen	*Saccharomyces cerevisiae*	8.5	Batch	(139)

at 25°C under sterile conditions. The gel was cut into cylindrical beads using a stainless steel cylinder 4 mm in diameter. 10% (w/v) of agar beads were added as inoculum to the medium to start up the cultivation. Biosynthesis of glycerol was conducted at temperature 37°C, pH 7.0 under constant agitation for 60 h. The maximum concentration of glycerol was 13.2 g/L; the yield from sugars was 28%.

2.14. Production of Fuel

2.14.1. Production of Ethanol

About 80% of global energy requirements are presently met by fossil fuels, which will lead to the eventual exhaustion of these energy resources. Thus, we've seen an increasing interest for a clean and renewable energy alternative. Petroleum comprises 97% of the energy consumed for transportation. Production of ethanol as an alternative liquid fuel for transportation can reduce oil consumption and protect environment. Compared to oil, the application of ethanol does not impact the amount of carbon dioxide in the atmosphere. The emission and toxicity of ethanol are lower than those of petroleum. All automobile manufacturers produce vehicles that can readily use 10% ethanol or 85% ethanol blends for fuel, and ethanol can replace diesel in heavy vehicles (141). Sales of ethanol worldwide exceeded $9 billion in 2002.

Brazil is the leader in ethanol production, followed by the United States. These countries had sales of $4 billion (44% of the worldwide market) and over $2.2 billion (24% of the worldwide market), respectively (www.bcintlcorp.com).

The biotechnological production of ethanol is based on enzymatic conversion of sugars in alcohol by microorganisms, usually by the yeast *Saccharomyces cerevisiae*. Starch, the most common used substrate, is a main cost element in fuel ethanol production. For example, fermentation of corn glucose is used for fuel ethanol production in the United States and from cane sugar in Brazil. Over 175 Mt of sugarcane (65% of 270 Mt harvested) was converted to ethanol fuel in 1996–1997 in Brazil. Alternative substrates include different food processing waste, agricultural residues, and the paper portion of municipal solid wastes. They can be used for fuel ethanol production to reduce costs, increase applications as a fuel additive, and provide a partial solution to their disposal (Table 8.12).

The largest source of lower-value substrate for fuel ethanol production is lignocellulosic waste. It is a complex substance consisting of cellulose, hemicellulose, and lignin. The contents of cellulose and hemicellulose account for 23–53% and 20–35% of plant material, respectively. Cellulose is a long chain polymer, made up of repeating units of glucose, which are connected by β-1,4-glycosidic bonds, whereas hemicellulose is a branched chain of xylose and arabinose that also contains glucose, mannose, and galactose. The composition of some lignocellulosic materials is shown in Table 8.13 (150).

To split cellulose and hemicellulose to monomers, the chemical hydrolysis by sulfuric acid or enzymatic hydrolysis by cellulase can be used. Production of ethanol from acid hydrolizates of agricultural–industrial residues containing cellulose is a known process, but new achievements in biotechnology continue to generate significant process improvement and create the possibility of bioethanol commercial production (141). The main steps in biotechnological conversion of lignocellulosic waste into fuel ethanol are: (a) release of cellulose and hemicellulose from their complex with lignin; (b) production of sugar solution from cellulose and hemicellulose; (c) fermentation of sugars to produce ethanol; (d) ethanol recovery from cultural medium. There are two ways to produce solution of sugars: (a) completely hydrolyze lignocellulose to monomers by mineral acid; (b) treat lignocellulose to solubilize hemicellulose and lignin, but leave cellulose to be saccharified enzymatically.

Cellulose can be separated from solution and hydrolyzed with cellulases (usually from the fungi *Trichoderma reesei*) followed by ethanol production. This hydrolysis may be conducted as a separate step or concurrently in a process known as simultaneous saccharification and fermentation. In the case when hydrolyzed hemicellulose and the solid cellulose are not separated after pretreatment and ethanol is produced from hemicellulose sugars concurrently, the process is called simultaneous saccharification and co-fermentation (SSCF) (150). Yeast *Saccharomyces cerevisiae*, which are conventionally used for production of ethanol from C_6 sugars cannot utilize arabinose, xylose, or cellobiose, which are produced enzymatically from hemicellulase or cellulose. Use of cellulase with sufficient β-glucosidase activity to produce pure glucose increases the cost of fuel ethanol. Application of the microorganism, which can utilize the above mentioned compounds and produce ethanol, can eliminate this problem.

Genetically engineered microorganisms for ethanol production from pentose, namely yeasts of genus *Saccharomyces* (147) and Gram-negative bacteria of genera *Escherichia*

Table 8.12
Solid waste for production of ethanol

Waste and pretreatment	Microorganism	Process	Productivity	Reference
Pineapple cannery waste	*Saccharomyces cerevisiae*, M	Continuous, 30°C, pH 4.5, dilution $0.05\,h^{-1}$	3.75 g/L h	(142)
Jerusalem artichoke tubers	*Kluyveromyces marxianus*	Batch, pH 3.5	73.0 g/L	(143)
Guava pulp	*Saccharomyces cerevisiae*	Batch, 30°C, pH 5.0	58.0 g/L	(144)
Sugar cane bagasse, 16%, pretreated with cellulase	*Klebsiella oxytoca* P2, GE	Batch, 35°C, pH 5.2	40.0 g/L	(145)
Cellulose, 10%, pretreated with cellulase	*Klebsiella oxytoca* P2, GE	Batch, 35°C, pH 5.2	35.7 g/L	(146)
Cellulose, 10%, pretreated with cellulase	*Zymomonas mobilis*, *GE*	Batch, 35°C, pH 5.2	36.2 g/L	(146)
Sugarcane bagasse pre-treated by steam explosion and hydrolyzed with cellulases	*Saccharomyces cerevisiae*, GE	Batch, 30°C, pH 5.5	0.13 g/g dry bagasse	(147)
Corn fiber hydrolyzates	*Escherichia coli*, GE	Batch	21.0–34.0 g/L	(148)
Wheat straw hydrolyzated by sulfuric acid	*Pichia stipitis*	Batch, 28°C, pH 6.5	0.41 g/g	(45)
Cotton gin waste pretreated by steam explosion and hydrolyzed with cellulases	*Escherichia coli*, *GE*	Batch, 435°C, pH 5.0	270 mL/kg	(149)

M mutant; *GE* genetically engineered.

(148, 151), *Klebsiella* (145, 146), and *Zymomonas* (152) were constructed for ethanol production from lignocellulosic wastes. For example, the cellobiose-fermenting recombinant *Klebsiella oxytoca* P2 containing chromosome-integrated pyruvate decarboxylase and alcohol dehydrogenase genes from the ethanol-producing bacterium *Zymomonas mobilis* can produce ethanol from glucose and cellulose and was recommended for simultaneous saccharification

Table 8.13
Composition of various lignocellulosic raw materials(150)

	Lignocellulosic material								
	Corn stover	Wheat straw	Rice straw	Rise huts	Bagasse fiber	Cotton gin trash	Newsprint	Populus tristis	Douglas fir
Carbohydrate (% of sugar equivalent)									
Glucose	39.0	36.6	41.0	36.1	38.1	20.0	64.4	40.0	50.0
Mannose	0.3	0.8	1.8	3.0	NA	2.1	16.6	8.0	12.0
Galactose	0.8	2.4	0.4	0.1	1.1	0.1	NA	NA	1.3
Xylose	14.8	19.2	14.8	14.0	23.3	4.6	4.6	13.0	3.4
Arabinose	3.2	2.4	4.5	2.6	2.5	2.3	0.5	2.0	1.1
Non-carbohydrate (%)									
Lignin	15.1	14.5	9.9	19.4	18.4	17.6	21.0	20.0	28.3
Ash	4.3	9.6	12.4	20.1	2.8	14.8	0.4	1.0	0.2
Protein	4.0	3.0	NA	NA	3.0	3.0	NA	NA	NA

NA not applicable.

and fermentation process (145, 146, 153). A description of the proposed technology is given in the following lines.

Genetically modified bacterial strain *Klebsiella oxytoca* P2 supplied by Professor L.O. Ingram (University of Florida) was used for ethanol production on pure cellulose by simultaneous saccharification and fermentation process (146). Bacterial culture was stored as freeze-dried biomass in ampoules. To produce inoculum, *K. oxytoca* P2 cells were grown on nutrient agar containing 600 mg/L chloramphenicol and the biomass was transferred to a liquid medium containing: 10 g/L of tryptone; 5 g/L of yeast extract, 5 g/L of sodium chloride, and 40 g/L of glucose. Inoculum was incubated under static conditions for 16 h at 30°C. Cells were separated by centrifugation and used for fermentation (initial concentration was of 320 mg dry weight/L. The medium for ethanol production was the same as for inoculum, but cellulose (100 g/L) was used as a carbon source instead of glucose. Saccharification and fermentation were performed under self-supported anaerobic conditions at 35° C and pH 5.2. Cellulase, which was used for saccharification of cellulose, contained 105 filter paper units (FPU)/mL and 20 IU/L β-glucosidase. It was added to the medium with 100 g/L cellulose in quantity 15 FPU/g. The fermentation medium and enzyme were sterilized by filtration. Cellulose was suspended in a small amount of distilled water, autoclaved for 20 min at 121°C, and added separately to the medium. Ethanol concentration was 30 g/L in 71 h. Specific ethanol production rate of *K. oxytoca* P2 was 1.21 g/g cell dry weight per hour.

There are some known projects for the industrial application of ethanol production from organic waste. For example, a large-scale plant converting municipal solid wastes, sewage sludge, and organic waste into fuel ethanol was planned for construction by the Masada Resource Group (http://www.solidwastemag.com) in Middletown, NY. Masada OxyNol™ concentrated acid hydrolysis technology could be used for converting recyclable garbage with the discarded food and other carbon-based wastes into ethanol. BC International Corporation constructed a plant near Jennings, Louisiana, which was projected to produce 30 million gallons of fuel and industrial-grade ethanol per year using BCI's proprietary technology with the almost complete conversion of the five carbon (C_5) sugars (arabinose and xylose) by

genetically modified microorganism from low cost cellulosic biomass (sugarcane bagasse), which resulted in superior economics of ethanol production (www.bcintlcorp.com).

2.14.2. Production of Hydrogen

Hydrogen is a clean substance, has a high energy yield (142 kJ/g) and can be a promising energy source in future. Microbial production of hydrogen may be one of the ways to solve the energy problem. Microbial hydrogen production is represented by two main methods: application of phototrophic bacteria cultured under anaerobic conditions and light and application of chemotrophic bacteria. If hydrogen is produced by photodecomposition of organic compounds, benefit from production of hydrogen will be coupled to waste bioremediation. Some liquid organic wastes, which were used for hydrogen production by phototrophic bacteria *Rhodobacter sphaeroides*, are shown in Table 8.14.

At the present time, phototrophic bacteria are considered the most promising method for biological production of hydrogen (158). For example, *Rhodobacter sphaeroides* was used for the treatment of olive mill wastewater (154). Olive mill wastewater contained in 100 g suspended solid 36.02 g of carbon; 5.26 g of hydrogen and 0.96 g of nitrogen was diluted by distilled water to a 2% concentration, and filtrated. pH was adjusted to 6.8–7.0 by the addition of NaOH, and medium sterilization was provided at 121°C for 15 min. Production of hydrogen gas was conducted at 32°C under constant stirring at a rate of 300 rpm. Light intensity was kept at 200 W/m^2 at the outer surface of the photobioreactor. The reactor was flushed with pure argon in order to create an anaerobic atmosphere. Afterwards, 10% inoculum of preactivated bacteria was added. The maximum hydrogen production was 13.9 L H_2/L of 2% olive wastewater. Along with hydrogen production, chemical oxygen demand decreased from 1,100 to 720 mg/L. In addition, valuable by-products including carotenoid (40 mg/L of olive mill wastewater) and polyhydroxybutyrate (60 mg/L of olive mill wastewater) were obtained.

The fermentative method for hydrogen production has an advantage over photosynthetic bacteria in that hydrogen can be continuously produced in a reactor without light. Low-cost substrates like renewable organic waste can also be used for hydrogen production by dark fermentation with chemotrophic bacteria (Table 8.15).

Hydrogen production from organic waste was accomplished using 103–336 L H_2/kg of dried solids for fermenting glucose-containing solution, or 22.4–184 L H_2/kg of dried solids from protein-containing solution (159). Pure cultures of anaerobic bacteria such as *Clostridium* sp. (159, 161) or a mixture of anaerobic microorganisms taken from anaerobic digestion sludge or sludge compost (162, 163) can serve as an inoculum for the bioconversion of organic waste into hydrogen. For hydrogen generation from anaerobically treated organic wastes, the activity of hydrogen consumers must be inhibited. Addition of acetylene, 1% v/v, in batch anaerobic composters contained 25% (w/v) total organic solids and inoculated with an undefined cellulolytic consortium derived from anaerobic digesters, inhibited methanogenic activity, but had no effect on the rate and amount of hydrogen produced by pure culture of *Clostridium thermocellum* grown under the same conditions (161). The example of hydrogen production from organic waste is described in the following lines (160). Organic waste collected from a dining hall was mixed with night soil sludge and sewage sludge from wastewater treatment plant in a ratio by total solids 46.3:37.3:16.4. The moisture content of this mixture

Table 8.14
Liquid waste for production of hydrogen by phototrophic bacteria *Rhodobacter sphaeroides*

Waste	Microorganism	Productivity	Rate of H_2 production, L/(L h)	Reference
Olive mill wastewater	*Rhodobacter sphaeroides*	13.9 L H_2/L of 2% wastewater	0.009 (1% wastewater)	(154)
Acidic stream after acidogenesis of selected municipal solid wastes	*Rhodobacter sphaeroides*	35 mL H_2/(g dry weight h)	0.048	(155)
Wastewater from lactic acid fermentation plant	*Rhodobacter sphaeroides*	4.5 L/L of 10% wastewater	0.0058 (50% wastewater)	(156)
Distillery wastewater	*Rhodobacter sphaeroides*, suspended cells	1.0 L/L of 10% wastewater	0.0007	(156)
Distillery wastewater	*Rhodobacter sphaeroides*, immobilized cells	3.0 L/L of 10% wastewater	0.006	(156)
Wastewater from tofu (a product from soybean) industry	*Rhodobacter sphaeroides*, immobilized cells	1.9 L/L of wastewater	0.016	(157)
Pretreated sugar refinery wastewater	*Rhodobacter sphaeroides*	8.6 L/L of 20% wastewater	0.001	(47)

was 85%. Anaerobic sludge was boiled for 15 min to inhibit the hydrogen consumers and to harvest spore-forming anaerobic bacteria used as seed. A mineral solution was added to obtain the following salt content: %: NH_4HCO_3, 0.2; KH_2PO_4, 0.1; $MgSO_4 \cdot 7H_2O$, 0.01; NaCl, 0.001; $Na_2MoO_4 \cdot 2H_2O$, 0.001; $CaCl_2 \cdot 2H_2O$, 0.001; $MnSO_4 \cdot 7H_2O$, 0.0015; and $FeCl_2$, 0.00028. A headspace gas contained 80% N_2 and 20% CO_2. The anaerobic digesters were incubated at 37°C under rotating at 1.5 rpm. According to the results, high hydrogen production potentials of 140 mL H_2/g of total volatile solid can be achieved with the hydrogen content in a biogas greater than 60%.

It was shown that carbohydrates are a much more effective substrate than proteins for hydrogen production. Promising results were obtained from the cultivation of hydrogen-producing anaerobic microflora from fermented soybean-meal in a silo on bean curd

Table 8.15
Solid waste for production of hydrogen by chemotrophic bacteria (modified from ref.(159))

Waste	Microorganism	Productivity	Reference
Organic municipal solid waste	Sludge from anaerobic digester	140 L H_2/kg TVS	(160)
Lignocellulosic waste	*Clostridium thermocellum*	11.2 LH_2/kg DS	(161)
Lignocellulosic waste	Consortium from anaerobic compost	16.8 LH_2/kg DS	(161)
Cellulose	Natural anaerobic microflora from anaerobic digestion sludge	149 LH_2/kg hexose	(162)
Household waste	Mixed anaerobic bacterial flora		(163)
Wastewater sludge	*Clostridium bifermentas*	20.2LH_2/kg DS	(159)
Bean curd manufacturing waste	Anaerobic microflora from fermented soybean-meals	316 LH_2/kg hexose	(164)

manufacturing wastes after filtration. Bean curd manufacturing waste containing 210 g of volatile solids was placed into 4 L of distilled water, filtrated, and used as organic substrate in a batch process. This medium contained: 7.76 g/L of total carbohydrate; 2.66 g/L of soluble carbohydrate and 5.01 g/L of protein. The organic substrate was supplemented with 142 mg/L of $FeCl_2 \cdot 4H_2O$ and 1 mL/L of 0.2% resazurin. An anaerobic inoculum with a biomass concentration of 1.63 g volatile suspended solids was added to the organic substrate at a ratio of 1:3. The initial pH was adjusted to 6.0, and the process occurred at 35°C under constant stirring for 60 h. The final hydrogen content in the headspace was 63% and the hydrogen yield was 2.54 mol of H_2/mol of hexose utilized (316 L H_2/kg hexose).

3. VALUE-ADDED BY-PRODUCTS OF ENVIRONMENTAL BIOTECHNOLOGY

3.1. Composting

The aim of environmental technologies for waste treatment is to reduce the volume and toxicity of waste. Simultaneously, the wastes can be converted into such value-added products as fuel or fertilizer. Composting is one of the main environmental technologies that can be applied for bioconversion of large quantities of organic waste. Composting is an aerobic mesophilic or thermophilic microbial decomposition of organic constituents into a humus-rich, safe, and relatively stable product. Aerobic decomposition is an exothermic process, with temperatures increasing in the compost material during the process. Composting is an ancient technology that was used by farmers for centuries to convert biodegradable horticultural, agricultural, gardening, and kitchen wastes into nutrient-rich material for further use as fertilizer.

The main objectives of composting are as follows: production of stabilized organic matter; odor reduction; volume reduction; inactivation of pathogens and parasites. Composting is a microbiological process during which different groups of microorganisms take part in the biodegradation of organic waste. Microbial succession during the composting process consists of: (a) a latent phase (ambient temperature 20°C) of microorganisms adapting to composting conditions, such as temperature, moisture content, aeration etc; (b) a mesophilic phase (20–40°C) of intensive microbial growth, resulting in temperature increasing due to oxidizing of organics; (c) an initial thermophilic phase (40–60°C) that sees growth of thermophilic bacteria, actinomycetes, and fungi; (d) a thermophilic phase (60–80°C), that sees growth of thermophilic and spore-forming bacteria, sulfur- and hydrogen-oxidizing autotrophs, and aerobic non-spore-forming bacteria; at the end of this phase temperature drops to 40°C; and (e) a cooling and maturation phase (40°C to ambient temperature), in which bacteria are involved in nutrient cycling, mesophilic/thermotolerant actinomycetes, and fungi.

This succession of the different phases of microbial development is typical for composting in the windrows or in the aerated static piles. The mesophilic phase lasts for some days, the thermophilic phases last from a few days to several months, and the duration of cooling and maturation phase consists of several months.

There are typically three main types of composting systems: static pile, windrow, and in-vessel. Static pile composting involves stacking the organic waste into piles for natural biodegradation without turning. In aerated static piles, air is supplied through perforated piles by blowers. It is the least expensive method of composting, which can be used for small-scale processes. The biodegradation rate depends on weather conditions and does not ensure the reduction of pathogens due to poor mixing. Windrows are long narrow parallel rows with 1–2 m height of mixed organic waste, which are periodically turned to provide aeration. Turning is provided more frequently at the beginning of composting when more oxygen is demanded for biodegradation of organics. Due to turning, temperatures above 60–70°C cannot be reached in composted wastes. Composting in windrows lasts 50–80 days. Windrows usually are used for large volumes of wastes; thus they are situated under cover outdoors and require a lot of space. While windrow composting has low capital costs and produces good quality compost, disadvantages include odor and leachate problems, cost of turning, loss of ammonia, and potential spreading of allergic spores of fungi in air during turning.

In-vessel systems, which use a closed reactor for the bioconversion of organic waste, ensure constant temperature control and proper air supply, require little space, minimize odor problems, and are not weather sensitive. The duration of this process is 14–19 days. Maturation of the product is provided in piles outside the reactor. The cost of in-vessel composting is higher than composting in piles and windrows, and is not always suitable for large volumes of organic wastes.

The essential parameters for composting are moisture content, C/N ratio, aeration, temperature, pH, particle size, additives, and processing time. The optimal moisture content of the composting material must be 60%, but the process can be performed in the range from 40 to 70%. Moisture content higher than 70% decreases the rate of organic decomposition, creates anaerobic conditions and odor problem. The moisture content in rice, fruit, and vegetable food waste is approximately 90%, in orange peels it is 76%, and in sawdust it is 25%. The

C/N ratio of the material must be between 25:1 and 35:1. The higher C/N ratio reduces the rate of process, but lower C/N ratio leads to nitrogen loss. Different wastes have different C/N ratio, for example, the C/N ratio is as follows: sewage sludge, from 6:1 to 8:1; food waste, 15:1; fruit waste, 35:1; green vegetable wastes, weeds, from 11:1 to 20:1; sawdust, 500:1. To receive a desirable C/N ratio, the mixing of waste can be used. Additional aeration can improve the process. Aeration is provided by turning, mixing, the use of fans, blowers, and compressors. The optimal air supply is considered to be from 0.6 to 1.8 m^3 air/(d kg of volatile solids) during the thermophilic phase of composting (55). Bulking agent, sawdust, wood chips, can be added to improve the mass transfer of oxygen and carbon dioxide between air and material. A temperature of at least 55–60°C must be maintained for several days to inactivate the pathogens, parasites, and weed seeds. However, temperatures higher than 70°C may cause inactivation of microorganisms and slow or stop the composting process.

Optimal pH levels of composting are from 7.0 to 8.0. Particle size greatly influences the biodegradable rate. Before composting begins, wastes are shredded to particles less than 5 cm in size. The smaller size of particles ensures the greatest surface area and enhances mass transfer between biodegradable material and microorganisms. However, if the particles are too small, the oxygen transfer can be negatively impacted. Additives such as another waste can be added for successful composting to provide optimal C/N ratio (sewage sludge to decrease, and sawdust to increase C/N ratio), water to maintain optimal moisture content, additional sources of nitrogen, phosphorus, and bioessential mineral elements if compost materials lack these elements, lime to provide optimal pH.

Processing time depends on the nature of material and conditions of composting. Food waste can be composted during some months, but horticultural waste composting may last from 9 to 12 months. Composting of food waste may take a year in static piles, several months in windrows, and several weeks for in-vessel composting. The final compost must be stable, rich in available plant nutrients, relatively free from pathogens, weed seeds, and plant inhibitors product, dark brown or black in color, similar to humus in smell, and touch. The pH must be around 7.0, the preferable C/N ratio is approximately about 10:1 (usually it is from 10:1 to 25:1), the moisture content is between 35 and 50%, and organic matter contents between 40 and 65%. Compost can be applied as a soil amendment to improve physical properties such as soil structure, water holding capacity, and porosity. It can be used as fertilizer to provide plants with essential nutrients such as nitrogen, phosphorus, potassium, and microelements. Compost can also be used as mulch for trees, landscapes, and gardens. Composting of biodegradable waste is an important element in sustainable waste management, and there is a trend towards rapid development of source segregated organic waste composting (165). In Europe, composting has an important role in the processing of the biodegradable waste, which will have to be diverted totally from landfill in the future. It was estimated that around 60 million tons of recoverable organic waste is produced in Europe each year. Approximately 15% (9 million tons) of the recoverable organic fraction is currently recovered through home composting or source separation and centralized composting throughout the EU countries. Economic analysis of composting in the United States indicated that municipal waste composting generally costs around $50 per ton, but it may be competitive with land disposal where the cost of landfilling is high (166).

Vermicomposting may be used for recycling small quantities of organic waste, such as food waste in an apartment or composting yard wastes in the backyard. Vermicomposting uses earthworms to consume the fragments of vegetable and fruit (not meat products) food waste. The earthworms' life activity ensures aeration and mixing of substrate, microbial decomposition of substrate in the worm's intestine, which leads to organic matter stabilization and production of high quality compost. It has been observed that 1 kg of worms can eat 4 kg of waste per week. The process takes place in containers or bins. The addition of excess waste can produce anaerobic conditions.

3.2. Aerobic Intensive Bioconversion of Organic Wastes into Fertilizer

Composting of organic wastes occupies much space and is not suitable for large-scale applications in countries with a shortage of land. To minimize the process duration and the space required for the large-scale bioconversion, an intensive in-vessel bioconversion of organic waste can be used. An aerobic thermophilic treatment to convert a mixture of sewage sludge and food waste or food waste into fertilizer was proposed (167–170). This process includes biotreatment of organic waste at 60°C under controlled aeration, stirring, and pH. To maintain a neutral pH at the beginning of the bioconversion, $CaCO_3$ was added at 5% to the total solids of organic waste. Addition of artificial or natural bulking agent improved the bioconversion and increased the stability of the final product. Addition of starter bacterial culture *Bacillus thermoamylovorans* SW25 enhanced the bioconversion of the mixture of sewage sludge and food waste, but no starter culture was needed in aerobic bioconversion of food waste into organic fertilizer (167, 170, 171). The final products contained stable organic matter, nitrogen, phosphorus, and potassium. Its application to subsoil resulted in faster growth and development of agricultural plants.

3.3. Recovery of Metals from Mining and Industrial Wastes

Wastewaters of mines and metallurgical industry contain many metals that are both a source of environment contamination and a source of value-added products which can be recovered for further use. For biological recovery of metals from waste, two main methods may be employed: (a) bioleaching and (b) biosorption (172, 173). In bioleaching technology, metals are solubilized into a dilute acid solution from waste rocks due to microbial activity. In the presence of sulfur and iron compounds, bacteria from genera *Thiobacillus*, *Sulfolobus*, and *Leptospirillum* can produce acid and oxidizing media caused in the oxidation of metals and their dissolution in the leachates (174). The solubilized metals are then recovered from leachates by solvent extraction or electroplating. In biosorbtion technology, metal ions can be sorbed by precipitation, adsorbtion, chelation, or ion exchange mechanisms on the surface of microbial cells or can be "trapped" by diffusion through cells membrane and concentrated inside the microbial cells. For example, bioleaching is essential for copper recovery from dumps of waste rocks. At least 25% of the world copper production comes from biorecovery. Bacterial leaching of agglomerated ore in heaps was responsible for 200,000 tons/year of cathode copper in 1998 (175). The content of copper in such dumps is low, approximately 0.1–0.5%. Diluted sulfuric acid is sprinkled or sprayed onto the dump surface and percolated through the dump creating low pH around 2–3, which is favorable for acidophilic

microorganisms development such as *Thiobacter ferroxidans*. These bacteria produce ferric iron that oxidizes copper chemically according to the following equations:

$$4Fe^{2+} + 4H^{+} + O_2 \rightarrow 4Fe^{3+} + 2H_2O \quad (Thiobacillus\ ferroxidans) \tag{1}$$

$$Cu_2S + 2Fe^{3+} \rightarrow CuS + Cu^{2+} + 2Fe^{2+} \quad \text{(Chemical reaction)} \tag{2}$$

$$CuS + 2Fe^{3+} \rightarrow Cu^{2+} + 2Fe^{2+} + S^{0} \quad \text{(Chemical reaction)} \tag{3}$$

The oxidized copper accumulates in leachate and is then recovered from it. More than 200,000 tons of cathode copper are produced annually using bacterial leaching of agglomerated coper ore in heaps (175). To increase the rate of leaching, the blowing of low pressure air can be used.

Biosorption is used to recover metals from different industrial effluents. The presence of metals in liquid wastes causes environmental pollution. To remove the metals from wastewater, it is possible to use inexpensive microbial biomass as a biosorbent, which can be later treated for metals recovery. Thus, there are two advantages of the biosorption process: clean up the industrial effluent and recovery of value-added elements. Many agricultural wastes can be used directly as biosorbents, including rice husk, wood charcoal, sunflower stalks, sawdust, pine bark, and canola meal. Microbial biomass was also successfully applied for metals recovery from industrial effluents. This biomass may be the waste after different biotechnologies, for example, the biomass of the yeasts *Saccharomyces cerevisiae*, waste from beer fermentation industry, biomass of *Penicillium chrysogenus*, waste of antibiotic production, biomass of *Aspergillus niger*, waste from citric acid production, or obtained by microbial cultivation on food processing wastes, and agricultural residues. To enhance the adsorption capacity of biomass and metal uptake, it can be modified by chemical treatment. Different applications have been reported: the use of the yeast biomass *Saccharomyces cerevisiae* to remove copper (176), application of mycelium of the industrial steroid-transforming fungi *Rhizopus* and *Absidia* to remove lead, cadmium, copper, zinc, and uranium (177), use of yeast biomass of *Rhotorula rubra* as biosorbent for cadmium and lead (178), application of biomass of *Saccharomyces cerevisiae* and *Pseudomonas aeruginosa* as biosorbent for uranium (179), use of fungi *Penicillium digitatum* as biosorbent for uranium, nikel, zinc, cadmium, lead, ferric, and copper (180), and use of *Aspergillus niger* biomass for cadmium, copper, lead, and nickel removal (181).

3.4. Recovery of Metals from Waste Streams by Sulfate-Reducing Bacteria

Metals in the waste streams are pollutants of the environment and must be removed from wastewater. Some metals can be not only removed but also recovered for further reprocessing. Biosorption of metals on living cells, non-living biomass, or microbial extracellular polymers as described earlier is one biotechnological method of metals recovery. Another suitable approach is the precipitation and recovery of carbonates, hydroxides, or sulphides of metals. A typical case is the recovery of metals (Cu, Ni, Cr, Zn) from electroplating wastewater containing usually sulfate as well. Precipitates can be used as pigments, raw material for chemical transformations, or metal concentrate.

Precipitation of metals after reaction with sulfide-producing sulfate-reducing bacteria (SRB) is the most practical biotechnology. Sulfate-reducing bacteria couple the oxidation of diverse organic compounds with the reduction of sulfate. Preferable carbon sources are such organic acids and alcohols as lactate, pyruvate, propionate, formate, succinate, fumarate, malate, benzoate, ethanol, propanol, phenol. There is a large phylogenetic and physiological diversity of SRB. They can be found in any anaerobic environment (redox potential must be lower than -100 mV), rich in organic matter, living at temperatures from 0 to 70°C, and pH of 5–9.5. H_2S produced by the biological reduction of sulfate can react with heavy metal ions to form an insoluble metal sulfide:

$$SO_4^{2-} + CH_3COOH + 2H^+ \rightarrow H_2S_2 + 2CO_2 + 2H_2O \quad (4)$$

$$H_2S + Fe^{2+} \rightarrow FeS \downarrow + 2H^+ \quad (5)$$

The biotechnology process is inexpensive and can be used for selective recovery of metals such as copper or zinc (182–185). Activity of SRB is inhibited by metals (Table 8.16). Therefore, their concentration must not exceed certain levels. Metal sulfides also inhibit the process and must be removed from the bioreactor (186).

To increase process efficiency, the production of sulfide and precipitation of metals can be separated (183, 187, 188). Bioreactors with fixed film or suspended aggregates are used to maintain high concentration of SRB biomass (182, 185, 189–191). To give SRB an advantage over methanogens competing for same donors of electrons, the ratio of COD/SO_4^{2-} must be below 1.2–1.7. The optimal ratio for domination of SRB in anaerobic environment is usually 0.2–0.3 under concentration of sulfate exceeding some g/L, which restricts the application

Table 8.16
Heavy metal toxicity to sulfate-reducing bacteria (from(181))

Metal	Culture of SRB	Toxic concentration, mg/L
Cu	*Desulfovibrio* spp.	20–50
	Desulfovibrio spp.	3
	Desulfovibrio spp.	2–20
	Mixed culture	4–20
	Mixed culture	12
Zn	Mixed culture	25–40
	Mixed culture	20
	Desulfovibrio desulfuricans	13
Pb	Mixed culture	75–80
	Pure culture strain L-60	125
Cd	Mixed culture	4–20
	Pure culture strain L-60	54
Ni	Mixed culture	10–20
	Desulfovibrio desulfuricans	10
Cr	Mixed culture	60
Hg	Pure culture strain L-60	74

areas of SRB for metal recovery. Solid organic waste such as spent mushroom compost, organic material, rice stalks, manure, can be used as donors of electrons for sulfate reduction. Efficiency of metal removal/recovery from wastewater using SRB can reach 98–99% and is significantly less expensive than chemical precipitation of the metals.

Recovery of chromium can be performed by the reduction of Cr(VI) to Cr(III) associated with its precipitation (192, 193). Chromate, containing Cr(VI), is soluble and toxic, whereas Cr(III) is less toxic and tend to form insoluble hydroxide. Chemical Cr(VI) reduction by H_2S is mediated by SRB. Biological reduction of Cr(VI) is mediated by chromium-reducing bacteria. Some sulfate-reducing bacteria are able also to reduce Cr(VI) to Cr(III), Mn(IV) to Mn(II), Fe(III) to Fe(II), or U(VI) to U(IV) (194).

3.5. *Recovery of Phosphate and Ammonia by Iron-Reducing and Iron-Oxidizing Bacteria*

Aluminum and iron salts, usually alum, $Al_2(SO_4)_3 \cdot 18H_2O$, sodium aluminate, $NaAlO_2$, polyaluminum chloride, ferric chloride, $FeCl_3$, ferrous sulfate, $FeSO_4$, ferric sulfate, $Fe_2(SO_4)_3$, are used for the precipitation of phosphate from wastewater (195, 196). The stoichiometric composition of phosphate precipitates differs from the mole ratio of Al: P and Fe: P both 1:1 because of the formation of hydroxide particles with the parts which are not accessible for phosphate binding. Therefore, to ensure a phosphate removal level of less than 1 mg P/L, an excess dosage of metal salts is required. Calcium and magnesium compounds also can be used for precipitation of phosphate from wastewater (197–200).

Inexpensive sources of iron like iron ore, wetland ore, iron-containing clay can be used for phosphate and ammonia recovery from wastewater using iron-reducing bacteria (IRB) and iron-oxidizing bacteria (IOB). This biotechnology combines the following steps: (a) bacterial reduction of iron (III) under anaerobic conditions from such inexpensive sources of Fe(III) as iron ore, wetland ore, iron-containing clay; (b) chemical precipitation of phosphate by Fe^{2+} ions or Fe(II) hydroxide; (c) formation of Fe(II) chelates with organic acids; (d) bacterial oxidation of these chelates and free Fe^{2+} by iron-oxidizing bacteria (IOB) under microaerophilic conditions; (e) co-precipitation of ions of NH_4^+ combined with fine regular particles of $Fe(OH)_4^-$ (201).

Fe(II) salts suitable for chemical recovery of phosphate are expensive and not stable at neutral pH. Fe(II) ions can be formed from Fe(III) in an anaerobic reactor due to the activity of iron-reducing bacteria (IRB). The dissimilatory iron-reducing bacteria (DIRB) can use ferric iron as an electron acceptor and reduce it to Fe (II) by the oxidation of H_2 or organic substrates under anaerobic conditions (202–204). These bacteria are able to gain energy for growth by coupling the oxidation of organic matter or H_2 to reduction of Fe(III):

$$4Fe^{3+}(\text{undissovled}) + CH_2O(\text{carbohydrates or acetate}) + H_2O \rightarrow 4Fe^{2+}(\text{dissolved}) + CO_2 + 4H^+ \quad (6)$$

IRB can be isolated from a variety of sources (202, 204, 205), for example from anaerobic digester (206). It is a phylogenetically diverse group including *Geobacter metallireducens*, *G. ferrireducens*, *G. acetoxidans*, *Desulfuromonas acetoxidans*, *D. palmatatis*, *Pelobacter*

carbinolicus, *Desulfuromusa kysingii*, *D. bakaii*, *D. succinoxidans*, *Shewanella alga*, *S. putrefaciens*, *Ferrimonas balearica*, *Geovibrio ferrireducens*, *Geothrix fermentans*, *Bacillus infernos*, and other species. They can reduce dissolved salts of Fe(III) and such iron minerals as ferrihydrite, lepidocrocite, maghemite, magnetite, hematite, and goethite. The IRB can reduce amorphous and poorly crystalline iron oxide easier than that of crystalline phase such as goethite and hematite. In general, the least expensive and suitable source of Fe(III) for the environmental applications of IRB are iron hydroxide, iron-containing clay, and crushed iron ore (207). The phosphate can be recovered from the solution by precipitation with the Fe^{2+} ions and Fe(II) hydroxide. The related reactions are listed below:

$$4Fe^{3+} + CH_2O + H_2O \rightarrow 4Fe^{2+} + CO_2 + 4H^+ \tag{7}$$

$$X_1Fe^{2+} + X + 2H_2O \rightarrow X_3Fe(OH)^+ + X_4Fe(OH)_2 + X_5Fe(OH)_3^- + X_6H^+ \tag{8}$$

$$(4 - X_1)Fe^{2+} + (4 - X_1)HPO_4^{2-} \rightarrow (4 - X_1)FeHPO_4 \tag{9}$$

$$X_3Fe(OH)^+ + X_3H_2PO_4^- \rightarrow X_3FeHPO_4 + X_3H_2O \tag{10}$$

Chemical recovery of ammonium can be performed by the formation of struvite, magnesium ammonium phosphate hexahydrate ($MgNH_4PO_4 \cdot 6H_2O$), but it requires the addition of Mg salt and must be performed at a pH higher than 9 (197). The recovery of ammonium by new biotechnology can be performed by the application of iron-reducing bacteria and iron-oxidizing bacteria (201). It was shown that slow oxidation of Fe(II) in water and soil model systems prevents nitrification due to the binding of ammonium ions with negatively charged ferric hydroxide, which was formed under microaerophilic conditions (208). The existence of ferric hydroxide as anions $Fe(OH)_4^-$ at a pH from 6 to 8 is well known (209). These anions could bind and precipitate positively charged ammonium ions. The removal of ammonium from solution occurred during chemical oxidation of Fe(II) (208). The molar ratio ammonium/iron in sediment was 0.77, while theoretically, this ratio is 1 for $(NH_4)Fe(OH)_4$. The reason was probably due to the large size of the iron hydroxide particles.

Ammonium concentration in the effluent of anaerobic digester of food waste or activated sludge is generally from 500 to 2,000 mg/L. To recover this ammonium, co-precipitation with negatively charged Fe(III) hydroxides formed by iron-oxidizing bacteria in the aerobic treatment of wastewater was proposed (201). Iron-oxidizing bacteria could be found in sites where groundwater containing ferrous ions or chelates of Fe(II) with humic acids has contact with oxygen. Fe(II), produced in an anaerobic digester may be further oxidized chemically. However, if Fe(II) was chelated with organic acids, its oxidation can be significantly accelerated by so-called iron-oxidizing bacteria. These bacteria actually degrade the organic "envelope" of the iron atom, which is chemically oxidized. In experiments, the average rate of biological oxidation of Fe(II) chelates was 4.5 mg/L h, while the rate of chemical oxidation was 1.0 mg/L h (201).

The sequence of iron transformations during anaerobic–aerobic treatment of strong nitrogenous wastewater may be shown by the following equations:

$$8Fe^{3+}(\text{undissolved}) + CH_3COO^-(\text{acetate}) + 4H_2O \rightarrow 8Fe^{2+}(\text{dissolved}) + 2HCO_3^- + 9H^+ \tag{11}$$

(anaerobic reduction of Fe(III) by iron-reducing bacteria);

$$4Fe^{2+}(\text{dissolved}) + O_2 + 4H^+ \rightarrow 4Fe^{3+} + 2H_2O \quad (12)$$

(aerobic oxidation of Fe(II));

$$Fe^{3+} + 4H_2O \rightarrow Fe(OH)_4^- + 4H^+ \quad \text{(hydrolysis of Fe(III))}. \quad (13)$$

The main product of ferrous oxidation under neutral pH is iron hydroxide $Fe(OH)_4^-$ (209). If ammonium is present in the water, Fe(II) oxidation follows the co-precipitation of $Fe(OH)_4^-$ with ammonium:

$$4Fe^{3+} + O_2 + 12H_2O + 4NH_4^+ \rightarrow 4Fe(NH_4)(OH)_4 + 12H^+ \quad (14)$$

Theoretically, the ratio of consumed oxygen to removed ammonium is 0.57 g O_2/g NH_4^+-N and 4.57 g O_2/g $NH_4^+ - N$ for the co-precipitation process and for the conventional nitrification process, respectively. Therefore, the co-precipitation process may be eight times more effective in consumption of oxygen than that of the nitrification process. However, the negative effect of these reactions on the anaerobic treatment of wastewater may be caused by the decrease of pH due to the release of protons. Therefore, the addition of iron in the process must be accompanied by pH control or by the addition of the buffering substance. Efficiency of ammonia removal can reach 98% (201).

A by-product of the co-precipitation process is the slow-releasing ammonium fertilizer. The precipitate, containing iron and ammonia, being suspended in water, released ammonium with an average rate of 1.6% of N per day. An additional advantage of precipitation of negatively charged iron hydroxides was the removal/recovery of such nutrients as phosphate and potassium from wastewater. Plant growth experiments showed that this precipitate could be used as an effective fertilizer. Haricot beans were grown for 15 days in sandy soil (control 1) with the addition of 140 mg of ammonium sulfate/kg of soil (control 2) or the equivalent by nitrogen quantity of iron–ammonium precipitate (experiment). The dry plant matter was $0.04 \pm 0.01\,g$, $0.36 \pm 0.04\,g$, and $0.47 \pm 0.02\,g$ in control 1, control 2, and experiment, respectively. The nitrogen content in dry biomass was $0.26 \pm 0.02\%$, $3.64 \pm 0.08\%$, and $4.63 \pm 0.17\%$ in control 1, control 2, and experiment, respectively.

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9
Anaerobic Digestion in Suspended Growth Bioreactors

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CONTENTS

Abstract This chapter concerns the principles of suspended growth anaerobic digestion. The fundamentals of anaerobic metabolism of organic matter are first presented. This is followed by presentation of anaerobic process stoichiometry and energetics as well as kinetics as these are prerequisites for the design of anaerobic processes. The importance of the feed characteristics that make a particular substrate medium or feedstock suitable are then discussed. In particular, the biodegradability of the feed under anaerobic conditions, the presence of inhibitory or toxic compounds, the availability of nutrients and the impact of flow variations in the feed are discussed. The various alternative reactor configurations are presented including conventional, high-rate, two-stage, and natural systems. In the sequel, the most important suspended growth bioreactor design considerations are presented. This includes the effect of operating parameters, sizing bioreactors, collection and exploitation of biogas and finally, startup and acclimation. Monitoring, process control, and process optimization issues are presented as these considerations are very important for developing and implementing anaerobic biotechnology. Finally selective applications are given. These include anaerobic digestion of sewage sludge, a comparison between alternate bioreactor configurations for the anaerobic digestion of an agroindustrial wastewater (dairy), the production of biogas from an energetic

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crop (sweet sorghum) and, finally, anaerobic digestion of solid wastes. Thus, the wide span of applications that this important process finds is well exemplified.

Key Words Anaerobic digestion • biogas • methane • bioreactor • energy crop • solid waste.

1. INTRODUCTION

Environmental biotechnology involves biotechnological processes that can be used to prevent pollution and/or save energy, thus allowing for sustainable development. In the absence of oxygen, many microbial processes are possible, depending on medium composition, environmental conditions, such as pH and temperature, and the availability of particular organisms. Environmental biotechnology processes are high in volume, but low in value. For this reason, feed sterilization is seldom applied. This means that the microbial ecology of the processes will be a strong function of the prevailing operational conditions. In general, a mixed "acclimated" culture will spontaneously develop depending on the conditions.

Oxygen serves as the terminal electron acceptor for many microbes. Processes that occur in the absence of oxygen include those that involve other electron acceptors such as nitrate and sulfate. Such "anoxic" processes are very useful for the removal of nitrogen and sulfur from wastewater. In this chapter, we will restrict the discussion to "truly" anaerobic processes, i.e., processes characterized by the complete absence of oxygen or other electron acceptors such as nitrates and sulfates.

During the fermentation process, both strictly anaerobic and facultative organotrophic microbes can grow in the absence of oxygen, utilizing organic compounds as an energy source, and produce a variety of metabolic products such as alcohols, fatty acids, methane, and hydrogen. The generation of a particular product will strongly depend on the operating conditions, the feed characteristics, and the species involved. From an environmental biotechnology viewpoint, the aim of using fermentation is to (a) remove the organic load that a feed stream contains and (b) produce a fuel such as ethanol, biodiesel, hydrogen or methane.

At this stage, "plant biomass" and "waste" have both served as candidate feeds for fermentation processes. Ethanol and biodiesel production from biomass is an important process for the bioconversion of this renewable energy source, whereas production of "biohydrogen" is an emerging possibility, especially in view of the development of "fuel cell" alternative (1). Our focus, however, will be on anaerobic digestion, the most widely used anaerobic environmental biotechnological process.

Anaerobic digestion (AD) is defined as the biological process that produces a gas mixture (called biogas) that contains methane (CH_4) and carbon dioxide (CO_2) as its primary constituents, through the concerted action of a mixed microbial population under conditions of oxygen deficiency. Biological methane production was first noticed by Volta in 1776, who described the release of methane from a swamp.

Anaerobic digestion is the most widely used and one of the oldest methods for sewage sludge stabilization. It was first used for high-solids municipal wastewater treatment toward the end of the nineteenth century by Louis H. Mouras, who designed and constructed sewage sludge digesters in Vesoul, France (2).

The method is also preferred for treating medium to high organic content liquid wastes (3). As a result, it has found many uses in treating high organic strength industrial wastewaters such as those generated by the agroindustries (4).

Anaerobic digestion also takes place spontaneously in a solid waste sanitary landfill, whereas in the recent years, anaerobic digestion of the organic fraction of municipal solid waste is gaining more ground as the method of choice for solid waste management (5).

Finally, anaerobic digestion has been considered as a method for energy crop biomass conversion (6).

The advantages of anaerobic digestion as a wastewater treatment method over its aerobic oxidation counterpart are numerous (7):

- Stabilization of high organic strength wastes.
- Generation of reduced amounts of sludge.
- Reduced nutrient (N and P) requirements.
- Low energy consumption.
- Biogas production (which may be used as an energy source).
- Anaerobic microorganisms can be maintained for extended periods without feeding.
- The generated stabilized biosolids can be suitable as a soil amendment.

The main relative disadvantages of conventional anaerobic digestion processes are:

- The sensitivity of methanogens to a variety of toxic compounds (7). In recent years, approaches and methods to withstand such toxicities have been developed (8).
- The control problems frequently exhibited by anaerobic bioprocesses.
- The relatively long startup times required for anaerobic digesters (often 8–12 weeks).

The objective of this chapter is to present the most fundamental issues regarding suspended anaerobic digester design and operation.

2. FUNDAMENTALS OF ANAEROBIC BIOPROCESSES

Enzymes in a live cell catalyze a large number of chemical reactions. The sum total of chemical reactions, called metabolism, consists of catabolic reactions, through which complex organic compounds are metabolized to simpler compounds generating energy, and anabolic reactions that are responsible for biosynthesis. Depending on the chemical composition of the growth medium, different microbial species prevail, which consume organic substrates while generating microbial products and the necessary energy for the cellular functions. In a nonsterile environment, as is the case for most applications of anaerobic digestion, multiple microbial species may grow simultaneously. The interactions between the different species are a function of the prevailing environmental conditions of the digesting medium, which includes physical parameters, such as temperature and chemical parameters such as pH, alkalinity, organic compounds, etc. The different species coexist in a symbiotic relationship, and "exchange" chemical compounds, a process that is responsible for their coexistence (i.e., the product of one species is the substrate for another).

Before discussing anaerobic bioreactor design, we must first consider the individual microbial processes that take place and the microorganisms responsible for each step, as well as their interactions.

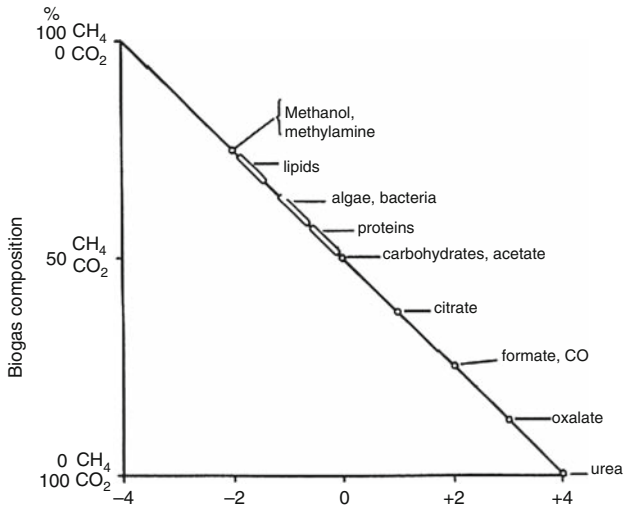

Fig. 9.1. Biogas composition as a function of the oxidation state of the organic substrate (12).

2.1. Microbiology and Anaerobic Metabolism of Organic Matter

In general terms, under completely anaerobic conditions, organic compounds are converted according to the overall reaction (9, 10):

$$C_nH_aO_b + \left(n - \frac{a}{4} - \frac{b}{2}\right) H_2O \rightarrow \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4}\right) CO_2 + \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4}\right) CH_4 \qquad (1)$$

For example, carbohydrates ($n = 1$, $a = 2$, $b = 1$) give a 50–50 mixture of carbon dioxide and methane. From this simplified reaction (which ignores important details such as microbial mass generation, as they will only slightly influence the stoichiometry), it becomes apparent that the constitution of the generated biogas will depend on the redox state of the organic carbon. Therefore, hydrocarbons generate equal amounts of methane and carbon dioxide, methanol and lipids generate biogas rich in methane, oxalic acid will produce biogas low in methane and urea will produce no methane (11). Figure 9.1 gives the composition of biogas as a function of the oxidation state of the organic carbon contained in the substrate (12).

The conversion of organic matter to biogas proceeds as shown in Fig. 9.2. We distinguish four stages: hydrolysis (or more precisely depolymerization) of organic macromolecules (lipids, polysaccharides, proteins, and nucleic acids) to the corresponding monomers (fatty acids and glycerol, monosaccharides, aminoacids, nitrogenous bases). The stage of acidogenesis follows. During this stage, the monomers are converted to fatty acids, producing also amines and alcohols to some extent, as well as hydrogen and CO_2. During the third stage (termed acetogenesis), higher fatty acids are converted to acetate and hydrogen. In the final stage of methanogenesis, methanogenic microorganisms (archaea) generate methane out of acetate (acetoclastic methanogens) and hydrogen (hydrogenotrophic methanogens).

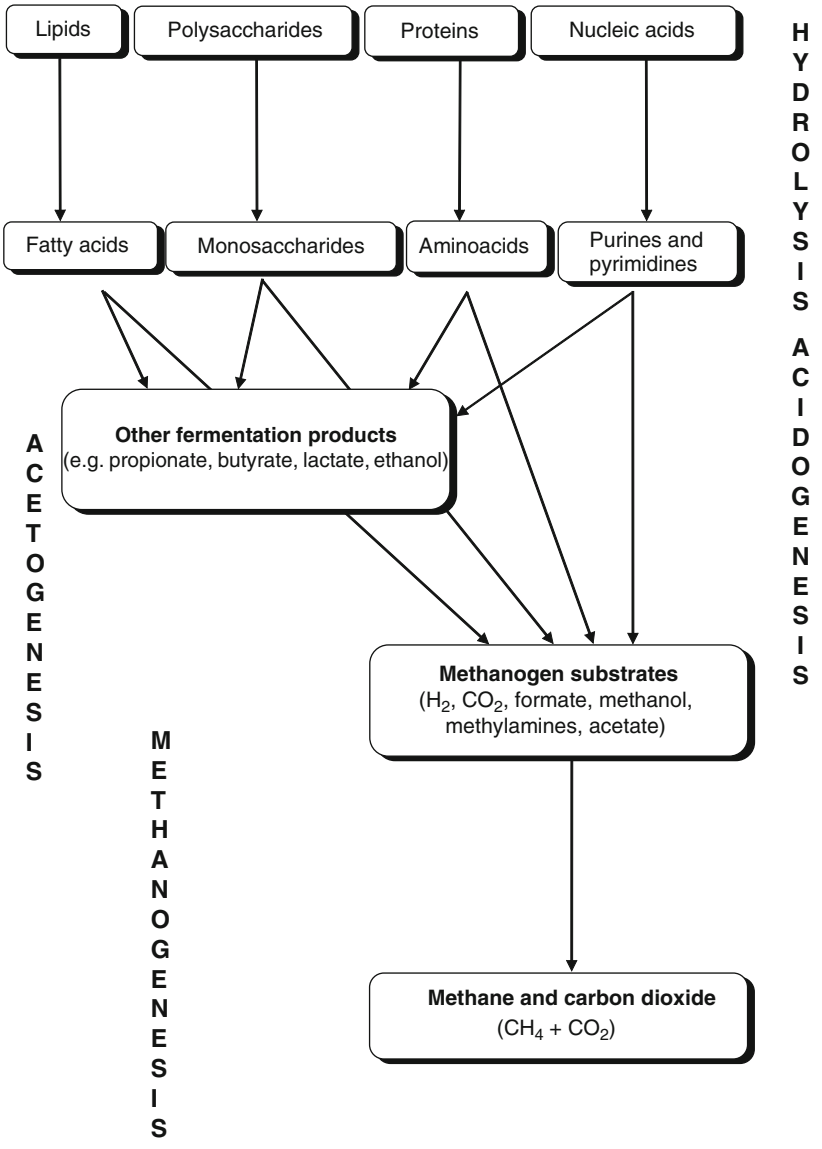

Fig. 9.2. Microbial steps for anaerobic digestion.

2.1.1. Hydrolysis

Microorganisms cannot directly metabolize particulate organics, since they cannot permeate the cellular membrane. The organics need to be "hydrolyzed" before they may be taken up by the microbilia (12, 13). Traditionally, hydrolysis is defined as "the chemical transformation of a compound to simpler ones through the reaction with water molecules." A more recent and technically more accurate description of the microbial process of converting particulate organics to simple soluble organic compounds is depolymerization. Hydrolysis is carried out

by the action of specialized enzymes called hydrolases, which act extracellularly on polymers or intracellularly on smaller molecules such as the dimer lactose.

2.1.2. *Acidification*

During acidogenesis, soluble organic compounds, which have been generated through the action of hydrolytic enzymes originating from the same or other species of the microbial consortium are biodegraded, generating a mixture of volatile fatty acids (VFAs) (mainly acetate, propionate, butyrate, and isobutyrate) in relative amounts that depend on (a) the organic composition, (b) the available species, and (c) the operational parameters of the reactor. The dominant microbes for this stage are bacteria, although small populations of protozoa, fungi, and yeasts have also been reported to carry out acidogenesis (14). Until 1965, only very few bacterial species had been isolated from anaerobic digesters and it was thought that facultative microbes were larger in numbers than obligate anaerobes (15). The acidogenic population accounts for approximately 90% of the total microbial population in a digester (16).

2.1.3. *Acetogenesis*

Acetic acid is an important intermediate in the anaerobic metabolism of organic substrates, since it is further utilized for the generation of methane. We generally distinguish between two different mechanisms for acetic acid production: acetogenic hydrogenations and acetogenic dehydrogenations. Acetogenic hydrogenations take place during the growth of two microorganism groups: (a) obligate proton-reducing or obligate hydrogen-producing species and (b) those that can utilize various electron acceptors during the degradation of organic substrates. The latter, depending on the prevailing hydrogen concentration, regulate their metabolism, producing more or less reduced products (facultative proton-reducing). Acetogenic dehydrogenations for the production of acetate include reactions of carbon dioxide with hydrogen, of carbon monoxide and water, and of methanol and carbon dioxide (Table 9.1). Usually, these species have the ability to grow on other organic substrates such as sugars, lactic acid, etc. also (17).

Measurements in anaerobic environments, such as in swamps and sludges, showed that the population of species responsible for acetogenic hydrogenations may be smaller by two orders of magnitude in comparison with the methanogens present in the same samples.

2.1.4. *Methanogenesis*

Methanogs are obligate anaerobic microorganisms that may be found in natural environments such as the rumen, the interior part of the stem of certain trees and in freshwater sediments. Methane has also been found to be released from high-salt environments as well as

Table 9.1
Acetogenic dehydrogenation reactions (18)

$2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O$
$4CO + 2H_2O \rightarrow CH_3COOH + 2CO_2$
$4CH_3OH + 2CO_2 \rightarrow 3CH_3COOH + 2H_2O$

Table 9.2
Methane producing reactions

Reaction	$\Delta G^{\circ\prime}$(kJ/mol CH_4)
$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$	−131
$4HCOOH \rightarrow CH_4 + 3CO_2 + 2H_2O$	−304
$4CO + 2H_2O \rightarrow CH_4 + 3CO_2$	−210
$4CH_3OH \rightarrow 3CH_4 + CO_2 + 2H_2O$	−319
$CH_3NH_3Cl + 2H_2O \rightarrow 3CH_4 + CO_2 + 4NH_4Cl$	−230
$CH_3OH + H_2 \rightarrow CH_4 + H_2O$	−113
$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-$	−31

high-temperature environments (e.g., *Methanothermus fervidus* isolated from thermal springs has an optimal growth temperature of 83°C) (19).

Methanogens may use a relatively small number of organic compounds as an energy source, including carbon dioxide, fomic acid, acetic acid, methanol, methylamines, and dimethyl sulfide. Some methanogens may also use carbon monoxide (19). Table 9.2 presents the methane-producing reactions on the basis of the use of these compounds and the corresponding free energy change (20).

Until recently, it was believed that all methanogens can generate methane from hydrogen and carbon dioxide. However, it was shown that although most methanogenic species have this ability, there are some that use acetic acid as a substrate and thus they have been divided into two groups: (a) acetotrophs such as *Methanothrix soehngenii*, *Methanosarcina* TM-1, *Methanosarcina acetivorans* and (b) obligate methylotrophs such as *Methanolobus tindarus*, *Methanococcoides methylmutens*, *Methanococcus halophilus*, which metabolize only methanol, methylamines, and dimethyl sulfide. It has been shown that both acetotrophs and methylotrophs produce methane directly out of methyl groups ($-CH_3$) and not through CO_2. Among hydrogen-utilizing methanogens, there are quite a few species that metabolize formic acid (e.g., *Methanococcus thermolithotrophicus*, *Methanobacterium formicicum*) and carbon monoxide (e.g., *Methanobacterium thermoautotrophicum*). Finally, some methanogens are capable of metabolizing almost every substrate among those referred to in the above discussion. *Methanosarcina barkeri* and *Methanococcus mazei*, for example, utilize everything, but formic acid (18–20).

2.2. Stoichiometry and Energetics

Designing an anaerobic biotechnological process first requires the assessment of quantitative relationships between the nutrients used for microbial growth and the products of microbial metabolism (including cellular mass), i.e., the stoichiometry of microbial growth.

Stoichiometric relationships often are determined by fitting experimental data (21, 22). Such constants are of course directly applicable only for the particular experimental conditions, since microbial growth and metabolite production is a very complex "overall" biochemical reaction, the stoichiometry of which will depend strongly on the relative rates of the simple bioreactions.

In order to extend the applicability of the employed stoichiometric coefficients, several workers have made appropriate assumptions that allow for variable stoichiometric "constants" that are a function of operating conditions, e.g., hydrogen partial pressure (23–26).

McCarty (27–29) used energetics for developing an overall general "microbial reaction" as the sum of individual redox reactions. Thus, the energy required for microbial synthesis is considered as the sum of three parts: (a) the free energy change for converting the electron donor to pyruvate (metabolic intermediate), (b) the free energy change for converting the nitrogen source to ammonia, and (c) the free energy change for biomass synthesis from pyruvate and ammonia. Taking into account the energy losses, the number (A) of electron equivalents of electron donor that need to be utilized for synthesizing one electron equivalent of cells is determined through an energy balance. Based on the fact that one electron equivalent corresponds to 8 g COD, the microbial yield factor may be determined (Y, g VSS/g COD). Although this representation still leads to a rough description of the relevant biochemical processes taking place in a pure culture, it is certainly superior to a simplistic "constant stoichiometry" model, and is often capable of capturing observed variations in the process stoichiometry as a function of operating conditions.

As already discussed, anaerobic digestion is a microbial process that involves a mixed microbial community, the relative populations of which are changing in the long run and are affected by many factors such as pH, temperature, organic loading rate (OLR), retention time, etc. This makes the whole effort of portraying the overall process as a single overall bioreaction even more unreliable. Nevertheless, in the absence of a better alternative, this approach may be used to express anaerobic bioconversion as an overall "bioreaction" during which substrates are converted to products and biomass. Following Rittmann and McCarty (30), the overall reaction may be written as follows:

$$\mathrm{C}_n\mathrm{H}_a\mathrm{O}_b\mathrm{N}_c + \left(2n + c - b - \frac{9df_\mathrm{s}}{20} - \frac{df_\mathrm{e}}{4}\right)\mathrm{H_2O} \rightarrow \frac{df_\mathrm{e}}{8}\mathrm{CH_4} + \left(n - c - \frac{df_\mathrm{s}}{5} - \frac{df_\mathrm{e}}{8}\right)\mathrm{CO_2}$$

$$+\frac{df_\mathrm{s}}{20}\mathrm{C_5H_7O_2N} + \left(c - \frac{df_\mathrm{s}}{20}\right)\mathrm{NH_4^+} + \left(c - \frac{df_\mathrm{s}}{20}\right)\mathrm{HCO_3^-} \tag{2}$$

where $d = 4n + a - 2b - 3c$.

This "bioreaction" describes the conversion of a waste (electron donor) with an "average" composition described by the empirical formula $\mathrm{C}_n\mathrm{H}_a\mathrm{O}_b\mathrm{N}_c$. The generated microbial mass is assumed to have an average composition described by the empirical formula $\mathrm{C_5H_7O_2N}$. This bioreaction considers the organic substrate as the electron donor, and assumes that the electrons are used partly for cell synthesis (a fraction f_s) and partly for energy (a fraction $f_\mathrm{e} = 1 - f_\mathrm{s}$). It is obvious that the exact stoichiometry can be determined from knowledge of the constants n, a, b, and c, as long as the parameter f_s is known. This parameter will depend on conditions such as temperature, biomass decay rate, and solids retention time (in a continuous bioreactor) through the relation:

$$f_\mathrm{s} = f_\mathrm{s}^\circ\left[\frac{1 + (1 - f_\mathrm{d})\mathrm{b}\theta_\mathrm{x}}{1 + \mathrm{b}\theta_\mathrm{x}}\right] \tag{3}$$

where f_d is the biodegradable fraction of the waste (typically 0.8), b is a decay rate constant (e.g., 0.05/day), and θ_x the retention time. The influence of temperature is reflected through the dependence of the free energies on temperature. Depending on the substrate, the fraction f_s° takes a different value. Thus, a typical value for carbohydrates is 0.28, whereas a typical value for municipal sludge is 0.11, implying that a much smaller yield of cellular mass is anticipated when sludge is used as the substrate than when carbohydrates are the main carbon source.

2.3. *Kinetics*

Overall, stoichiometry is certainly useful for assessing the anticipated relationship between reactants (nutrients and cellular mass) and products. The procedure outlined above may be used for answering questions such as:

- How much cellular mass may be obtained from a given amount of organic substrate?
- What is the anticipated biogas composition?
- Is the available nitrogen sufficient, or do we need to add some, and if yes, how much?
- Do we need to add alkalinity?

In order to be able to size and operate an anaerobic digestion system, the kinetics of microbial growth and biogas production are necessary. If we had a simple microbial process with a single microbial species growing on a limiting organic substrate, a simple Monod model would adequately describe the needed relationship between conversion and holding time (which for a given flow rate is translated into the required bioreactor volume).

For a single species growing in a well-mixed medium, the rate of change of cellular concentration is given by:

$$\frac{dX}{dt} = \mu X \tag{4}$$

where X is the cellular mass (M/L^3), t the time (T), and μ the specific growth rate (T^{-1}), which is generally a function of various operating conditions, such as temperature and pH, and is usually assumed to be a function of a limiting nutrient (usually the carbon source). The most commonly used expression is that of Monod (31):

$$\mu = \frac{\mu_{max} S}{K_S + S} \tag{5}$$

where μ_{max}: is the maximum specific growth rate (T^{-1}), S: is the concentration of the limiting substrate (M/L^3), and K_S: is the saturation constant (equal to the substrate concentration for which the $\mu = \mu_{max}/2$) (M/L^3).

Endogenous respiration, cellular maintenance, and cell lysis are all processes that lead to reduction of cellular mass. They are usually assumed to be adequately described by a first-order reaction with a "decay" constant $b(T^{-1})$. Then the Monod equation is modified to:

$$\frac{dX}{dt} = (\mu - b)X \tag{6}$$

Substrate consumption is then assumed to be described by:

$$\frac{dS}{dt} = -\frac{1}{Y}\frac{dX}{dt} = -U\,X \tag{7}$$

where Y is the biomass yield coefficient (M/M) and U is the specific substrate utilization rate (M/M/T).

If cellular decay can be ignored, Eqs. (4) and (7) (with μ defined by Eq. (5)) may be integrated to determine the following relationship between holding time t and final substrate concentration S, for a batch reactor with a starting substrate concentration S_0 and a starting biomass concentration X_o:

$$\ln S = \ln\left[S_0 + Y(S_0 - S)\frac{S_0}{X_0}\right] + \left(\frac{X_0 + Y S_0}{Y K_s}\right)\ln\left[\frac{X_0 + Y(S_0 - S)}{X_0}\right] - \mu_{\max} t\,\frac{[X_0 + Y S_0]}{Y K_s} \tag{8}$$

From Eq. (8), it becomes apparent that the required holding time to achieve a ceratin conversion is inversely proportional to the maximum specific growth rate. Anaerobic digestion, however, is a multistep process involving the action of multiple microbes, which grow in a symbiotic manner. In order to answer questions such as: "What is the required volume to achieve a certain conversion?," a more detailed mathematical model is needed that adequately describes all key species interactions, and numerical methods must be used to integrate the resulting complex coupled material balances. A way around this difficulty is based on the fact that multistep processes contain a particular step, the so-called rate-limiting or rate-determining step, which, being the slowest, limits the rate of the overall process (32). This allows simplification of the kinetic analysis, as we may assume spontaneous conversion of all matter to the reactants of the rate limiting step. The first attempts for modeling anaerobic digestion led to models describing only the limiting step. The limiting step is defined as "that step which will cause process failure to occur under imposed conditions of kinetic stress" (33). Kinetic stress will be relevant when the reactor volume is small enough so that complete conversion is not observed. For obvious reasons of economy, we always want to operate under kinetic stress conditions, since complete conversion would only be possible for infinite reactor volume.

It should be stressed that the steps preceding the last slow step may still influence the overall conversion. Thus, for a given retention time, the rate of hydrolysis of particulate organics will influence the maximum available substrate concentration for methanogens (assumed to be acetate) and thus the maximum observable specific growth rate of these organisms. During a wide range of operating conditions, the limiting step is not always the same. It may depend on wastewater characteristics, hydraulic loading, temperature, etc. (34). For example, the limiting steps may be acetogenic methanogenesis (35, 36), the conversion of fatty acids to biogas (37), or the hydrolysis of biodegradable suspended solids (38). Indeed, it is not uncommon to have a different step as limiting depending on the operating conditions.

Below is a brief description of the key anaerobic digestion models that have been developed so far for describing suspended growth systems. The Graef & Andrews model (39) involves only the acetoclastic methanogens. The conversion of fatty acids into biogas is considered

limiting. Volatile fatty acids are expressed as acetic acid and the methanogen composition is assumed to be $C_5H_7NO_2$. The overall reaction, according to this model may be represented as follows:

$$CH_3COOH + 0.032\,NH_3 \rightarrow 0.032\,C_5H_7NO_2 + 0.92\,CO_2 + 0.92\,CH_4 + 0.096\,H_2O \quad (9)$$

Monod kinetics with substrate inhibition are assumed (35), i.e.,

$$\mu = \frac{\mu_{\max}}{1 + \frac{K_s}{S} + \frac{S}{K_i}} \quad (10)$$

where μ (in per day) is the specific growth rate of methanogens. Undissociated acetic acid is considered as the limiting substrate S (spontaneous conversion of all steps preceding methanogenesis is assumed). Its concentration is determined based on the equilibrium assumption of the acetic acid dissociation reaction. The constants K_s and K_i are the saturation and inhibition constants, respectively. The pH is estimated by a total ion balance. According to this model, a digester is expected to fail whenever, for some reason, the fatty acid concentration is increased. This causes a drop in the pH, a rise in the concentration of undissociated acetic acid concentration. This in turn causes a drop in the growth rate of the methanogenic population, until they are washed out, if the situation is prolonged.

An anaerobic digester is essentially a three-phase system. The model of Graef and Andrews (as many other later models) assumes a gas phase in contact, but not in equilibrium with the liquid phase. Gas phase is assumed to obey the ideal gas law. Methane is assumed to be water insoluble and directly transferable to the gas phase, whereas the generated CO_2 partially dissolves in the liquid phase giving carbonic acid, which depending on the pH is dissociated giving bicarbonate and carbonate ions, and partly escapes to the gas phase at a rate given by the equation:

$$T_G = K_L \left(K_H P_{CO_2} - [CO_2]_D\right) \quad (11)$$

where K_L is a mass transfer coefficient, K_H is Henry's constant, P_{CO_2} is the CO_2 partial pressure, and $[CO_2]_D$ is the dissolved CO_2 concentration.

The "limiting step hypothesis" leads to simple and readily usable models for designing anaerobic digestion systems. Such models, however, do not describe the digester behavior very well, especially under transient operating conditions, and consequently may prove of limited use for process control and optimization. In addition, they may fail to describe situations in which there is a different "rate-limiting" step depending on the operating conditions. Other models that also assume substrate inhibited Monod kinetics of the methanogens and with additional features such as hydrolysis, acidogenesis, and ammonia inhibition (40) are as follows; hydrolysis of biodegradable solids, acetogenesis, and methanogenesis, dependent on pH and temperature (41); acidogenesis step, that form acetate from glucose, and are inhibited by undissociated acetic acid (42); a slow and a fast hydrolysis step, acidogenesis of the soluble intermediates and methanogenesis (43) have also been developed.

Although simplified models based on the rate-limiting hypothesis are quite useful for the design of suspended growth anaerobic processes, these models are generally not in a position to describe the dynamic behavior of digesters under varying loading conditions, which often

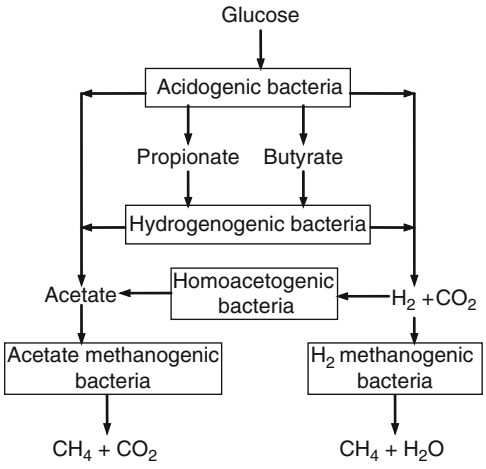

Fig. 9.3. Flow chart of Hill (44) model.

may lead to digester failure. They are thus inadequate for control and dynamic simulation purposes. Several models have been developed in recent years that reflect an understanding of the key microbial and physicochemical interactions that take place in a digester. Hill (44) allowed for inhibition of methanogenesis by the total fatty acid concentration. The following bacterial groups were assumed to participate in the overall digestion process (Fig. 9.3): (a) acidogenic, which grow on glucose (considered as the dissolved organics less the VFAs) form a mixture of acetic, propionic and butyric acids, (b) hydrogenogenic, which have a slow growth rate, convert propionic, and butyric acid into acetic acid and H_2, (c) homoacetogenic produces acetate from H_2 and CO_2, (d) H_2-methanogenic reduce CO_2 into CH_4, and (e) acetate-methanogenic convert acetic acid into biogas (CH_4 and CO_2). All the five steps were assumed to be inhibited by high fatty acid concentrations. The model was based on specific stoichiometric reactions for each of the five key reaction steps.

Mosey (23) was the first among several modelers to consider the hydrogen partial pressure as the key regulatory parameter of anaerobic digestion of glucose. This influences the redox potential in the liquid phase. The model considered four bacterial groups (Fig. 9.4) to participate in the conversion of glucose to CO_2 and CH_4: (a) the acid-forming bacteria, which are fast-growing and ferment glucose to produce a mixture of acetate, propionate, and butyrate, (b) the acetogenic bacteria convert the propionate and butyrate to acetate (c) the acetoclastic methane bacteria convert acetate to CO_2 and CH_4, and (d) the hydrogen-utilizing methane bacteria reduce CO_2 to CH_4.

The fatty acid relative production was assumed to depend on the redox potential or equivalently, on the ratio [NADH]/[NAD^+]. This ratio was made a function of the hydrogen partial pressure in the gas phase. Apart from the acidogenic bacteria, hydrogen's partial pressure also influences the acetogenic growth rate, since high values inhibit (thermodynamically) the generation of propionic and butyric acid. Finally, low pH values pH (< 6) are expected to be inhibitory to all bacterial species. According to the Mosey model, a sudden increase in the

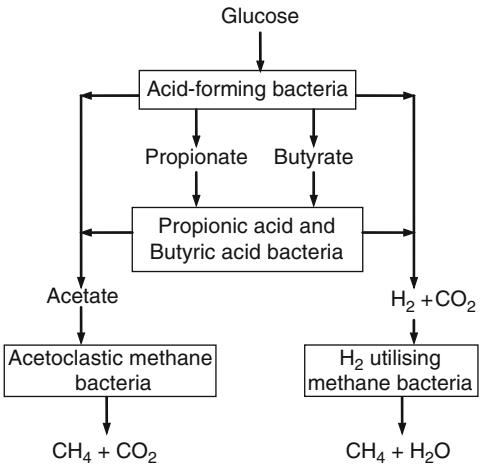

Fig. 9.4. Flow chart of Mosey (23) and Pullammanappallil et al. (26) models.

OLR is expected to cause an accumulation of VFAs, since the acetogens grow at a slower rate than the acidogens. The subsequent drop in the pH inhibits in turn the hydrogen-utilizing methanogenic bacteria, causing a rise in the hydrogen partial pressure, which causes further accumulation of propionic and butyric acids. Methane generation is stalled when the pH drops to particularly low levels (< 5.5). Other models, based on the work of Mosey, followed (24–26).

All the models described thus far are capable of predicting digester failure, caused by a specific disturbance, either through a drop in the pH and/or through accumulation of VFAs. This is a commonly observed behavior in digesters treating municipal sludge and/or high organic content industrial wastewaters. None of these models, however, can adequately describe the anaerobic digestion of manure (45), as this exhibits a self-regulation of the pH, attributed to the generated ammonia. The model by Angelidaki et al. (46) considers hydrolysis, acidogenesis, acetogenesis, and methanogenesis, and is capable of adequately describing such ammonia generating digesters. A more complicated model by Siegriest et al. takes into account ammonia inhibition, lysis and hydrolysis of cell biomass, description of a physical–chemical system of pH level including the main buffer systems (47).

The models described above consider organic matter as a whole, and do not account for the nature of the organic macromolecules in the feed composition. A modeling approach that considers the complex feed composition (breakdown in carbohydrate, protein, VFAs, and other organics) was proposed (4). This model describes the codigestion process of agroindustrial wastewaters. It is assumed that the wastewaters consist of carbohydrates and proteins (undissolved and dissolved) and other dissolved organic matter. The conversion of organic matter to biogas is carried out by the simultaneous action of three groups of bacteria: acidogens, (hydrolysis and acidogenesis), acetogens, and methanogens. In the hydrolysis step, the undissolved carbohydrates and proteins are hydrolyzed to dissolved carbohydrates and proteins, respectively; in the acidogenesis step, the dissolved carbohydrates, proteins, and other organic matter are converted to acetate and propionate, while in the acetogenesis

step, propionic acid is converted to acetate. Finally, methane is produced by acetoclastic methanogens. Hydrolysis of undissolved proteins and carbohydrates is assumed to proceed with first-order kinetics while Monod kinetics are assumed for the acidogenesis, acetogenesis, and methanogenesis step. The consumption of propionate and acetate proceeds under substrate inhibition. The model is capable of predicting adequately the COD and fatty acids dependence on the operating conditions and should be useful for designing codigestion processes (48). We will complete this survey of models for suspended growth anaerobic digestion by mentioning the so called Anaerobic Digestion Model 1 (ADM1), which was developed by an IWA task force in 2002 (49). This modelling framework provided a platform for discussion and was based on all previous modeling efforts for suspended growth anaerobic digestion systems.

The kinetic models discussed thus far may be used to describe "truly" suspended growth systems, that is, systems for which microbes are present as a loosely defined sludge (flocculant sludge). As presented later, high-rate anaerobic processes often develop sludge granules, that is, specific, well-structured, high-density particles, typically 1 mm in size, for which the observed conversion rates may be significantly different due to the heterogeneity introduced by the granular sludge. In other words, concentration gradients are likely to develop, and transport limitations may significantly influence the observed biodegradation kinetics. These systems are not truly suspended systems, and they are often referred to as hybrid systems (2).

During the last 20 years, significant research efforts have been invested in the understanding of granule formation in high-rate systems such as the Upflow Anaerobic Sludge Blanket (UASB) reactor. Although the precise mechanism of granule formation still remains unknown, their composition and the factors influencing their formation are understood to a great extent. The granules contain bacteria in a 3-D array. The exact bacterial types depend on the wastewater composition (50–57). The factors that influence the formation of granules are (7, 50, 52, 54):

- Digester startup conditions
- Degree of acclimation to the feed wastewater
- Hydraulic loading
- Organic loading
- Biogas production per unit volume
- Concentration of inhibitors
- Availability of nutrients
- Cation concentration, especially Ca^{2+} and Mg^{2+}
- Concentration and type of suspended solids contained in the wastewater.

These factors should be evaluated from a modeling point of view, and the effect of the significant ones should be properly accounted. Kalyuzhnii and Fedorovich suggested a general approach for modeling of a UASB reactor (58). A detailed review of existing anaerobic digestion models is provided by Lyberatos and Skiadas (59).

3. EFFECT OF FEED CHARACTERISTICS ON ANAEROBIC DIGESTION

The suitability of a particular organic feed for anaerobic digestion in terms of technical and economic feasibility should be addressed before designing an anaerobic digestion process for this particular waste stream.

The chemical composition of the substrate is one of the most important parameters that determine the anaerobic process characteristics. The prevalence of particular microbial species takes place through natural selection as the fittest survive in a competing environment, depending on their ability to grow in the medium with the specific organic and inorganic constituents. In addition, their ability to coexist with other species may have a decisive role on the survival of a species in such an environment. A feed candidate for anaerobic digestion should ideally be (a) rich in organic compounds that are biodegradable under anaerobic conditions, (b) nontoxic to the desirable anaerobic populations, and (c) containing sufficient nutrients. Of those conditions, (a) and (b) are the most important since they determine technical feasibility.

3.1. Anaerobic Biodegradability

Some complex substrate constituents may be nonbiodegradable, in which case the organics may be only partially mineralized. This then limits the applicability of AD as an effective method for complete removal of organic pollutants. In addition, since AD (as will be discussed later) requires elevated temperatures (35°C or 50°C), wastewaters with low organic contents may require too much additional energy to heat up the wastewater, so that AD may be technically feasible, albeit uneconomical. The ideal feed then is one that contains high concentrations (typically above 2,000 mg COD/L) of readily biodegradable organic compounds.

3.2. Inhibition and Toxicity

Anaerobic microorganisms, especially methanogens, are especially sensitive to many substances. Methanogen inhibition leads to reduced methane production, a buildup of VFAs, a drop in the pH and further inhibition. As already mentioned, this "positive" feedback often leads to bioreactor failure. The following are the main inhibitors:

- Oxygen: Methanogens are strict anaerobes, so that even traces of oxygen seriously inhibit their metabolism. Therefore, it is important to secure tight covers to keep air out of the reactors.
- Nitrite and Nitrate: these compounds are also inhibitory to AD. As they may serve as electron acceptors for facultative anaerobes, they are usually reduced first, before methanogenesis can take place.
- Ammonia: Ammonia, in its nonionized form (NH_3), is very toxic to methanogens. Smaller toxicity is observed at neutral pH (nonionized fraction increase with pH). Inhibition is observed for a (total) concentration above 1,500–3,000 mg/L and pH $>$ 7.4, whereas above 3,000 mg/L it becomes toxic for all pH. Ammonia, although not present in the original feed, may increase during biodegradation, if the feed has a very high protein content.
- Higher fatty acids: Higher fatty acids (e.g., lauric, myristic, oleic, etc.) when present in high concentrations, have also been known to inhibit the action of acetoclastic methanogens (60).
- Heavy metals: As already mentioned, heavy metals, such as Ni, Co and Mo, are required nutrients when present at low concentrations. However, at higher concentrations, heavy metals, usually encountered in industrial wastewaters are inhibitory to anaerobic digestion in the order: Ni $>$ Cu $>$ Cd $>$ Cr $>$ Pb. During anaerobic digestion, sulfates are typically reduced to H_2S. As this gas reacts with the heavy metals, insoluble sulfides may be generated leading to a reduction of the toxicity.
- Chlorinated hydrocarbons: Chlorinated aliphatic hydrocarbons are toxic to methanogens, and chloroform ($CHCl_3$) is particularly toxic in concentrations exceeding 1 mg/L.

- Aromatic compounds and Formaldehyde: Pure cultures of methanogens (e.g., *Methanotrix concilii*, *Methanobacterium espanolae*, *Methanobacterium bryantii*) are inhibited by aromatic compounds (e.g., benzene, toluene, phenols), whereas formaldehyde (HCHO) is toxic to methanogens in concentrations exceeding 100 mg/L.
- Sulfides, hyposulfides, and sulfates (HS^-, S^{2-}, $SO_4{}^{2-}$): Hydrogen sulfide (H_2S) and sulfide anions (HS^-, S^{2-}) are among the strongest inhibitors of anaerobic digestion, in concentrations exceeding 150–200 mg/L.

Toxicity is a very important issue that may well limit the applicability of anaerobic digestion processes. The frequent presence of toxic compounds in industrial wastewaters may reduce the effectiveness of the process, whereas in some cases, it might render this alternative completely infeasible from a technical point of view. In some cases, toxicity may be removed. For example, olive-oil debittering wastewater was pretreated with white-rot fungi to remove the phenols that were inhibiting anaerobic digestion of this organics-rich wastewater (61). In other instances, appropriate acclimation can solve the inhibition problem. This approach was used for treating wastewaters containing long chain fatty acids (62). In other instances, however, removal of toxicity may be impossible or uneconomical.

3.3. Availability of Nutrients

Bacteria require other nutrients in addition to a carbon source. Trace elements, such as Fe, Co, Mo, Mg, Ca, Na, Ba, Se, and Ni, are all required. Most wastes usually have such constituents present in abundance. Nitrogen and phosphorus, referred to as nutrients, are also necessary for microbial metabolism. Recommended proportions for anaerobes varied from 700:5:1 for C:N:P (63) to 25–30:1 for C:N. Possible nitrogen deficiencies may be made up by the addition of urea (NH_2CONH_2), or $NH_4{}^+$, while phosphorus is usually added in the form of $PO_4{}^{3-}$. Thus, nutrient deficiencies are generally a solvable issue.

3.4. Flow-Rate Variations

Seasonal variations present a major challenge for the applicability of AD to many industrial wastewaters. Industries processing agricultural products, for example, use as raw materials various fruits, vegetables, meat, milk, etc., a large number of which are not produced on a year-round basis. Some of these industries, such as olive-mills and dairies, have a specific product that is seasonally produced. Other industries vary their production during the year, as the raw material that they process depends on the season. Canneries for instance, may be processing carrots, celery, potatoes, etc. during different periods in the calendar year. In either case, the immediate consequence is that the wastewaters generated from these industries vary significantly during the year, both in quantity and characteristics. The high organic load that agroindustrial wastewaters carry makes anaerobic digestion essentially the only viable alternative.

Long digester start-up times may be particularly limiting for agroindustries that operate only during part of the year. Individual treatment units can be prohibitively expensive for small-scale agricultural products processing industries when compared with the total investment associated with these enterprises.

The scattered and seasonal nature of such agroindustries suggest that a truly viable option would be to have a centrally located anaerobic digestion plant that would be servicing a whole area, providing thus the required economies of scale to make any treatment process possible. Such a plant might be receiving different wastewaters during the year, ensuring that no downtimes will be necessary, and thus avoiding the costs associated with long startup times (48). In addition, mixed wastewaters may provide a more balanced "mix" that may be more readily digested, without the need for external nutrient additions. Thus, for instance, an industrial wastewater with nitrogen deficiency that would normally require constant correction of its alkalinity may be well digested if treated together with a waste exhibiting nitrogen surplus such as cow manure.

4. REACTOR CONFIGURATIONS

The selection of appropriate bioreactor configurations is very important for the development of effective anaerobic bioprocesses. In the recent years, various digester types have been developed, each with its own advantages and disadvantages. The selection of the appropriate reactor for treating a particular waste or, more generally, feed should be a function of economic considerations, but will in every case depend strongly on waste characteristics (organic load, solids content, presence of toxic substances). Lettinga (64) suggested five conditions that should be met by an effective anaerobic system:

- High retention of biomass
- Good contact of biomass with the substrate
- High reaction rates and lack of transport limitations
- Ability of biomass to acclimate to various waste types
- Prevalence of favorable environmental conditions for all microorganisms during a variety of operational conditions.

Depending on the feeding strategy, a bioreactor may be batch, fed-batch, or continuous. Since anaerobic digestion is a relatively slow process that is characterized by long startup time requirement, batch reactors are practically excluded. Fed-batch reactor operations may be a viable startup strategy. This discussion will be limited to continuous bioreactor configurations that are characterized by an effluent liquid stream equal in flowrate to the influent stream, and a constant reacting fluid volume. We generally distinguish two broad categories of anaerobic digesters: conventional and high-rate systems.

4.1. Conventional Systems

Conventional anaerobic digesters are the simplest bioreactors from a construction point of view. Microorganisms grow suspended in the "reacting" liquid medium, and mechanical stirring secures the homogeneity of the liquid phase (Fig. 9.5a). For these systems, the solids retention time (SRT) coincides with the hydraulic retention time (HRT). In order to increase the system's ability to retain the generated biomass (first criterion from above), a recirculation of biosolids following sedimentation has been proposed (Fig. 9.5b). This may allow a significantly higher rate since a high biomass concentration will lead to a high overall rate, and thus to a smaller volume requirement, as seen in Eq. (4). Such systems are called anaerobic contact

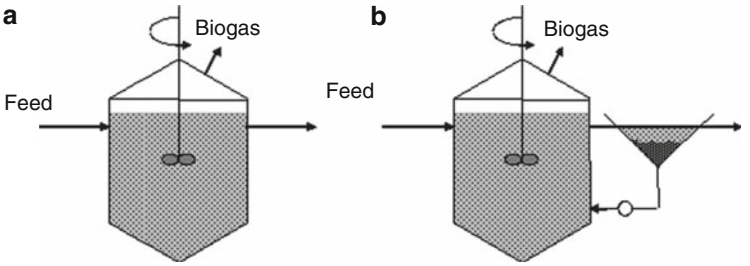

Fig. 9.5. Conventional anaerobic digesters (**a**) CSTR, (**b**) CSTR with recycle of settled sludge (anaerobic contact).

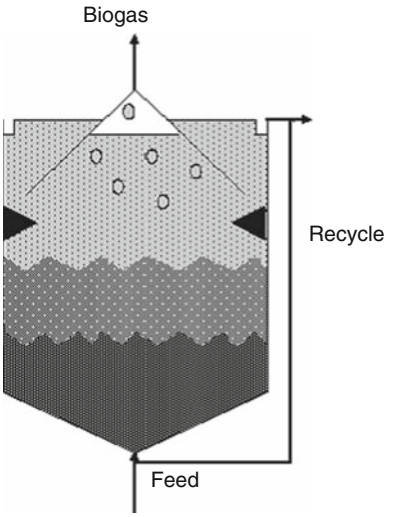

Fig. 9.6. The upflow anaerobic sludge blanket reactor (UASBR).

systems. Solids separation could also be effected using membranes or through centrifugation. However, these are generally more expensive alternatives.

4.2. High-Rate Systems

High-rate anaerobic reactors aim at achieving a high rate of substrate consumption. They are equipped with appropriate systems for biosolids retention. We distinguish two types of high-rate systems: (a) suspended growth high-rate anaerobic reaction systems and (b) attached growth high-rate anaerobic reaction systems. Representative suspended-growth reactors include the UASB reactor (Fig. 9.6) and the Anaerobic Baffled Reactor (ABR) (Fig. 9.7). Representative attached growth reactors include the upflow and downflow anaerobic filters and the fluidized bed anaerobic bioreactors.

UASB reactors are usually cylindrical vessels in which the waste moves upward through a sludge blanket at a linear velocity of the order of 3 m/h. The top of the reactor has a gas

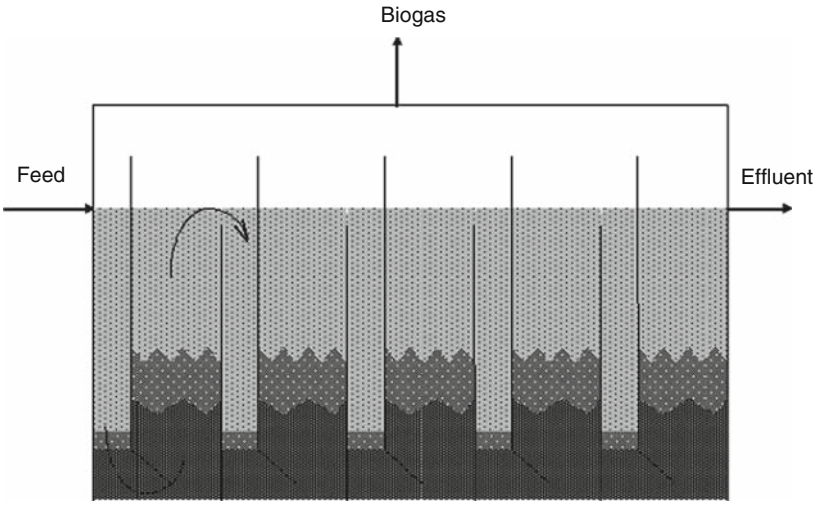

Fig. 9.7. The anaerobic baffled reactor ABR.

collector shaped like an inverted funnel, over which liquid overflows. The region between the funnel and the top of the reactor serves as a settling zone, which ensures that the solids that have entrained during the overflow have the opportunity to settle and remain inside the reactor. UASB reactors do not exhibit any dead volume or uneven flow problems, as long as the biogas production rate exceeds 1 m^3/m^3/day for a 4–6 m high digester. For low organic content wastewaters, biogas recirculation is recommended. The liquid phase of the UASB reactor will generally maintain a uniform temperature, pH, and composition throughout its height. The UASB reactor is most suitable for medium-strength wastewaters (2,000–20,000 mg COD/L) and low suspended solids concentration. Typical values of the HRT are 12 h, and typical OLRs are 16 kg COD/m^3/day and 1 kg COD/kg VSS/day respectively (7, 50, 64).

UASB reactors have the following advantages:

- They contain high biomass concentration and high SRTs
- Their design is simple
- Mass and heat transfer are favored
- A small bioreactor volume is required (when compared with the conventional counterpart)
- They are robust to feed variations
- Granule formation is favored

The main disadvantages of UASB reactors are:

- Settling may limit the process efficiency
- High solids containing wastewaters render them nonsuitable as granulation is impeded and operating conditions limit hydrolysis
- Granule formation requires the control of too many operational parameters

The ABR (65, 66) is an alternate suspended growth system that also allows for high biomass retention. This is accomplished by forcing the wastewater to move through successive

downflow and upflow compartments. The downflow part is small in volume and serves practically for transporting the wastewater to the bottom of the next compartment. The slow upflow movement of the wastewater allows for sludge formation and retention in the upflow compartment. Thus, the wastewater has sufficient contact with sludge on its way to the effluent. The ABR may be described by a series of UASB reactors (67). Hydrodynamically, the UASB reactor resembles a continuously stirred-tank reactor (CSTR) (68), and an ABR a series of CSTRs, provided that biogas production is sufficient to maintain good mixing in each compartment (66, 67).

The ABR is suitable for the treatment of medium-strength wastewaters ($\sim$ 8 g COD/L) up to an organic loading of 36 kg COD/m^3/day.

The ABR has the following advantages:

- Simple design and construction
- No need for a gas–solid separator
- Biomass retention is maximized by the alternating downflow and upflow wastewater movement
- It may be operated for long times without sludge retention
- It can handle organic loading disturbances well

The main disadvantages of the ABR are:

- Limited applicability to low-medium strength wastewaters
- Biomass retention depends on settling characteristics
- Solids retention is largely dependent on the HRT
- Little practical experience beyond the lab-scale experimentation

Existing data are rather insufficient for an effective design and optimization of ABR reactors. On the other hand, the UASB reactor technology has matured and there is a large number of full-scale plants in place today.

A variation of the ABR is the Periodic Anaerobic Baffled Reactor (PABR) developed by Skiadas and Lyberatos (69). This reactor consists of two concentric cylinders (Fig. 9.8). The area between the cylinders is compartmentalized so that the reactor resembles an ABR with the compartments arranged in a circular manner in the annular region (Fig. 9.8a). The wastewater enters the digester at the downflow section of the feeding compartment, comes up at the upflow section of the same compartment and passes on to the next compartment through the outer tubing. The flow pattern is repeated at the third and fourth compartments. Wastewater eventually leaves the system after passing through the upflow part of the effluent compartment, which is the fourth one in counterclockwise order.

The role of the four compartments is periodically changed by proper switching (on or off) the twelve valves of the outer tubing, in a clockwise manner (Fig. 9.8b). At the zero switching frequency, the reactor behaves as an ABR. At an infinite switching frequency, the compartments become identical so that the reactor should behave like a UASB reactor. By setting the switching frequency, a great flexibility is obtained, which takes advantage of the optimal reactor configuration (UASB reactor, ABR or "something in between"), depending on the loading conditions.

It has been demonstrated that for low loading rates, the ABR mode (no switching) is superior, whereas for high loading rates, the UASB reactor mode (valve switching) is preferable.

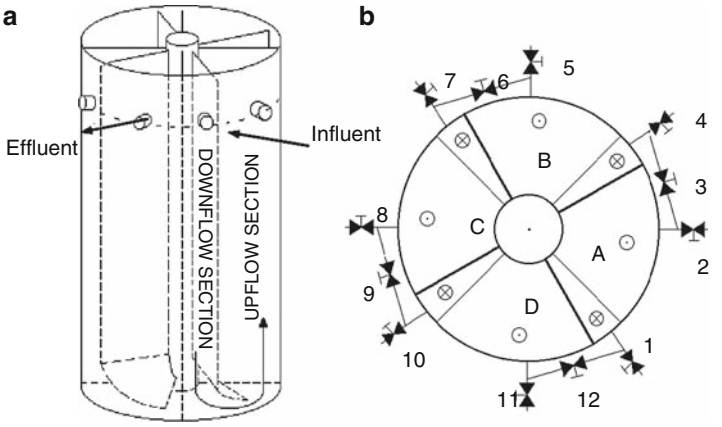

Fig. 9.8. (**a**) Front view of a four compartment PABR, (**b**) Top view of a four compartment PABR. 1, 2,. . . ,12 valves, ⊙ Upflow, ⊗ Downflow.

Consequently, the PABR is best suited for handling time-varying loading rates since it allows for maximal conversion rates at all times. This reactor type needs to be tested at a pilot scale before such systems may be reliably designed.

A combination (hybrid) of the basic bioreactor configurations may also be used. Thus, following (70) we may have two or more digesters operating in parallel or in series and two-phase (or as are sometimes called two-stage) systems that aim at carrying out acidogenesis and methanogenesis in separate tanks that may be connected through recirculation.

4.3. Two-Stage Systems

Two-stage anaerobic digestion of solid wastes or semisolid wastewater has been considered more efficient than single-stage systems (e.g., (71, 72)), since the conditions that optimize each phase can be effectively regulated. Typically, the first stage involves solid hydrolysis and acidogenesis, while in the second stage, the dissolved organic matter is converted to biogas by methanogens.

Inhibition of hydrolysis and acidogenic fermentation has been reported to be caused by VFA accumulation (73) with the pH dropping below 6.0 (74). For this reason, several researchers proposed the recirculation of the second stage effluent (which has very low organic matter) to the first stage (75, 76). In this way, the rate of the hydrolysis–acidogenesis step (and consequently the rate of the overall process) can be increased, since a liquid portion, rich in dissolved organic matter, is removed to the second stage and is replenished with the methanogenic effluent of low organic content and a pH close to 7.5.

4.4. Natural Systems

Natural methanogenic ecosystems, which include cattle rumen, municipal solid waste landfills, and sediments, contribute significantly to greenhouse gas emissions. The rumen is a unique ecosystem found in many species of herbivorous mammals known as ruminants,

examples of which include sheep, cows, goats, and deer. The primary role of the rumen is to provide pregastric digestion of structural polysaccharides (such as cellulose, hemicellulose, and lignin). The microbial ecosystem of the rumen is made up of a complex symbiotic community of protozoa, bacteria, and fungi. The system is mainly anaerobic, although facultative bacteria have been identified around the rumen wall associated with the oxygen movement across the wall from nearby capillaries. Substrates consumed by the population include fiber, starch, proteins, nucleic acids, and lipids. The maintenance of the microbe population is achieved using variable retention times with many microorganisms, generating quickly to keep concentrations high in the liquid. Organisms with longer generation times or long lag phases survive through adhesion with feed particles or entrapment in the solid digesta, which has a throughput significantly slower than the liquid contents. Some microbes do not leave the system at all and it is believed that these organisms play a vital role in the degradation of fibrous feeds (77). Products of the fermentation include VFAs such as acetate and propionate and gases such as hydrogen, carbon dioxide, and methane. Gas removal is critical to protecting the health of the animal.

5. SUSPENDED GROWTH ANAEROBIC BIOREACTOR DESIGN

5.1. Operating Parameters

As previously discussed, anaerobic digestion involves many groups of microorganisms, which because of their physiological differences, have a different sensitivity and dependence on temperature, pH, organic substrate composition, nutrient requirements, and presence of inhibitors. The impact of these factors is critical to the anaerobic bioreactor design and operation. For this reason, they are considered in the following discussion.

5.1.1. pH and Alkalinity

On most occasions, the bioconversion of organics is best accomplished in pHs ranging between 7.0 and 7.2. Many species can grow effectively in a pH range between 6 and 9. The two key parameters that influence the pH of a digester are the VFA concentration and alkalinity (expressed as mg $CaCO_3$/L).

Alkalinity is attributed mainly on HCO_3^- ions (bicarbonate alkalinity), which originate from the evolution of CO_2 during the biodegradation of organics. Fatty acids produced during acidogenesis tend to lower the bioreactor pH. Under normal operating conditions, this decrease in pH is regulated by HCO_3^-, as well as by acid consumption during acetogenesis and methanogenesis. Under unfavorable conditions (e.g., inhibition of methanogenesis followed by VFA accumulation), a small buffering capacity of the system (low alkalinity) may lead to a drop in the pH with further inhibition and bioreactor failure. The following relationship shows that partial pressure of CO_2, pH, and alkalinity are directly related:

$$\mathrm{pH} = \mathrm{p}K_{\mathrm{a1}} + \log\left(\frac{\mathrm{alkalinity(bicarbonate)}/50,000}{\mathrm{CO_2(g)}/K_{\mathrm{H}}}\right) \tag{12}$$

where $K_{\mathrm{a1}} = 5 \times 10^{-7}$ (35°C) is the dissociation constant for carbonic acid and $K_{\mathrm{H}} = 38\,\mathrm{atm/mol}$ (35°C) is Henry's constant for CO_2. Bicarbonate alkalinity is expressed in mg

$CaCO_3$/L. Thus, for a carbon dioxide partial pressure of 0.25 atm and a bicarbonate alkalinity of 2,800 mg/L, the above equation gives a pH of 7.2. If during an overload, VFAs increase, the bicarbonate alkalinity will decrease, leading to an immediate drop in the pH. If the alkalinity is high, the digester may withstand a substantial increase in the VFA concentration without a detrimental decrease in the pH. A way to ensure a pH within the desirable region is the increase of alkalinity through the addition of ammonia (57).

Wastes that have a small buffering capacity may require the addition of alkalinity in the form of $Ca(OH)_2$ (lime), NH_3, NaOH, $NaHCO_3$, or KOH. Lime, although cheap, is generally undesirable since it forms precipitates causing fouling. Sodium salts are more expensive. Also, very high amounts of sodium bicarbonates may lead to the accumulation of Na to inhibitory levels. This may be circumvented by the addition of a mixture of sodium and potassium salts. The addition of ammonia is another alternative, although as we have seen, ammonia may also be inhibitory. Codigestion with cow manure, however, might be an inexpensive alternative.

5.1.2. Temperature

Many researchers have investigated the effect of temperature on anaerobic digestions. Methane production rate increases with temperature, in particular, methane production at 25°C is 25% lower to that at 60°C (78–81). Furthermore, thermophilic anaerobic digestion leads to higher reaction rates, and organic solids biodegradation yields a better separation of solid and liquid phases (important for biosolids settling and recycling) and a higher destruction of pathogens (important for sewage sludge stabilization). The increased energetic requirements, however, coupled with an increased sensitivity to toxic compounds, and reduced stability, render thermophilic digestion less applicable and less economical in practice (82).

5.1.3. HRT

The HRT of a bioreactor is perhaps the most important design parameter. For a given feed flow rate Q, the HRT is simply V/Q, where V is the liquid volume in the reactor. Consequently, the HRT is immediately related to the size of the bioreactor. The HRT of a digester (and any continuous bioprocess to this effect) must be sufficiently large so that the desirable bacterial populations may be retained in the system rather than being washed out. Its recommended value will depend on the type of digester used, as well as the feed characteristics (57). Anaerobic species that are particularly sensitive to the HRT are the methanogens and the acetogens (83). In general terms, typical value ranges for conventional suspended growth bioprocesses are 10–60 days (57).

5.1.4. Mixing

The benefits of mixing are (84–87): minimization of solid deposition and dead space, uniform substrate distribution and reduction of short-circuiting, elimination of scum formation, intimate contact between microorganism and substrate, uniformity of temperature, even distribution of alkalinity and removal and dilution of metabolic products, which may be inhibitory from the site of its formation. Mixing was found to improve the rate of biodegradation by breaking up gas bubbles surrounding the microbial aggregates and enhancing the contact between liquid and biomass (88). Generally in fermentors, mixing promotes gas removal by increasing the rate of nucleation for bubble formation (89). It was demonstrated

that Salmonella organisms survive longer in the stagnant zones of a digester, indicating the importance of mixing in pathogen destruction (90).

Mixing may not be essential for digesting feed streams that contain solids, which contribute significantly to the total biodegradable matter, and if the solids exhibit good settling properties. It was shown that in a single-stage digester treating cattle manure slurry, the intensity of mixing did not affect the extent and rate of degradation; rather, unmixed digesters exhibited a better extent and rate of degradation (91, 92). For a given HRT, unmixed digesters were able to retain the solid fraction for a longer duration, thereby increasing the extent of degradation. Moreover, the formation of microbial aggregates was enhanced in unmixed digesters. The concentration of extracellular polymeric substances was higher in unmixed digesters.

Mixing within a digester can be powered externally or internally, mediated through turbulence created by introduction of feed and gas generation. Externally powered mixing systems can be divided into four types (93): mechanical stirring, mechanical pumping, unconfined gas injection, and confined gas injection.

Mechanical stirring systems consist of either low-speed turbines or low-speed mixers. Mixing is accomplished by rotating impellers. This type of stirring is not suitable for digesters with a gas holder cover since submergence is reduced as the cover rises.

Mechanical pumping systems consist of propeller-type pumps mounted in draft tubes, which can be located either inside or outside the digester. Mixing is accomplished by creating a fluid pumping action. Another type of mechanical pumping is pumped recirculation, which consists of pumps and associated pipe work to withdraw slurry from one part of digester and inject into another. In unconfined gas injection systems, gas is collected at the top of the digester, compressed and then discharged through bottom diffusers or mounted lances. Mixing is accomplished by gas bubbles that rise to the surface, carrying and moving the sludge.

In a confined gas injection system, gas is released within a draft tube. In high-rate systems such as the UASB reactor, Internal Circulation (IC), and expanded granular sludge bed (EGSB), mixing is accomplished internally through turbulence created by the biogas generated within the system.

5.1.5. Toxicity Prevention and Removal

Wastes to be treated by anaerobic digestion could potentially contain different compounds that may be toxic to anaerobic populations. As we have seen, several different chemicals are quite toxic especially to methanogens, thus potentially limiting the treatability of a given waste or wastewater. Once the reason for toxicity has been determined, there are several possible actions that could be taken to alleviate the effects.

First of all, since toxicity is a function of concentration, it is always possible to dilute the wastewater to an extent that the toxicity is removed. Generally, this is not a very viable approach since dilution will imply higher waste volumes, which in turn imply higher digester volumes and capital cost.

A second possibility is the removal of the toxicant through precipitation or complex formation. Thus, for toxicity caused by metals, the addition of sulfate may alleviate the problem. In the low redox potential prevailing in the digester, sulfates will be reduced to sulfides, which will react with metals forming insoluble precipitates. Needless to say, care must be taken that

the amount of sulfate added is not excessive, or inhibitory H_2S may be formed. Inhibition by detergents (LAS) may be alleviated through addition of cationic quaternary ammonia compounds.

Another possibility is that the toxicity of a compound may be pH-dependent. Thus, ammonia becomes significantly more toxic in the free ammonia form, which prevails for high pH values. Consequently, maintaining a lower pH may alleviate ammonia inhibition. Inhibition of fatty acids is more pronounced when they are undissociated. This then implies that for VFA inhibition, an increase in the pH is favorable in removing toxicity. On the other hand, low pH leads to the stripping of hydrogen sulfide, removing the toxicity that is caused by excessive H_2S production. Thus, depending on the possible cause of inhibition, a certain pH correction may prove beneficial.

On some occasions, organic toxicants may be removed by an appropriate biological pretreatment. Pretreatment of olive-mill wastewaters with a white-rot fungus removes selectively the phenolic compounds that are responsible for poor performance of anaerobic digestion (61).

5.2. Sizing Bioreactors

5.2.1. Conventional Systems

Because of the complexity of anaerobic digestion, their design is largely done empirically. The main approaches to sizing CSTR digesters are described in the following section.

5.2.1.1. Sizing Based on Retention Time

The steady-state material balances for a CSTR, in which a pure microbial culture grows on a limiting substrate S yield:

$$\frac{1}{\theta} = \frac{\mu_{\max} S}{K_s + S} - b \tag{13}$$

where θ is the retention time, which is equal to V/Q, with V being the useful reactor volume and Q the volumetric flow rate. Consequently, if some valid microbial kinetics are available, Eq. (13) may be used to find the retention time required to achieve reduction of the substrate from the feed concentration S_0 to the desired effluent concentration S. Once the retention time is decided, it may be multiplied by the wastewater flowrate to give the required liquid volume. Notice again that small S implies large V, i.e., large reactor volumes. Also, small specific growth rates imply large reactor volumes.

From Eq. (13), it becomes apparent that as the reactor volume decreases (the retention time increases), the effluent substrate concentration increases. For $S = S_0$, microbial washout occurs. This defines the minimum retention time, which may be calculated from:

$$\frac{1}{\theta_{\min}} = \frac{\mu_{\max} S_0}{K_s + S_0} - b \tag{14}$$

It is clear that depending on the temperature and the organic substrate in the feed, there is a minimum retention time at which the microbes responsible for the rate-determining step are washed out. Typically, we want to operate with a retention time that is at least 2.5 times larger than $\theta_{\min}$ (a safety factor 2.5). Temperature is very important as it influences the kinetic

Table 9.3
Anaerobic digester retention times

Temperature (°C)	θ_{min} (days)	Design θ (days)
18	11	28
24	8	20
35	4	10
40	4	10

parameter $\mu_{\max}$. Mesophilic digesters are then bound to be larger than thermophilic digesters if they are to achieve the same conversion.

From our previous discussion regarding the kinetics of anaerobic digestion, it should be clear that once the rate-determining step and its kinetics are known, we may assume complete stoichiometric conversion of the organic substrate to the substrate of the rate determining step S_0 (usually methanogenesis). Then Eq. (14) may be used to determine the minimum retention time, multiply by 2.5 to obtain the design retention time, which then defines the required reactor volume. Quite often, however, we do not have reliable kinetics. Table 9.3 may be used to determine the recommended retention time for a suspended growth CSTR.

Table 9.3 may be used for digestion of municipal sludge or similar wastes. In the event that we want to size a digester for a new and largely untested wastewater, it is recommended that a lab-scale digester is used for assessing the required retention time in order to achieve a certain conversion. Such an experiment will also provide useful information on other requirements specific to the particular wastewater such as need for alkalinity, need for toxicity removal, etc. as well as the anticipated biogas production.

5.2.1.2. Sizing Based on the Organic Loading Rate

An alternative procedure for sizing a conventional anaerobic digester is based on the organic loading rate, defined by:

$$\text{OLR} = \frac{QS_0}{V} \tag{15}$$

where S_0 is the influent organic concentration expressed in COD. Clearly, having chosen OLR for a particular wastewater, Eq. (15) may be used to determine the required reactor volume. Usually, the OLR is chosen based on lab-scale or (even better) pilot-scale experiments so that a satisfactory performance can be anticipated. Typical ranges for the OLR in conventional digesters are 1–10 kg COD/m^3/day.

5.2.1.3. Anaerobic Contact Systems

Anaerobic contact systems allow for the retention of biomass and hence higher volumetric rates. In other words, the retention time of the solids is much higher than the HRT. If we assume that the solids separator that follows the bioreactor allows for retention of biomass, so that only a fraction δ of the biomass entering the separator leaves with the effluent stream

(which implies that the ratio of HRT to SRT is equal to δ), then Eq. (14) is modified to:

$$\frac{1}{\theta_{\min}} = \frac{1}{\delta}\left(\frac{\mu_{\max} S_0}{K_S + S_0} - b\right) \tag{16}$$

Clearly, a small δ allows for low retention times and hence digester volumes. Anaerobic contact reactors may have a biomass concentration of 4–6 g VSS/L and typically lead to high organic removal efficiencies with a HRT, which is of the order of 0.5–5 days (2) and an OLR 0.5–10 kg COD/m^3/day. A word of caution is appropriate here. Depending on the wastewater, the settling characteristics of the anaerobic biosolids may not be good. Consequently, experimentation at a pilot-scale before full-scale implementation is strongly recommended if one deals with a new wastewater type.

5.2.2. Upflow Anaerobic Sludge Blanket Reactors

In principle, sizing a UASB reactor is similar to sizing a CSTR. Because of granulation, however, the observed volumetric bioconversion rates are much higher. Also, the upflow velocity is crucial for such reactors. The upflow velocity depends on the soluble fraction of the COD. For wastewaters with a high dissolved COD (dCOD) fraction, the mean upflow velocity can be up to 3 m/h (2), whereas for low dissolved COD fraction approximately 1 m/h is more appropriate to minimize problems with sludge settling. This means that for a given volumetric flow rate, higher reactors (e.g., 6 m) may be used for high (dCOD/total COD) wastewaters than for low (dCOD/total COD) wastewaters. Permissible OLRs are a function of temperature. For 15°C, guideline OLRs range between 1.5 and 3 kg/m^3/day, for 25°C between 3 and 8 kg/m^3/day and for 35°C between 9 and 18 kg/m^3/day, depending on dCOD/total COD. Generally, higher OLRs may be applied once granulation is satisfactory. As far as HRTs are concerned, typical design values are 10–14 h for 16–20°C, 8–10 h for 20–25°C, and 6–8 h above 25°C.

The general procedure then for sizing is as follows. Depending on the temperature and the fraction of dCOD/total COD, a volume is chosen that gives acceptable OLR and HRT. For example, for a 120 m^3/day(= 5 m^3/h) of a wastewater with high dCOD/total COD and with a total COD of 5 g/L, choosing a 35°C a reactor volume of 40 m^3 gives an HRT of 8 h and an OLR of 15 kg/m^3/day, which are both acceptable. Having found the volume, the height and surface area are determined so as to give a satisfactory upflow velocity. Thus, for our example, an 8 m high reactor implies a surface area of $40/8 = 5$ m^2, which then gives an upflow velocity of 5 (m^3/h)/5 m^2 = 1 m/h.

Having sized the UASB reactor, another important design issue concerns the gas–solid separator device (GSS). This device serves the following main purposes:

- Separate and discharge of biogas.
- Prevent washout of viable biomass through thorough settling of the sludge back to the main reactor body.
- Prevent washout of floating sludge.
- Clarify effluent.

To this end, the design typically is done as follows:

- The diameter of the gas exhaust must be sufficient to secure satisfactory removal of the biogas through the cap.
- The height of the gas collector must be approximately 20–25% of the total reactor height.
- The area of the apertures around the gas collector that allows for liquid upflow should be approximately 15–20% of the total surface area of the reactor.
- The funnel must have a slope of 45–60°, to provide good settling.
- Overlapping baffles should be installed below the apertures to prevent gas bubbles from entering the settler compartment.

Finally, it should be stressed that UASB reactors are not proper reactor choices for wastewaters that have high total solids. For treating such wastes, a liquid/solid separation, such as a presettler or filter, must necessarily precede the UASB reactor.

5.3. Biogas Collection and Exploitation

Anaerobic digesters need to have a well-sealed cover, since the possibility of oxygen entering the reactor may significantly inhibit methanogenesis and cause process failure. The covers are often floating, allowing thus a constant head pressure in the reactor.

The biogas generated in anaerobic digesters must be collected and if possible utilized. Methane should be collected for three reasons:

- It is a greenhouse gas, significantly worse than CO_2.
- A mixture of methane and O_2 can lead to explosions and hence threaten the safety of the plant.
- Methane is a useful fuel (calorific value is $35,800\,kJ/m^3$).

The collected methane gas must be cleaned and separated from other biogas constituents such as carbon dioxide, hydrogen sulfide, and excess moisture. Hydrogen sulfide is corrosive to metal piping and must be removed by scrubbing the gas with iron oxide sponge or a gas scrubber. To the extent that the waste contains metal cations to start with, hydrogen sulfide is reduced as insoluble metal salts may be formed. Any carbon dioxide that is not stripped during the removal of hydrogen sulfide may be removed to reduce the total volume and increase the gas heating value. The removal of carbon dioxide is expensive and is only economically feasible when the gas is to be sold commercially. Otherwise, it is preferable to simply burn the biogas as is, despite the fact that the heating value is reduced. The most common method of carbon dioxide removal is absorption through a chemical or aqueous solution, as in a scrubber.

After cleaning and purifying the methane gas, it can either be stored for later use or used immediately. If it is not to be utilized, it can be simply flared. Alternately, it can be used as fuel for boilers providing sensible heat, which is needed to control the temperature of the waste at mesophilic or thermophilic conditions. If the economies of scale permit, it can be used in an internal combustion engine to generate electricity. Any excess gas that cannot be used by the treatment plan can be sold.

5.4. StartUp and Acclimation

When an anaerobic reactor is fed with a new feed medium, the various populations will gradually change through various mechanisms, until eventually an “acclimated” population

has stabilized. Complete acclimation (or adaptation) of an anaerobic continuously fed bioprocess typically requires at least 12 retention times (14).

Breure et al. (94, 95) studied the acidification of glucose and gelatine at 30°C and various pHs in two parallel anaerobic chemostats at a constant dilution rate (D = 0.12/h) that were inoculated with sewage sludge from a sewage treatment plant and were previously acclimated each to a different carbon source (one to glucose and one to gelatine). They observed that in the reactor acclimated to glucose, gelatine was converted by only 30%, whereas in the gelatine-acclimated bioreactor, glucose was completely utilized. This proves the impact of acclimation on digester performance. A thermophilic anaerobic sludge acclimated to lactic acid (181 days of operation at an HRT of 13 days) can convert this organic compound at much higher rates than nonacclimated sludge (96).

A safe startup of an anaerobic digester is done at a low OLR, which is gradually increased with time, permitting proper acclimation. An additional criterion for UASB reactors is that they should be started up at relatively low upflow velocities, (not exceeding 0.5 m/h), until adequate granulation has occurred, a process that may take several weeks.

6. CONTROL AND OPTIMIZATION OF ANAEROBIC DIGESTERS

6.1. Monitoring

For process optimization and control, it is necessary to evaluate the process status by appropriate measurements. The most readily used measurements are:

1. Gas production: the earliest and most commonly used parameter for monitoring and control of the anaerobic process (97). Reactor systems with high HRTs, however, are subject to biogas production delays that may cover a complete HRT period or even more.
2. Gas composition: By passing the biogas through a column/bed of soda lime, the CO_2 can be scrubbed off, and a simple volumetric measurement of the methane can be conducted. Alternatively, the CH_4 and CO_2 percentage can be measured using gas chromatographic methods or infrared spectroscopy.
3. Volatile fatty acids: It is well-recognized that monitoring the specific concentration of VFAs can give vital information on the process status (98). Often acetate and propionate will be dominating, which earlier led to the assumption that the ratio between propionate and acetate could be used as an indication of process imbalance. However, it has been shown that high VFA concentrations may not necessarily be the result of inhibition (99). Other studies have shown that the isoforms of butyrate and valerate are better indicators of changes in the process balance (98).
4. Redox potential: The monitoring of redox potential could detect inhibition by oxygen, but could not be used otherwise to give information about the system (100).
5. Ammonia: high ammonia concentrations, often the result of protein bioconversion, may lead to inhibition as discussed previously.
6. pH: The importance for monitoring pH is self-evident from the above discussion. It is a relatively inexpensive and reliable measurement. The only problem is that by the time it signals failure of the digester, it is too late for prevention.
7. Alkalinity: it is important to measure as it is directly related with the digester's ability to handle overloads.

In addition to these measurements, gas phase hydrogen (101) and dissolved hydrogen sulfide, carbon monoxide (102) have been used. Other parameters that have been monitored include:

- Total solids (TS) and volatile solids (VS)
- Total Organic Carbon (TOC)
- Biochemical Oxygen Demand (BOD) and COD
- Specific Methanogenic Activity (SMA): a test specifically developed for anaerobic conditions, estimates the potential gas production rate in a reactor system
- Molecular techniques, such as immunological techniques and techniques based on RNA and DNA probing
- Coenzyme F_{420} and NADH

6.2. Process Control

A major reason for the slow acceptability of anaerobic digestion has been that it has been perceived as difficult to control. The successful operation of a digester requires maintenance of a balanced population of acidogenic and methanogenic organisms. Disturbances, such as temperature fluctuations, organic overloading, or the introduction of toxins, can cause imbalance, which if not rectified can lead to a shutdown of the process.

To ensure proper control of the process, the onset of imbalance needs to be sensed very early. The main criteria of a good sensor for process control are sensitivity and speed in responding to the onset of imbalance. Measurements commonly used for process control are intermediate compounds (e.g., pH, volatile organic acid concentrations, bicarbonate alkalinity, gas concentrations/flow rates) during the digestion process.

A general approach to controlling the process is to adjust the manipulated variable so as to maintain the monitored variable at a predetermined set-point through a controller. The manipulated variable in most instances is the feed flow rate. Bicarbonate dosing has also been used in a few cases as the manipulated variable (103, 104). The control law applied may be a simple on/off control (105), proportional-integral control (106, 107), adaptive control (108–113), and neural networks (103, 112), or through rule/knowledge-based systems such as fuzzy controllers (114) and expert systems (97, 115–117). A complete survey of the state of the art in process control of anaerobic digestion may be found in (118).

6.3. Optimization

Currently, anaerobic digesters are operated utilizing simple control laws such as pH and feed rate control to maintain a preset pH set point and prevent overloading or overflow, respectively. There exists a considerable scope for optimizing the operation of these systems. Optimized operational strategies that need to be addressed include maintenance of stability of the process, minimization of the operating costs, and minimization of effluent discharge costs. These issues are discussed in the following section.

6.3.1. Stability

Methanogenic bacteria can be washed out if subjected to disturbances in the form of hydraulic overloads, organic overloads, and pH and temperature changes. Washout causes a reduced conversion of organic matter and the eventual shutdown of the treatment plant.

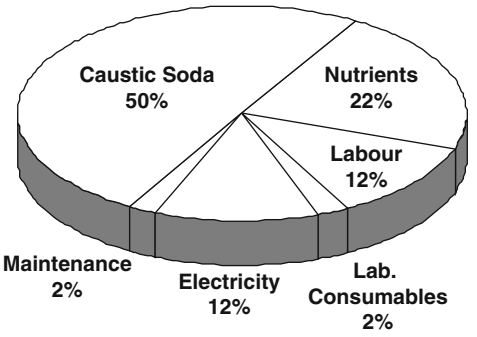

Fig. 9.9. Distribution of operating costs for a high-rate anaerobic treatment plant.

Addition of a healthy inoculum is then required to restart the system, resulting in significant downtime for the treatment plant. Active inoculum purchased and transported from other high-rate anaerobic treatment plants can also be enormously expensive. During the shutdown period, the effluent must be disposed into the sewer without treatment or transportation from the site, thereby exacerbating the costs. It is possible to avoid instability by proper manipulation of reactor feed flow recycle flow and pH set points.

6.3.2. Operating Costs

A major cost of operating high-rate anaerobic treatment systems is associated with the caustic addition for pH control. This cost can amount to more than 40% of the plants' total operating costs. However, it can be reduced by sensible manipulation of recycle flows and pH set points. Other operating costs are associated with nutrient addition, electricity, labor, etc. They depend on the wastewater characteristics, the local regulations and the treatment plant infrastructure. Figure 9.9 shows the distribution of operating costs of a typical anaerobic wastewater treatment plant treating brewery effluent in Australia.

6.3.3. Discharge Costs

These costs are associated with discharging effluent to sewer. The discharge cost depends on both the quantity and quality of effluent. Also, penalties apply if poorly treated wastewater is discharged into the sewer or local water catchment. These situations arise when the efficiency of the treatment plant decreases. In addition, if the buffer capacity of the equalization tank is exceeded, then the partially treated effluent may also require discharge.

Current operating strategies center on maintaining process stability and fail to account for minimizing operating and discharge costs. A cost-based objective function (J) can be used in identifying the optimal set points for operating the plant. The objective function includes five terms relating to the cost of operation of the plant and is the sum of these terms over some time period t:

$$J = \int_0^{\tau_s} \left(\frac{w_1}{|(L_h - L(t))|} + \frac{w_2}{|(L_1 - L(t))|} + w_3 F_{NaOH}(t) + w_4 \mathrm{TOC}_{out}(t) + \frac{w_5}{Q_{gas}(t)} \right) \mathrm{d}t \tag{17}$$

where w_1, w_2, w_3, w_4, and w_5 are weighting factors, L_h is the maximum and L_l the minimum allowable level in equalization tank, $L(t)$ is the level in equalization tank at time t, $F_{NaOH}(t)$ is the flow rate of caustic at time t, $TOC_{out}(t)$ is the filtered TOC of effluent from the methanogenic reactor at time t, and $Q_{gas}(t)$ is the total gas production rate from the methanogenic reactor at time t. The five terms in the objective function relate to the cost of accumulation of effluent in the equalization tank, the cost of emptying the effluent in the equalization tank, the cost of caustic addition, the cost of sewer-discharge, and the cost of loss of stability/gas production of the system, respectively.

7. APPLICATIONS

As discussed in the introduction, anaerobic digestion finds applications in several areas aimed at waste and/or wastewater stabilization as well as energy production from biomass. These situations are exemplified in the following discussion.

7.1. Anaerobic Sludge Digestion

Stabilization of the excess sludge generated in wastewater treatment plants is one of the best-established applications of anaerobic digestion. Usually, a mixture of primary and secondary sludges is dewatered and then led to conventional mesophilic anaerobic digesters. The generated biogas may be calculated as follows:

$$Q_{CH_4} = 0.35\frac{m^3}{kg}\left\{EQS_0 \times 10^{-3}\frac{kg}{g} - 1.42P_x\right\} \tag{18}$$

where:

Q_{CH_4}: methane production rate in m^3/day
0.35: theoretical yield of methane per kg BOD_L converted
E: sludge utilization efficiency (~ 0.8)
Q: sludge flow rate (m^3/day)
S_0: influent BOD_L (g/m^3)
1.42: BOD_L/VSS
P_x: cellular mass generated per day (kg VSS/day)

The amount of cellular mass generated during anaerobic digestion of sludge P_x may be computed using the equation:

$$P_X = \frac{YQ(ES_0)}{1 + b\theta} \times 10^{-3}\ \text{g/kg} \tag{19}$$

where

Y: biomass yield factor (g cells/g BOD) (~ 0.05)
b: decay constant (per day) (~ 0.03/day)
θ: HRT (day)

Methane accounts for approximately 2/3 of the generated biogas.

Example: Digester design for sludge stabilization.

Consider a plant that generates $120\,m^3$/day of sludge containing $44,300\,g/m^3$ BOD_L. We want to design a mesophilic digester (35° C) at which temperature $b = 0.03$/day, $Y = 0.05$, and $E = 0.8$.

From Eq. (19) we have:

$$P_X = \frac{YQ(ES_0)10^{-3}g/kg}{1+b\theta} = \frac{0.05 \times 120 \times 0.8 \times 44,300 \times 10^{-3}}{1+0.03\theta}$$

From Table 9.3, we see that at 35° C the recommended HRT is 10 days. For $\theta = 10$ days, the above equation gives $P_x = 164\,kg$/day. For $\theta = 10$ days, the required volume is:

$$V = Q\theta = 120\,m^3/d \times 10\,d = 1,200\,m^3$$

From Eq. (18) we then have:

$$Q_{CH_4} = 0,35 \times \{4,256\,kg/d - 1,42 \times 164\,kg/d\} = 1,408\,m^3/d$$

The percentage of stabilized sludge will then be:

$$PS = \frac{\text{rate of stabilization}}{\text{rate of addition}} \times 100 = \frac{QES_0 \times 10^{-3}\,kg/g - 1.42\,P_x}{QS_0 \times 10^{-3}\,kg/g} \times 100 = 76\%$$

7.2. *Comparison Between UASB and CSTR for Anaerobic Digestion of Dairy Wastewaters*

Wastewaters coming from cheese-producing industries in Greece are high in organic matter (about 40–60 g COD/L) since they generally contain discarded cheese-whey as well. These wastewaters are rich in easily biodegradable carbohydrates and have a relatively low content in suspended solids (1–5 g/L). Because of the high organic content of dairy wastewater, anaerobic digestion is essentially the only viable treatment method. Lab-scale experiments described in the following section compare the applicability of the UASB reactor versus a conventional digester for this wastewater type (119).

7.2.1. UASB Experiment with Dairy Wastewater

A 10 L UASBR was constructed of Plexiglas (total height of 113 cm with inner diameter of 11 cm), inoculated with anaerobic mixed liquor from dairy wastewater and glucose-fed digesters and fed with dairy wastewater. The digester was initially fed with diluted dairy wastewater (2,500 mg COD/L), subsequently the influent COD concentration was increased gradually (by reducing the dilution) while at the same time the HRT of the system was changed so as to allow a satisfactory COD removal. A recirculation rate of approximately 52 mL/min was used throughout. Figure 9.10 shows the digester influent and effluent COD concentration as well as the system efficiency (based on %COD removal) versus time. Figure 9.11 shows the HRT and the OLR changes over time. Finally, Fig. 9.12 shows biogas production and pH values versus time.

The maximum digester COD removal efficiency (98%) was reached at HRT = 6 days with an influent COD concentration of 37 g/L (OLR = 6.2 g COD/L/day). When increasing the influent COD concentration to 42 g/L (OLR = 7.5 g COD/L/day), the COD removal

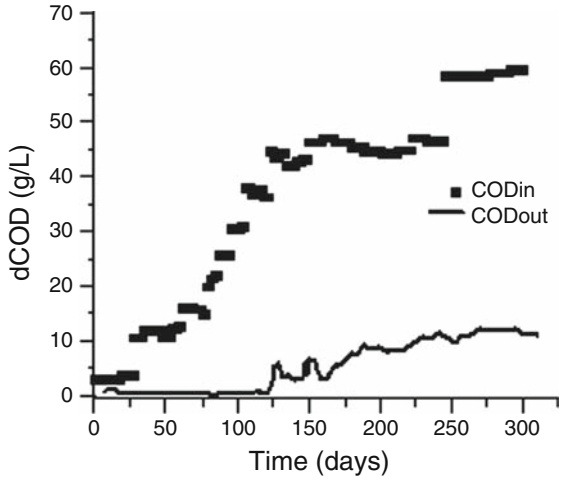

Fig. 9.10. Effluent and influent COD concentration and system efficiency (based on COD removal) versus time.

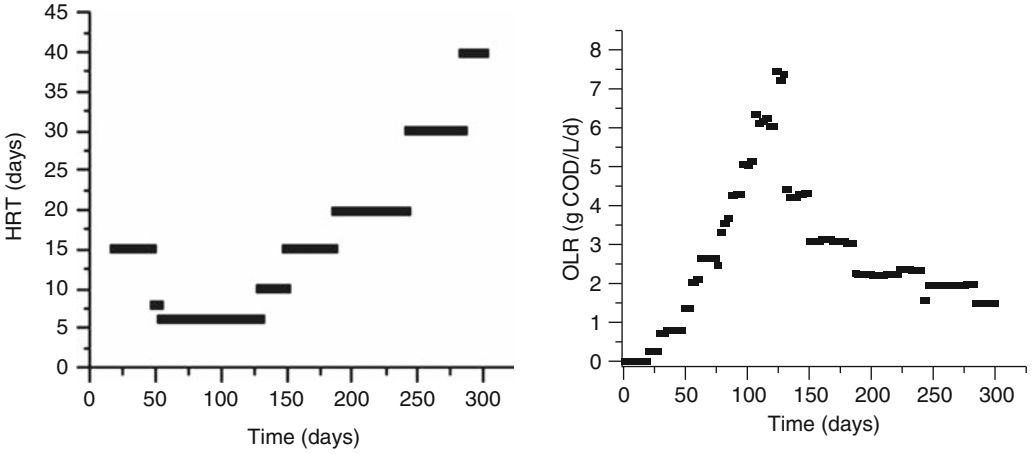

Fig. 9.11. Hydraulic Retention Time (HRT) and Organic Loading Rate (OLR) changes versus time.

efficiency was reduced to 85–90% with a mean COD effluent concentration of 5 g/L. After this point, the increase of influent COD resulted in even lower efficiencies accompanied by a sharp decrease in biogas production and pH values as well. Thus, an OLR for a UASB reactor treating dairy wastewater of 6.2 g COD/L/day may be safely used and could be increased up to 7.5 g COD/L/day. For nondiluted dairy wastewater, an HRT in excess of 30 days is required followed by a dramatic decrease in COD removal efficiency.

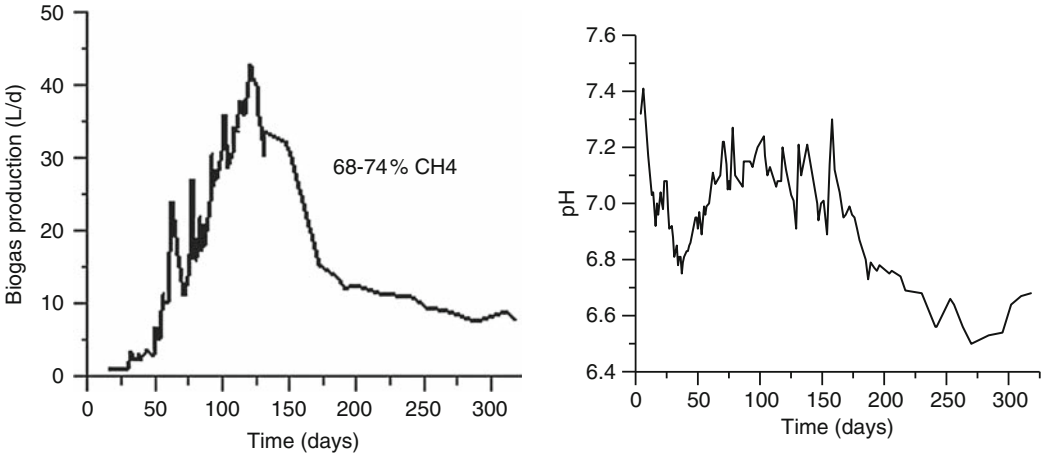

Fig. 9.12. Biogas production and pH values versus time.

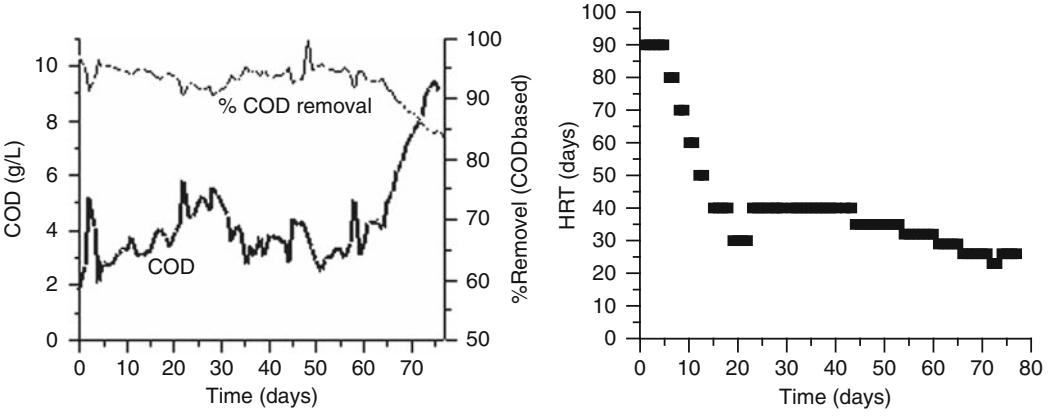

Fig. 9.13. Effluent COD concentration, % COD removal and HRT versus time in conventional digester.

7.2.2. Conventional Digester Experiment

For comparison purposes, an 8 L (useful volume) draw and fill digester was fed with nondiluted dairy wastewater (60 g COD/L) at progressively lower HRT as shown in Fig. 9.13. The highest possible OLR was found to be 2.3 g COD/L/day at a HRT of 26 days.

7.2.3. Conclusion

The high retention times required for nondiluted wastewater do not justify the use of a UASB reactor, as a less expensive conventional reactor could equally well be employed for such a high COD wastewater (> 40 g/L).

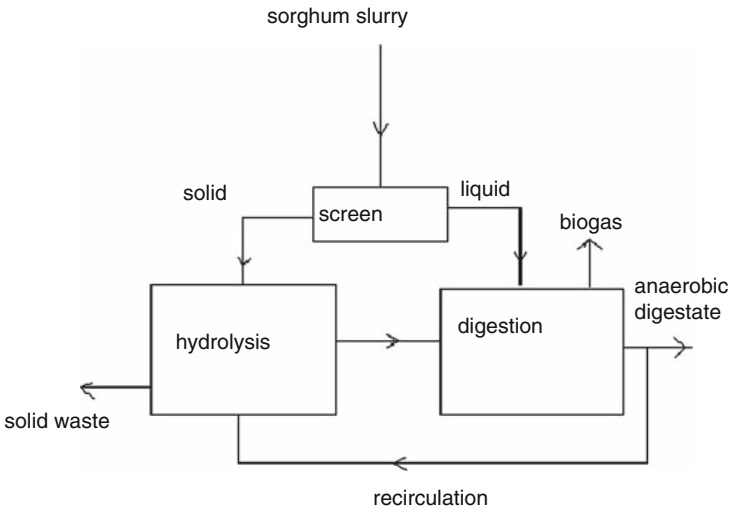

Fig. 9.14. A two-stage process for anaerobic digestion of sweet sorghum.

7.3. Biogas Production from Sweet Sorghum

There is an increasing interest in the technologies that focus on the energy recovery from biomass. Biomass (energetic plants) is a renewable source of energy, since bioprocesses can convert the captured solar energy to different forms of liquid and gaseous fuels, such as ethanol, hydrogen, methane, etc. The possibility of using sweet sorghum for biogas production in a two-stage system (Fig. 9.14) has been examined (120). Ground sweet sorghum was suspended in water and allowed to drain through a screen that separated it to a liquid extract and a solid portion.

The hydrolyzing reactor was a conventional 10 L continuously stirred tank reactor, maintained at 55° C, and fed with the solid portion. The HRT of the CSTR ($\sim$ 3 days) was significantly smaller than the SRT ($>$ 40 days). In order to achieve this, the effluent of the CSTR was gravity-separated into a solid and liquid phases. The solid part was immediately returned to the reactor to increase the solid retention time, while the liquid portion was directed to the second stage of the configuration. The second stage of the system was a 15 L, PABR (Fig. 9.8), operated at 35° C (69) and fed on the liquid effluent of the CSTR mixed with the liquid part of the raw sweet sorghum. Part of the effluent of the PABR was recirculated to the CSTR, in order to maintain the pH.

After 120 days of operation, the system stabilized at the following conditions:

1. Hydrolytic reactor: pH: 6.5, dissolved COD: 1.5–2 g/L, VSS: 1.5–2 g/L (85–90% of TSS), alkalinity: 1, 000 mg $CaCO_3$/L, biogas composition: 40–45% CH_4, and 50–55% CO_2, biogas production rate: 0.8 L/L/day.
2. PABR: pH = 6.5–7, dissolved COD = 0.4–0.5 g/L, VSS: 0.5 g/L (80% of TSS). alkalinity: 1,100 mg $CaCO_3$/L, biogas composition: 55–60% CH_4, and 40–45% CO_2, biogas production rate: 1.2–1.4 L/L/day.

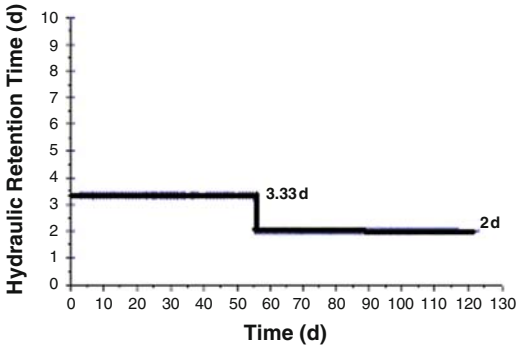

Fig. 9.15. Variation of the HRT of the hydolytic reactor for the digestion of sweet sorghum.

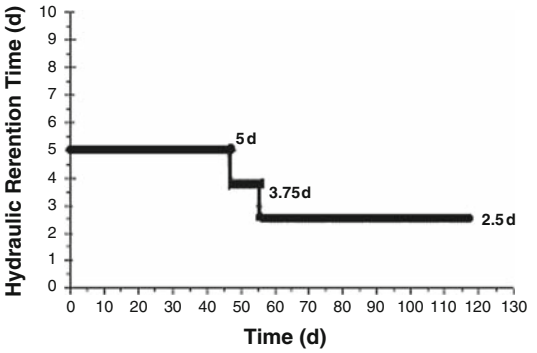

Fig. 9.16. Variation of the HRT of the PABR for the digestion of sweet sorghum.

Figures 9.15 and 9.16 present the variation in the HRT of the two reactors throughout the period of experimentation. The methane production rate is shown in Fig. 9.17. This example shows how a CSTR and high-rate anaerobic technologies may be used efficiently to generate biogas from an energy crop.

7.4. Anaerobic Digestion of Solid Wastes

Biogas from landfill may be energetically exploited, but at a very slow rate. Special anaerobic bioreactors can significantly speed up this process. Ammonia toxicity, a frequent problem in solid waste digesters, can be avoided by regulating the C/N ratio in the feed.

A typical digestion unit involves the following processes:

- Separation of organic fraction
- Suspension in water
- Anaerobic digestion
- Sludge filtering
- Aerobic composting

Pretreatment is required to remove metals and glass. Water is added to attain a 10–30% solids and the mixture is fed to the digester (retention of 2–3 weeks). The biogas generated is used for energy production, part of which is utilized for energy requirements. The residue (with

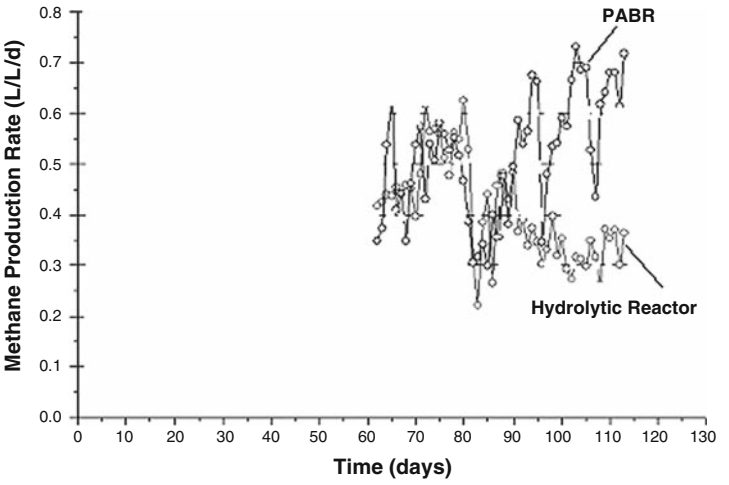

Fig. 9.17. Methane production rate by each reactor.

volatile solids reduced by 50–65%) is dehydrated to 60%, while the liquid fraction is reutilized for suspending the feed and the solid is composted.

Two system types may be used for anaerobic digestion: liquid systems with solids up to 15–20% and dry systems with solid contents above 20%, such as DRANCO, VALORGA, and BIOCEL. These systems are further distinguished as single or two-stage systems. Leach-bed reactors represent an alternative approach to such systems (121), which in a sense resembles high-rate landfills (122). Reactors operate under mesophilic (33–37° C) or thermophilic conditions (55–60° C). Also, some are continuous and some are batch-fed (e.g., BIOCEL). Finally, codigestion with manure and sludge has also been examined. Aerobic composting is the main competitive technology, but anaerobic digestion is gaining ground as:

(a) The technology is improved
(b) Aerobic compost products have received a reduced interest and increased objections
(c) Anaerobic digestion leads to reduced emissions of VOCs.

Table 9.4 summarizes the basic operating characteristics and the yields of various solid waste digestion facilities. For mixed wastes, Silvey et al. (123) estimate that 0.19 m^3/kgVS of methane is produced. Typically 100–200 m^3 of biogas are produced per ton of waste, with a methane content of 55–70% and 200–300 kg compost. Anaerobic digestion uses 20–40% of the generated energy. The net energy production is then 100–250 kWh/ton.

NOMENCLATURE

ABR = Anaerobic Baffled Reactor
b = decay rate constant (T^{-1})
BOD = Biochemical Oxygen Demand
$[CO_2]_D$ = dissolved CO_2 concentration
COD = chemical oxygen demand

Table 9.4
Anaerobic digestion units

Unit	Type	Capacity (tons/year)	Solid % in waste	% of volatiles	Retention time (days)	Produced biogas (m^3/ton)	Methane percentage	Energy production (kWh/ton)	Reference
Brecht, BE	DRANCO	20,000	40	55	15.3	103	55	165	(5)
Saltzburg, AU	DRANCO	20,000	31	70	?	135	?	220	(5)
Bassum, GE	DRANCO	13,500	57	51	?	147	?	245	(5)
Amiens, FR	VALORGA	85,000	60	63–65	18–22	145	55	566 (steam)-eq.198 elect.en	(124)
Tilburg, NL	VALORGA	52,000	45	45–50	20	92–110	55	152	(124)
Engelskirchen, GE	VALORGA	35,000	36	65	25	126	52	235	(124)
Lelystad, NL	BIOCEL	50,000			2–10	70 (est.)			(125)
Arnhem, NL	BIOCEL	35,000			22	100	55	165	(125)
Kahlenberg, GE	BIOPERCOLAT	500	50 (est.)	55	4	84	70		(126)
Design	BIOPERCOLAT	50,000			4	75	65–70		(126)
Vaasa, FI	Waasa	60,000			10–15	100–150	60–70		(126)
MinamiAshigara, Japan	Waasa					145	60		(126)
Verona, IT	Semidry	155,000	74	75	13	62–84 est.)	60–65	113 (est.)	(127)
Ave.12 facilities	KOMPOGAS	20,000			15–20	146	60	191	(128)
Zurich SWt	KOMPOGAS	5,000			15–20	95	55–60	110	(128)

dCOD = dissolved chemical oxygen demand
E = sludge utilization efficiency (~ 0.8)
f_d = biodegradable fraction of the waste (typically 0.8)
$f_e = 1 - f_s$ = fraction of electrons used for energy
f_s = fraction of electrons used for synthesis
K_{a1} = the dissociation constant for carbonic acid
K_i = inhibition constant
K_L = mass transfer coefficient
K_H = Henry's constant
K_S = saturation constant (M/L^3).
OLR = organic loading rate
PABR = Periodic Anaerobic Baffled Reactor
P_{CO_2} CO_2 = partial pressure
P_x = cellular mass generated per day (kg VSS/day)
Q = volumetric flow rate (L^3/T)
Q_{CH_4} = methane production rate in m^3/day
S' = concentration of the limiting substrate (M/L^3)
S_0 = feed substrate concentration
SMA = Specific Methanogenic Activity
t = time (T)
TOC = Total Organic Carbon
TS = Total solids
U = specific substrate utilization rate (M/M/T)
UASB = Upflow Anaerobic Sludge Blanket
V = useful reactor volume (L^3)
VSS = volatile suspended solids concentration
T_G = Gas transfer rate
Y = biomass yield coefficient (M/M)
X = cellular mass (M/L^3)
Greek Letters
δ = fraction of the biomass entering the separator that leaves with the effluent stream
θ = retention time (T)
θ_{min} = minimum retention time
θ_x = retention time
μ = specific growth rate (T^{-1})
μ_{max} = maximum specific growth rate (T^{-1})

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10
Selection and Design of Membrane Bioreactors in Environmental Bioengineering

Giuseppe Guglielmi and Gianni Andreottola

CONTENTS

Abstract The membrane bioreactor (MBR) technology is nowadays widely considered as one of the most important innovations in the field of wastewater treatment in the last decades. MBRs couple suspended growth wastewater treatment with membrane filtration, and early applications were presented in late 1960s. However, the actual popularity occurred during the 1990s, with a higher and higher interest in the relevant strength aspects of the process compared with conventional activated sludge (CAS) systems: High process compactness, excellent effluent quality (often suitable for water reuse) and lower sludge production. In urban sewage treatment, the most important advantage derived from using membrane filtration is the elimination of the secondary settling tank for the treated wastewater clarification. This can lead to some positive consequences summarized as follows: the obvious footprint reduction due to the lack of the secondary settling tank; the indirect footprint reduction due to the possibility to operate at higher mixed liquor suspended solids concentrations; the biomass selection is influenced by their degradation efficiency for pollutants rather than their ability to form well-settling flocs, as commonly happens in CAS plants. Two parts can be distinguished within the chapter. At first, a general description of membrane processes is provided through a structure- and geometry-based classification of membranes, a description of some constituent materials, a brief introduction of the most important membrane processes and of the most relevant factors affecting membrane performance. Then, the chapter focuses on membrane bioreactors for solid/liquid separation. The possible MBR configurations are described (side-stream,

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membrane immersed in the biological tank and membrane immersed in an external tank). Besides, the fouling phenomenon is discussed with special care for those MBR operational aspects, which play a relevant role in fouling mechanisms, mainly being the characteristics of the mixed liquor suspension, the membrane geometry, the hydrodynamic conditions and the hydraulic regime. Major strategies for fouling control are presented: wastewater pre-treatment facilities, air scouring, intermittent permeation and cyclic backwashes with either permeate or chemical solutions. Furthermore, the "critical flux" concept is introduced as a tool for the periodical assessment of membrane performances under various operating conditions; the most suitable version of the critical flux for MBRs (the sustainable flux) is proposed as possible fouling control strategy aimed to minimize aggressive chemical cleanings, thus extending the membrane expected lifetime. A specific section of the chapter is dedicated to some of the most diffused commercial applications of the MBR technology, ranging from the flat sheet geometry (Kubota, Huber) to hollow fibre (Zenon, Memcor-US Filter, Mitsubishi) and tubular ones (X-Flow), from submerged to side-stream schemes. A COD-based approach for the design of suspended growth wastewater treatment processes for total nitrogen removal under steady-state conditions is presented. The method is essentially based on the well-consolidated approach proposed by the University of Cape Town in the early 1980s and formalised by International Water Association with the well-known activated sludge models (ASM1, ASM2 and AMS3). The method is based on the COD fractionation according to the biodegradability of both its particulate and soluble aliquots. The design value of SRT (solids retention time) is determined as a function of the required ammonia nitrogen quality in the effluent, the nitrifiers biokinetics and the anoxic fraction of the overall biological process volume. An iterative determination of the anoxic fraction and of the recycle ratio is then suggested, in order to achieve the needed nitrogen concentration in the effluent. The method presented and the design example are mainly aimed to make explicit the conventional equations in terms of the required effluent standards for nitrogen forms as well as to show the possible differences between CAS systems and MBRs due to the different biological kinetics.

Key Words Wastewater treatment • membrane bioreactors • design criteria • fouling • critical and sustainable.

1. INTRODUCTION

As a consequence of more restrictive environmental legislations, during last decade, a significant change has been registered in the regulation of polluted discharges. One of the most important innovations can be identified in the introduction of the "environmentally sensitive areas" concept, according to which the sensitivity is referred to the eutrophication risk for the receiving water body. The new normative scenario generated an increased awareness regarding nutrient removal by setting new effluent quality standards for phosphorus and nitrogen. However, the need of most effective and complete biological processes often wrestles with the lack of available spaces required to conform and upgrade existing plants or to build new facilities; increasing costs of building areas and the socio-economic implications make this aspect very topical. On the other hand, the amount of surface area necessary for the conventional treatment schemes is further increased when a high efficiency in N and P removal is required.

The above-mentioned reasons explain the growing interest in innovative technologies by both scientists and designers. Such new processes can not only guarantee better removal efficiencies but they also provide significant reductions in terms of footprints and process volumes with respect to conventional activated sludge plants (CASPs) and trickling filters (TFs). In the last few years, several applications have been developed for moving bed biofilm reactors (MBBRs), aerated and anoxic submerged biofilters as well as other technologies, which are compatible with small space requirements. From this point of view, the best choice to compact the size of the plant is represented by membrane biological reactors (MBRs). MBR technology derives from coupling the conventional suspended growth process with membrane filtration; the membrane employed can be classified on the basis of the nominal pore size, the geometrical structure and the constituent material. On the whole, three different typologies of membrane bioreactors can be distinguished. First, MBRs can be used to obtain complete solids retention in a mixed phase flow. Second, a kind of MBR is called bubble-less aeration MBR, which is applied to the transfer of oxygen to the biological process. Finally, MBRs are used to extract priority organic pollutants form aggressive industrial wastewater.

In urban sewage treatment, elimination of the secondary clarifier (replaced by membranes) is the most evident advantage associated to MBRs. Hence, a drastic reduction in footprint can be obtained with respect to CASPs; a schematic example is shown in Fig. 10.1. This footprint recovery is further emphasized by the higher mixed liquor suspended solids (MLSS) concentration that can be kept in the biological tank. Besides, due to the lack of a secondary settling tank, the actual selective pressure of the biodegradation mechanisms is represented by the bacterial attitude for utilizing the pollutant compounds and not by their own ability to form well-settling flocs. The solid retention time (SRT, also referred to as sludge age) thus clearly defined from the hydraulic retention time (HRT), providing several benefits in terms of volume and operational conditions. At the same time, the full retention of solid particles can promote a wider diversity of the microbiological communities, and it also allows the free swimming bacteria to grow, differently from CASPs. From the environmental perspective, removing will eliminate any negative impact of filamentous bacteria on the effluent quality; these new biological conditions will allow for very good effluent quality, in terms of both carbonaceous compounds and nutrient pollution, making water reclamation and reuse possible for agricultural, recreational and industrial activities.

Notwithstanding the above-mentioned positive aspects, the widespread use of MBR technology is thwarted by two major problems: the scarceness of consolidated information about biokinetics and the membrane fouling. The first factor has a great impact on the designing of the biological tank because of the changes in bacterial activity and substrate removal rates. This can lead to overestimating or underestimating if too simple an approach is adopted in the calculations of the biological tank. Secondly, as all membrane processes, MBR technology is affected by fouling. Wrong choices of the design flux value can cause considerable variations in the capital costs related to the membrane installation. At the same time, inadequate operational fluxes can make maintenance cleaning frequent and very expensive.

This chapter focuses on the application of solid/liquid separation MBRs to wastewater treatment, that is the only kind of membrane bioreactor which has been applied until now on both pilot and full scales. It provides some basic membrane filtration concepts as well

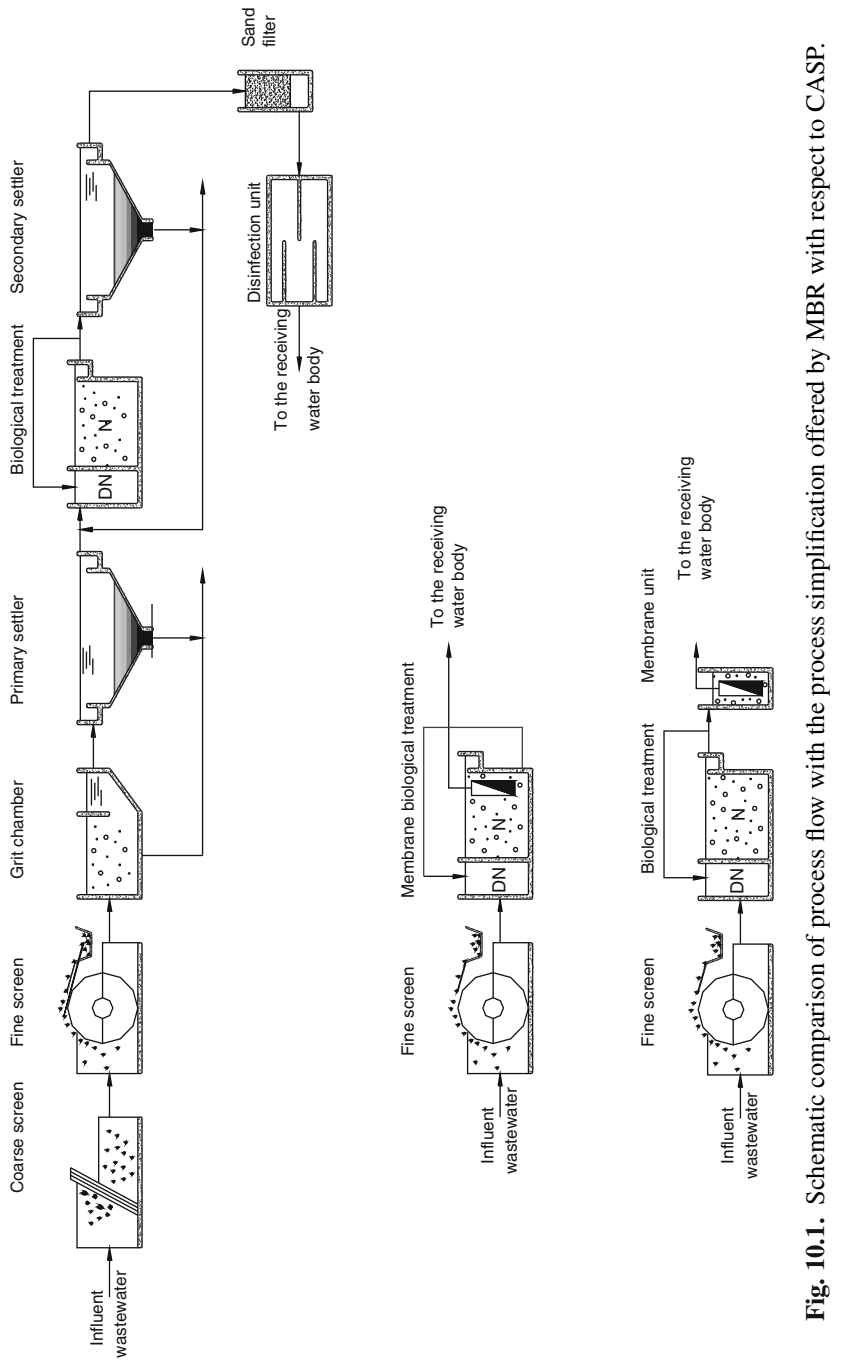


Fig. 10.1. Schematic comparison of process flow with the process simplification offered by MBR with respect to CASP.

as some basic concept about biological growth mechanisms. Therefore, the first part of the chapter shows

- A structure-based and geometry-based classification of membranes
- A description of some common constituent materials
- A short introduction of most important membrane processes
- Relevant factors that affect membrane performances and mathematical modelisation approaches typically used to predict flux and permeability

Following this, possible MBR configurations are described, and the fouling phenomenon is presented specifically in relation to mechanisms involved and common control strategies; the critical and sustainable flux concepts are also introduced. In order to provide a survey of most applied commercial membranes, some of them are reported with typical values for operational parameters. A special section of the chapter is dedicated to a steady-state approach to the biological tank design for N and C removal. The method is based on an easily applicable chemical oxygen demand (COD) fractionation, which allows for the assessment of the volume required, the daily sludge production and the daily mass of oxygen needed. Such a method can be used for designing all kinds of biological suspended growth processes once the influent wastewater characterization and the biological kinetics of both heterotrophic and autotrophic biomasses are known. Finally, in order to describe each calculation step, a design example is provided.

2. THEORETICAL ASPECTS OF MEMBRANE FILTRATION

Membrane processes are included in the general definition of filtration processes, according to which two or more components in a fluid stream can be separated on the basis of the pore size differences between the filtering medium and the component (or components) to be removed. This kind of separation usually produces two outflows (permeate flow and concentrate flow) from one inflow (feed flow). A simple schematization is shown in Fig. 10.2.

Membranes allow filtration applications to be extended up to dissolved solutes in liquid streams and separation of gas mixtures. Filtration processes can be divided into depth filters and screen filters. In the former case, the separation mechanism occurs within the whole thickness of the filtering medium. In the latter case, particles are retained on the filter's surface. Membranes are defined as screen filters. Table 10.1 resumes the most significant differences

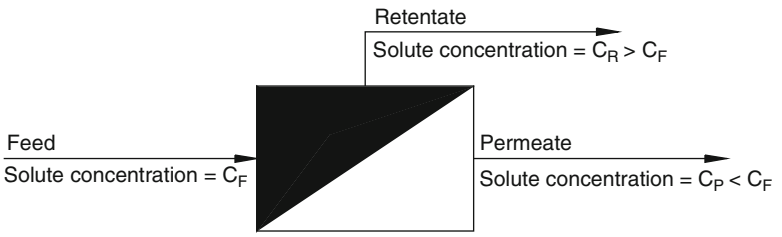


Fig. 10.2. Schematic representation of a membrane filtration process.

Table 10.1
Main differences between screen filters and depth filters

	Depth filters	Screen filters
Actual filtering portion	Whole thickness	Surface layer
Structure	Randomly oriented fibres, channels	Uniform and continuous, rigid
Filtering mechanism	Trapping, adsorption, impact, diffusion	Separation
Flux	Dead end	Dead end, cross flow
Retentate	Blocked in the filter thickness	Can be recovered
Microbial growth	High	Relatively low

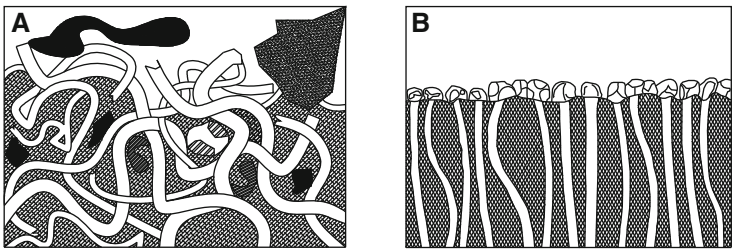


Fig. 10.3. Schemes of depth filters with randomly oriented fibres (**a**) and screen filters, which retains the particles on its surface (**b**). (Adapted from Cheryan (1)).

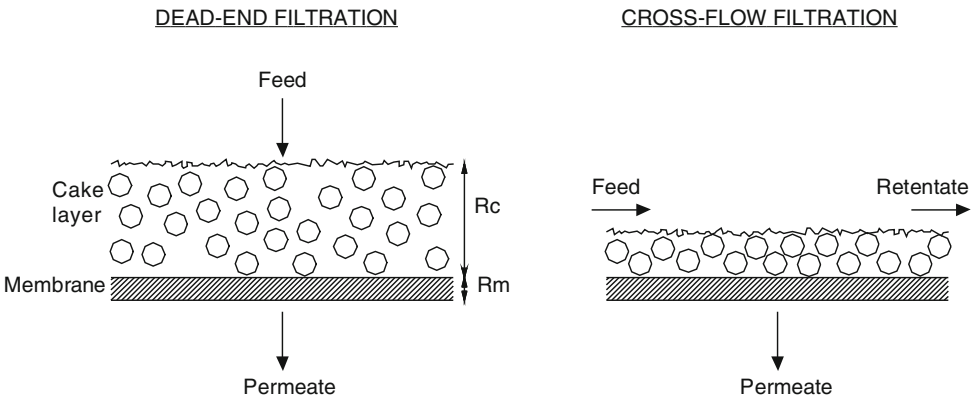


Fig. 10.4. Dead end filtration and crossflow filtration.

between depth filters and screen filters while the structures and the solids separation mechanisms are shown in Fig. 10.3.

According to the direction of feed, permeate and retentate, membranes can work under either dead-end condition (feed flows perpendicularly to the filtering surface) or crossflow condition (feed flows parallel to the membrane surface and orthogonal to the permeate flow); such a concept is schematically shown in Fig. 10.4.

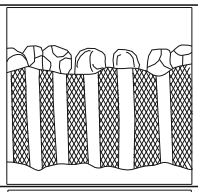
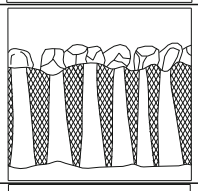
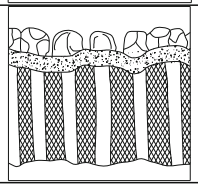
2.1. Membrane Classification

In considering membrane processes, several categorizations can be operated. Primarily, membranes can be classified according to their ultrastructure as either microporous or asymmetric. In turn, microporous membranes can be divided into isotropic or anisotropic; isotropic membranes feature a uniform pore size throughout the thickness of the membrane, whereas in anisotropic membranes, a change in pore size can be observed from the external to the internal surface of the membrane itself. Asymmetric membranes are also defined as skinned filters, being characterized by a thin skin on the surface facing feed flow; the skin layer represents the actual filtration layer, while the underneath layers just support it. Table 10.2 summarizes the main differences between microporous and asymmetric membranes.

The maximum equivalent pore diameter and the nominal diameter concepts refer to different ways used in defining the retaining attitude of microporous and asymmetric membranes. Particularly, the maximum equivalent diameter is related to the threshold size above which all particles with a larger diameter are retained, when a specified concentration limit is kept in the feed. However, "nominal" ratings are used for asymmetric membranes, where a nominal pore size is defined. It indicates the molecular size or molecular weight above which a certain percentage of the solute will be retained by the membrane, under controlled conditions.

Another membrane classification is based on the separation mechanism that is used by the membrane. In this sense, membranes can be either dense or porous. The former involve chemical–physical interactions between the permeating species and the membrane surface; they are typically used in high selectivity processes (reverse osmosis, nanofiltration and electrodialysis). Porous membranes rely essentially on mechanical separation; microfiltration and ultrafiltration (i.e. the filtration processes used in MBRs) use these membranes.

Table 10.2
Membrane structures

Microporous	Isotropic (constant pore size)		Maximum equivalent diameter
	Anisotropic (variable pore size)		
Asymmetric	Skinned layer + supporting layer		Nominal diameter

A description of the different membrane filtration processes is given in Sect. 2.3. Finally, a third classification, which is probably the most common, can be made according to the constituent materials, dividing membranes into organic and inorganic. Organic membranes are classified as either natural or synthetic, the former deriving mainly from cellulose (1). For instance, cellulose acetate is obtained from cellulose by a reaction with acetic acid, sulphuric acid and acetic anhydride. Cellulose is a polymer consisting of $C_6H_{10}O_5$ units that is available in nature in wood pulp and cotton liners. One of the most important advantages in utilizing cellulose acetate for membrane manufacturing is certainly represented by its hydrophilicity, which reduces the fouling propensity. Other advantages are a low cost, an easily feasible production scheme and a wide range of pore size obtainable. However, some limiting factors in the application of cellulose acetate membranes are a low value of the maximum operational temperature (about 40°C), a narrow pH range (preferably 3–6), an high biodegradability which makes them very exposed to microbial attack, and a low chlorine tolerance because of the chemical oxidation induced by chlorine and its derivatives. Polyamide compounds are also used in membranes manufacturing; they can be obtained by a polymerizing condensation of diamines and carboxylic acids. With respect to cellulose acetate, polyamide membranes feature not only a wider pH range, but also a lower resistance to chlorine and a stronger biofouling tendency. Polysulphone and polyethersulphone are characterized by repeating units of dyphenilene-sulphone. Their most important advantages are the wide temperature range (up to 75°C) and pH range, which ensure a good tolerance to acids and chlorine resistance, the extended range of pore size and the easiness of the manufacturing process. However, a major problem of polysulphone and polyethersulphone membranes is their low pressure limits, which make them only applicable in microfiltration and ultrafiltration processes. Polyvinylidene Fluoride, which is also referred to as PVDF, is a hydrophobic material. However, sometimes these membranes are externally covered with a thin layer of hydrophilic material in order to reduce their fouling propensity. Besides, PVDF is highly chlorine resistant. Basically, polymeric microporous membranes are made by a phase inversion process. First, the polymeric solution is cast to form a thin layer of material, and then the solvent evaporates, thereby determining an increase of the polymer concentration near the air–solution interface, which gives the typical asymmetric structure.

Composite membranes are conceptually similar to the asymmetric Sourirajan's membrane developed in the 1950s. The main difference lies in the method used to obtain the asymmetric structure; instead of using phase inversion, composite membranes are realized by covering a larger porous layer with a thin (0.2 μm) dense layer. Such membranes offer a very good chlorine tolerance and excellently wide operational ranges in terms of both pH and temperature but, because of the higher complexity of their manufacturing process and of the consequently higher costs, they are employed only when a considerable selectivity is required (reverse osmosis and nanofiltration).

Inorganic membranes are also referred to as ceramic or mineral membranes. A paste derived from a powder with a narrow particle distribution is extruded and thermally sintered in order to obtain a macroporous support, which is subsequently coated by casting a finer-grain powder. These membranes are typically packaged in single-channel or multi-channel tubular modules. They started to spread during the early 1980s bringing some relevant benefits such as tolerating

chemicals much more effectively than polymeric membranes, having large ranges of pH, temperature and pressure as well as extended lifetimes. However, the narrow range of pore size and above all the large installation costs definitively represent the main limitations of their application.

2.2. *Types of Packaging of Membranes*

A major role in the choice of a membrane is played by the module geometry, which should be able to ensure a large modular structure, a suitable turbulence near the membrane–liquid interface and a high specific surface (i.e. the filtering surface per unit of volume). Basically, four different kinds of membrane geometries can be distinguished:

- Plate and frame
- Hollow fibre
- Tubular
- Spiral wound

In plate and frame modules, a flat sheet membrane is laid on a plan support, and a net-like structure is put between the membrane and the support itself in order to avoid the filter collapsing on the support, thereby, allowing the permeate to flow throughout the channel. The whole system (support and membrane) form a cartridges; several cartridge are assembled to form a module. Plate and frame membranes are used for reverse osmosis, and above all electrodialysis, but there are also applications in the field of microfiltration. An example of microfiltration plate and frame module is the Kubota membrane (Fig. 10.5), which is further examined in Sect. 3.3.1.

Tubular membranes can be either organic or ceramic. Polymeric tubular membranes are manufactured by casting the polymeric solution on the internal surface of a porous paper or plastic cylinder. Usually, the internal diameter can vary between 4 and 25 mm. The feed is pumped throughout each channel, and therefore an "in-to-out" filtration is achieved. Various tubes are included within a shell, which has an inlet and an outlet for the feed and the retentate, respectively, as well as a side gate for the permeate flow. A major advantage of tubular membranes is certainly given by the easiness in keeping wellcontrolled hydrodynamic conditions as a consequence of the well-defined spatial region in which the feed flows. Thus, the required turbulence (i.e. the required Reynolds number) is easily obtained. Sometimes, in order to optimize the fouling control and to reduce power consumption at the same time, air supply is combined with feed recirculation, the latter being used only during peak flows; an example of such process is the scheme proposed by Wherle Werk AG for the landfill leachate treatment (Fig. 10.6).

Hollow fibre membranes are structurally very similar to tubular ones, but they are self-supporting, so no external additional support is required. This kind of membrane is used in a wide range of processes from microfiltration to reverse osmosis. The internal diameter of fibre usually varies between 0.2 and 3 mm, and the filtration process can occur both from inside to outside and vice versa. The actual filtering layer is 100–400 μm thick. Two cross sections of hollow fibre membranes are shown in Fig. 10.7.

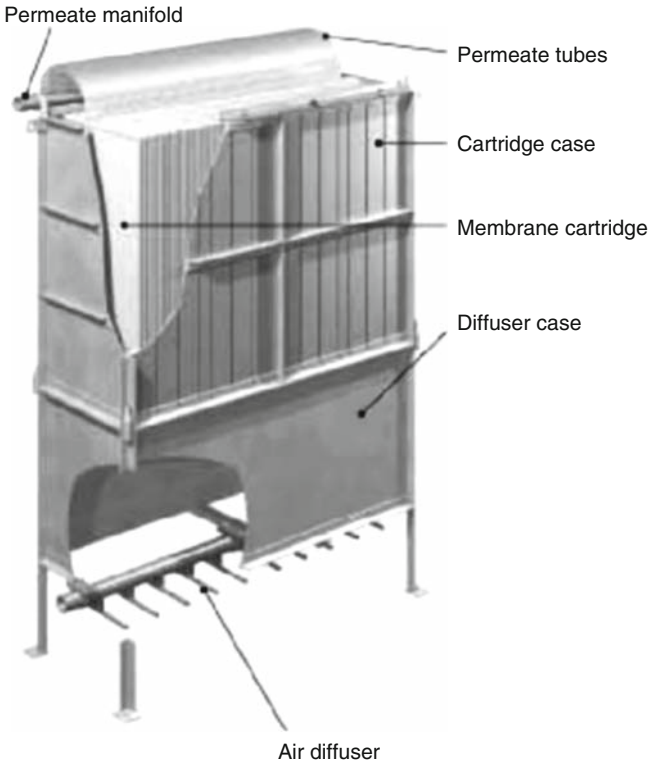


Fig. 10.5. A Kubota module with flat sheet membranes inside.

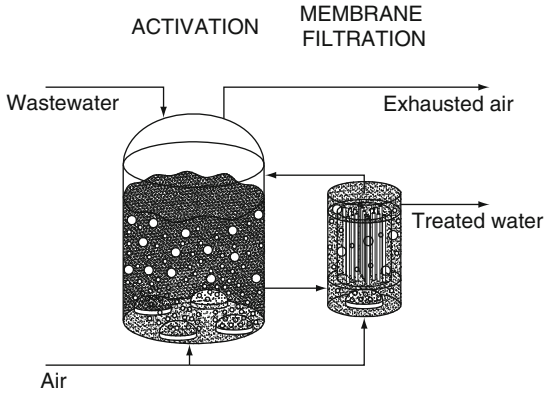


Fig. 10.6. Schematic of the Wehrle Werk process for leachate treatment Biomembrat-Loop®.

All fibres are usually assembled according to a U-shape configuration, and their tips are fixed in two epoxy resin baffles. In terms of hydrodynamics, an operational velocity of about 0.5 and 2.5 m/s is typically suggested. Due to the self-supporting structure, hollow fibres can be backwashed with permeate or chemical solutions.

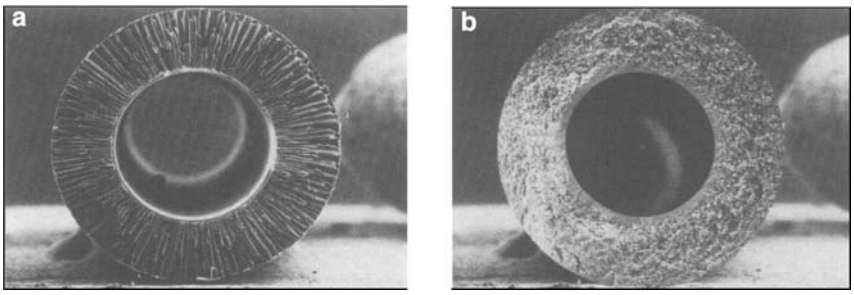


Fig. 10.7. Ultrastructure of the crosssection of two hollow fibres. Symmetric structure hollow fibre (**a**) and dense structure hollow fibre (**b**). (Adapted from Cheryan (1).)

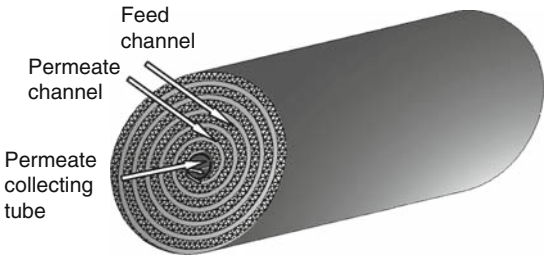


Fig. 10.8. Working principle of spiral wound membranes.

The working principle of spiral wound membranes is shown in Fig. 10.8. Two membranes are glued on three of four sides; the fourth side is linked to a pipe, which represents the axis around which the membranes are rolled up. In other words, a spiral wound module mainly consists of plastic bags with permeate spacers inside and actual filtering membrane outside. Such bags are then wound round a permeate collector and mutually separated mutually by the feed channel. The assembled module is then located within a stainless steel or PVC shell, the tips of which are connected to devices for feed and permeate pumping. The hydrodynamic condition is turbulent, and the velocity in the feed channel is between 0.1 and 0.6 m/s. The most significant advantages of spirals are the high specific surface (about $1,000\,m^2/m^3$) and the very easy manufacturing process. However, because of its own geometry, which results in a quite relevant propensity to foul easily, such configuration is adopted only for nanofiltration and reverse osmosis, and it is not used in membrane bioreactors.

2.3. Membrane Technologies

Usually, membrane processes are catalogued according to statistically determined pore sizes. In ultrafiltration, the discriminating factor is often expressed in terms of molecular weight cut off (MWCO), the unit of which is a Dalton (Da); MWCO refers to the weight cut-off of the solute which is retained in the percentage of 90%. Membrane processes can be

further classified according to the driving force that allows the permeate to pass through the membrane resulting in the following processes:

- Pressure driven
- Driven by a chemical potential difference
- Driven by an electromotive force

The natural mechanism during which a certain solute passes through a semipermeable membrane is called osmosis. This passage relies on the difference in the solute concentrations between the two sides of the membrane and thus in the chemical potential associated to the solute itself. Solvent passes from the lower concentration side to the higher one, balancing the solute concentration in both zones. On the other hand, the solute molecules are larger than the membrane pores, so they accumulate on one side. Chemical potential can be considered as a function of the molar fraction of the same solution; therefore, large particle sizes correspond to low molar fractions and low osmotic pressures. On the basis of above considerations, osmotic pressure is negligible when suspended particles, macromolecules or polymers are taken into account.

Figure 10.9 resumes the operational ranges of pressure driven membrane processes, as a function of separated particle size. Figure 10.10 shows the components, which can be separated by each of these technologies.

Microfiltration (MF) is used to separate inorganic particles, microorganisms, oils and colloids. The driving force is given by a pressure gradient applied between the feed and permeate sides; usually, pressure values lower than 1 bar are utilized. Because of both hydraulic and

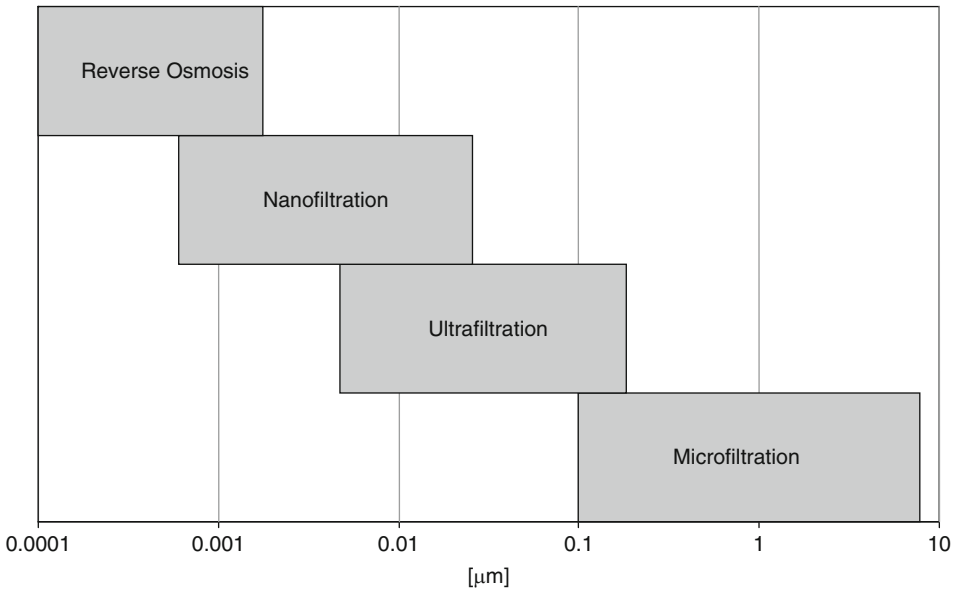


Fig. 10.9. Classification of membrane processes according to the size of the retentate components.

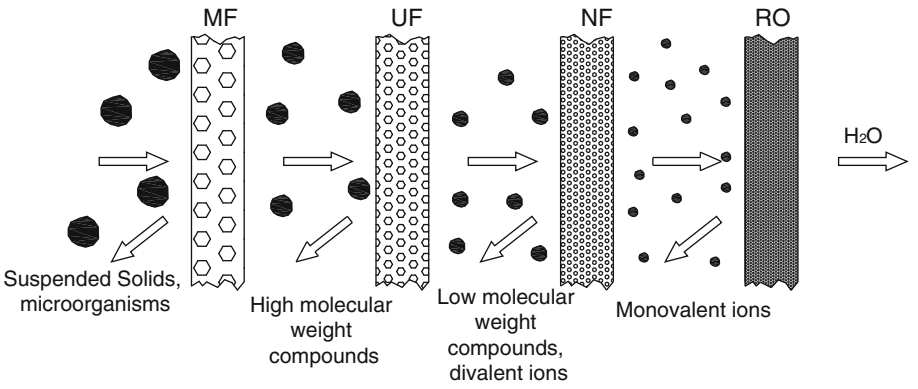


Fig. 10.10. Selectivity of pressure driven membrane processes.

structural reasons, pressure values higher than 0.7–0.8 bar should be avoided. In fact, with the fairly large pore size, too high pressure values initially result in an increase of the membrane productivity though a sudden drop in the flux is observed soon after as a consequence of some phenomena such as solute molecules building up at the liquid–membrane interface, pore clogging and superficial fouling. These mechanisms, also referred to as concentration polarization and membrane fouling, are discussed in depth in Sect. 2.5.

Ultrafiltration (UF) allows the separation of soluble macromolecules, removal of proteins, viruses and bacteria as well as both lipophilic and lipophobic polymers. The nominal pore size can vary between 20 and 200 Å (1 Å $= 10^{-10}$ m), which corresponds to a MWCO range between 0.5–1 and 100 kDa. The applied pressure is usually higher than 1 bar and lower than 10 bar; osmotic pressure is usually negligible although sometimes the extracted flux can diminish significantly, down to zero, when a very high osmotic pressure difference between feed and permeate is reached. Industrial applications of UF are widespread in both production cycle and deriving wastewater treatment. In Leiknes and Semmens, UF was applied to emulsified oil treatment (2); other typical application fields include protein and polymer concentration and chemicals reclamation. Sometimes UF is coupled with other technologies in order to obtain very high quality water. Water with a high content of humic acids has been successfully treated by means of UF. The experimental tests performed by Ødegaard et al. showed very high removal efficiencies and described UF as a possible alternative solution of conventional clarification processes (3).

Nanofiltration (NF) takes its place between ultrafiltration and reverse osmosis and typically features a 10^{-3} μm pore size and a 0.5 kDa MWCO. NF permits the removal of bivalent dissolved salts, endotoxins and other compounds as pesticides and herbicides. The separation mechanisms are not only mechanical, but also electrostatic, being that the membrane's surface is charged. Main applications of NF can be summarized as follows:

- Colour removal
- Disinfection
- Desalting of industrial wastewater with subsequent recovery of proteins and carbohydrates

- Softening
- Lignin removal
- Sugar concentration

Reverse osmosis (RO) is sometimes referred to as hyperfiltration. It separates solute species according to their different diffusion rates with respect to water. Therefore, RO ensures complete retaining action of all dissolved ions in the feed, including monovalent salts. Pore sizes range between 0.1×10^{-3} μm and 1×10^{-3} mm and the rejection coefficient (a parameter indicating the ability of the membrane to separate salts) is 99% for sodium chloride. The operational pressure can increase up to 70 bars, because of the very high difference of osmotic pressure between the two sides of the membrane which makes power consumption very higher than all the other membrane filtration processes. Usually, reverse osmosis is used for

- Sea water and brackish water desalination
- Final step of industrial wastewater treatment, with reuse of treated effluents
- Water production for electronic industry

2.4. *Factors Affecting Membrane Processes*

Performances of a given membrane filtration process are mainly influenced by the membrane itself, the feed suspension and the operational parameters inducing some mechanisms that, affect the membrane behaviour. For pressure driven processes, a basilar factor is represented by the TMP, which indicates the difference between the feed side pressure and the permeate side pressure, providing the actual driving force of the membrane process. TMP (Pa) is given by

$$\mathrm{TMP} = P_{\mathrm{feed}} - P_{\mathrm{permeate}} \tag{1}$$

In side-stream configuration, P_{feed} is determined as the mean value of inlet pressure and outlet pressure. Hence, it is

$$P_{\mathrm{feed}} = \frac{P_{\mathrm{in}} + P_{\mathrm{out}}}{2} \tag{2}$$

When submerged modules are used, P_{feed} is conventionally calculated as the hydraulic head at the mean depth:

$$P_{\mathrm{feed}} = \frac{\rho g h_1 + \rho g h_2}{2} \tag{3}$$

where ρ is feed density, kg/m^3; g is acceleration due to gravity, m/s^2; h_1 and h_2 are top and bottom module depths, respectively; m. P_{permeate} is measured on the extraction pipe line.

Permeate flux J (m^3/m^2/s) is the ratio between the permeate flow (Q; m^3/s) and the actual filtering surface area (A_{membrane}; m^2):

$$J = \frac{Q}{A_{\mathrm{membrane}}} \tag{4}$$

Commonly, J is expressed in L/m^2/h also indicated as LMH; sometimes it is given in terms of m^3/m^2/day. When periodical working conditions are adopted (i.e. relaxation or periodical

backwashes), a change in the net flux must be considered. Particularly, during relaxation mode the suction (i.e. permeate extraction) is stopped, making the applied hydrodynamic conditions capable of maximizing the mechanical cleaning. Therefore, the net flux is given by Eq. (5):

$$J_{\text{net}} = J_{\text{gross}} \frac{t_{\text{suction}}}{(t_{\text{suction}} + t_{\text{pause}})} \tag{5}$$

where J_{net} is net flux related to the whole process cycle, $\text{L/m}^2\text{/h}$; J_{gross} is gross flux measured during the extraction period, $\text{L/m}^2\text{/h}$; t_{suction} and t_{pause} are the durations of suction and pause periods respectively, s. If a permeate back-pulse strategy is implemented, the loss of permeate must be taken into account; the net flux is calculated according to Eq. (6):

$$J_{\text{net}} = J_{\text{gross}} \left\{ \frac{t_{\text{suction}} Q_{\text{suction}} - t_{\text{BP}} Q_{\text{BP}}}{(t_{\text{suction}} + t_{\text{BP}}) Q_{\text{suction}}} \right\} \tag{6}$$

where new terms are added to Eq. (5). In particular, Q_{suction} is permeate flow during suction phase, L/s; Q_{BP} is permeate back-pulse flow, L/s; t_{BP} is the duration of back-pulse phase, s.

The permeability is obtained by dividing the flux by the applied TMP. It is expressed as $\text{L/m}^2\text{/h/Pa}$ and is calculated by means of Eq. (7):

$$\text{Permeability} = \frac{J}{\text{TMP}} \tag{7}$$

Often permeability is indicated in $\text{L/m}^2\text{/h/bar}$.

Usually, both J and permeability are referred to a standard temperature in order to consider the influence of temperature on the permeate dynamic viscosity. Assuming the permeate as pure water (fairly acceptable for this case), Reid et al. (4) suggest the following expression to describe the temperature effect on the dynamic viscosity:

$$\eta_{\text{T}} = \text{e}^{\left(-24.71 + \frac{4{,}209}{T} + 4.527 \times 10^{-2} \cdot T + 3.4 \times 10^{-5} \cdot T^2\right)} \tag{8}$$

where η_{T} is the dynamic viscosity of permeate, Pa s; T is the temperature, K. Then, based on a reference temperature of 20°C, the permeate flux can be standardized with Eq. (9):

$$J_{20} = \frac{\eta_{\text{T}} J_{\text{T}}}{\eta_{20}} \tag{9}$$

Two major factors affecting filtration processes are concentration polarization and fouling. Concentration polarization (CP) is the term typically used to describe the solutes build-up on the membrane surface. Such a mechanism causes gradual formation of a concentration boundary layer near the membrane (the socalled "polarization layer") where the solute concentration is higher than in bulk; therefore, a concentration gradient tends to be created. At the same time, the hydrodynamic conditions enhance Brownian transport mechanisms, which promote a back-diffusive flow. Physically, this situation leads to an additional resistance to the filtration. Making reference to the Fig. 10.11, it is possible to notice the increasing trend of solute concentration nearby the membrane surface, from the bulk value (C_{B}; kg/m^3), up to a maximum value (C_{G}; kg/m^3) which remains constant in the gel layer.

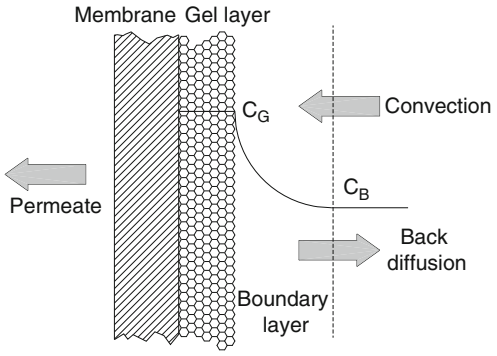


Fig. 10.11. Schematic representation of concentration polarization.

Polarization allows some barely soluble compound to precipitate on the membrane surface, which causes a gradual increase of both colloids and suspended particles near the membrane itself.

Fouling can be viewed as the main consequence of the mechanisms, which make the membrane dirty like pore clogging and pore blocking. The most important fouling causes in MBR applications are discussed in Sect. 3.2. Here, some generic factors involved in fouling are introduced and grouped according to the following categories:

- Membrane features
- Feed composition
- Operational conditions

2.4.1. Membrane Properties

The roughness of the surface is a major factor: uneven membranes are more subject to be fouled than homogeneous ones.

Hydrophilicity expresses a membrane propensity to attract water. A given material is defined as hydrophilic when it interacts with water and as hydrophobic when it is incompatible with water. Typically, the hydrophilic or hydrophobic behaviour of a material is assessed by measuring the contact angle of a water drop on the membrane surface (Fig. 10.12); the smaller the angle, the more hydrophilic is the membrane. Table 10.3 groups some materials according to their hydrophilicity degree.

When aqueous streams are filtered, the membrane should be hydrophilic. In fact, an hydrophobic material tends to adsorb hydrophobic compounds in the feed solution, so promoting fouling. However, in many cases hydrophobic membranes are covered with a thin layer of hydrophilic material in order to make optimum use of both the hydrophobic sturdiness and the hydrophilic low fouling propensity.

The membrane pore size and more specifically its relation with the particle size in the feed can also influence fouling. When large pore membranes are used, the pore blocking mechanism is enhanced. Therefore, higher values of permeability can be observed during the filtration start up, with a subsequent sudden decrease that leads to very low values under

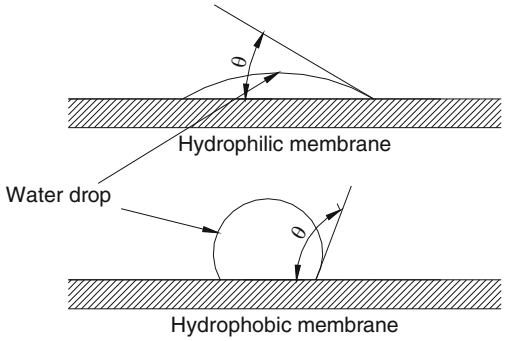


Fig. 10.12. Contact angle to assess the membrane hydrophilicity.

Table 10.3
Constituent materials classified according to hydrophobicity/hydrophilicity

Hydrophobic materials	Hydrophilic materials
Polytetrafluoroethylene	Cellulose esters
Polyvinyl difluoride	Polycarbonate
Polypropylene	Polyamide/polyetherimide
Polyethylene	Polyethertherketone
Polysulphone/polyethersulphone	

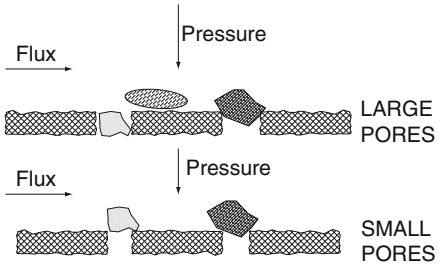


Fig. 10.13. Pore blocking effects on small pore membranes and large pore membranes.

steady-state conditions. On the contrary, if the pore size is small, the turbulence induced in crossflow filtration can easily remove particles on the membrane surface, resulting in a higher steady-state permeability (Fig. 10.13).

Electrically, many membranes have negative charges under normal conditions. The membrane charge is a key factor in fouling development when there are charged particles in the feed suspension. In wastewater treatment, the use of some chemicals that release positive ions (e.g. Al^{3+}) to achieve phosphorus precipitation can increase fouling tendency, because of the attraction between these positive ions and the membrane itself.

2.4.2. Feed Composition

Due to their typical charge density, proteins play a basilar role in membrane fouling. Such effect is further emphasized by their attitude to interact with other chemical species in the feed.

If salts are present in the suspension to be filtered, two phenomena occur: chemical precipitation also referred to as scaling and electrostatic attraction towards the membrane surface. pH also can influence fouling, primarily when proteins and salts are in the feed.

Preliminary removal treatments for oils and greases can improve membrane performances. For the same reason, also many silicone-based oily anti-foaming agents have a negative impact on the permeability. In any case, hydrophilic membranes are less influenced by oil than hydrophobic.

2.4.3. Operational Parameters

The temperature effect on the membrane behaviour is not yet completely clear, and it seems to be ambiguous. Under a certain TMP, an increase of temperature gives a higher flux as a consequence of the lower permeate dynamic viscosity. However, if there are salts present, the solubility of which decreases when the temperature rises, then higher temperature values can cause a decrease in permeability.

High shear rates induced by feed recirculation or feed-sided air pulsing remove deposited material resulting in the reduction of the hydraulic resistance associated to the fouling layer (see Sect. 3.2).

2.5. *Mathematical Models for Flux Prediction*

Under no polarization and negligible fouling, permeate flux can be expressed as a linear function of TMP, as shown in Eq. (10):

$$J = \frac{\varepsilon_{\mathrm{m}} d_{\mathrm{p}}^2 \mathrm{TMP}}{32 \Delta x \eta} \tag{10}$$

where, in addition to the already introduced symbols, d_{p} is the mean pore diameter, m; ε_{m} is porosity or fraction void volume in the membrane, dimensionless; Δx is membrane thickness, m. Equation (10) conceptually derives from the Hagen–Poiseuille law for laminar flow through channels, the channel being represented by the membrane pore. In order to apply Eq. (10), fluid passing through the membrane must be Newtonian and have constant density. Moreover, laminar flow has to be supposed, with steady-state conditions and negligible end-effects. Actually, the above expression is valid only when low TMP is applied and good turbulence conditions are assured constantly; otherwise, an asymptotic trend of J vs. TMP can be observed because of the concentration polarization effects (Fig. 10.14).

Under such conditions, a mass transfer model must be used to predict the flux. Therefore, considering the scheme in Fig. 10.11, two different solute flows must be taken into account: the convective flow from bulk towards membrane and the back-diffusive flow due to polarization.

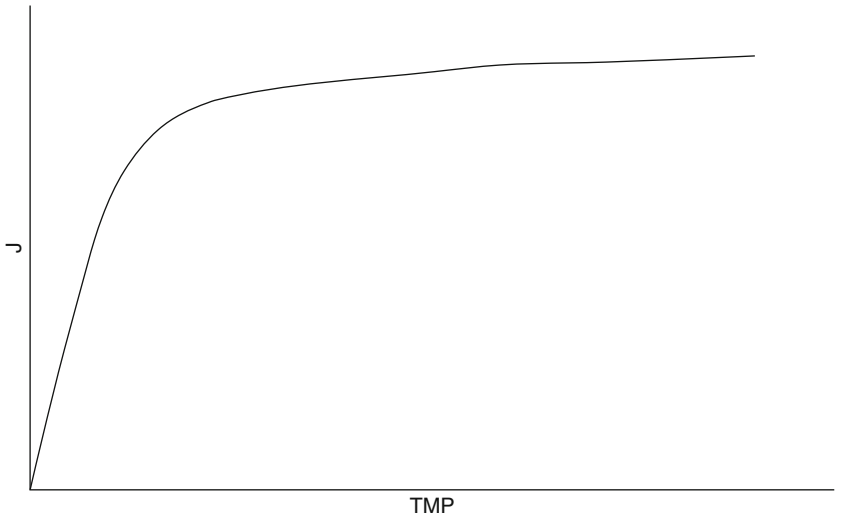


Fig. 10.14. Asymptotic trend of flux due to concentration polarization.

The convective flux J_{conv} of solute can be expressed according to Eq. (11):

$$J_{conv} = JC_B \tag{11}$$

The back-diffusive flow rate J_{bd} of solute can be obtained by

$$J_{bd} = D\frac{dC}{dx} \tag{12}$$

where D is the Brownian diffusion coefficient, m^2/s; dC/dx is the solute concentration gradient referred to a differential element in the boundary layer. Under steady-state conditions, these two terms are equal; thus, integration over the boundary layer gives

$$J = \frac{D}{\delta}\ln\frac{C_G}{C_B} \tag{13}$$

where δ is the concentration boundary thickness, m. The ratio D/δ represents the mass transfer coefficient and is indicated with letter k. Therefore, the permeate flux is given by

$$J = k\ \ln\frac{C_G}{C_B} \tag{14}$$

Several expressions of Eq. (14) are available in the literature. Most of them have been experimentally determined, but they cannot be applied in different contexts other than their own specific. An extended review of such models has been provided by Stephenson et al. (5).

Neither Eqs. (10) nor (14) is able to provide a complete description of J vs. TMP relationship. Hence, a simple model, known as the "resistance model," is generally assumed it

relies on the "resistance in series" concept, according to which the flux can be determined as a function of TMP with Eq. (15):

$$J = \frac{\text{TMP}}{\eta R_{\text{T}}} \tag{15}$$

where R_{T} is the overall resistance to filtration, 1/m. R_{T} can be calculated as the sum of all involved resistances by the following equation:

$$R_{\text{T}} = R_{\text{M}} + R_{\text{CP}} + R_{\text{F}} \tag{16}$$

In Eq. (16), R_{M} is the intrinsic membrane resistance, 1/m; R_{CP} and R_{F} are the resistances due to concentration polarization effects and fouling, respectively, both expressed in 1/m. The intrinsic membrane resistance physically represents the resistance offered by a clean membrane when pure water is filtered. According to Cheryan, once the pore size and the superficial porosity is known, the Hagen–Poiseuille expression for laminar flow in cylindrical channel can be applied (1), obtaining:

$$R_{\text{M}} = \frac{K_{\text{g}}(1-\varepsilon_{\text{m}})^2 S_{\text{m}}^2 \Delta x}{\varepsilon_{\text{m}}^3} \tag{17}$$

where K_{g} is a coefficient depending on the pore geometry, which equals 2 for cylindrical pores; ε_{m} is porosity or fraction void volume in the membrane, unitless; S_{m} is specific surface area/unit volume, 1/m; Δx is membrane thickness, m. Equation (17) is valid for membranes whose pores can be considered as cylindrical; in other words, it can only be applied directly to microporous isotropic membranes. However, it is possible to extend it also to different geometries, through modifications in K_{g} value. R_{CP} can be considered as a function of TMP, according to Eq. (18):

$$R_{\text{CP}} = \Phi \cdot \text{TMP} \tag{18}$$

where Φ is a coefficient depending on viscosity, shear rate and temperature.

Equation (15) is able to entirely describe the flux trend as a function of TMP. Under no polarization, the term R_{CP} can be neglected and a linear relationship is obtained; when the polarization effect becomes relevant, R_{CP} becomes predominant, and J can be calculated by

$$J = \frac{1}{\eta \cdot \Phi} \tag{19}$$

Under fixed conditions, J tends to be a constant value, so giving the asymptotic trend described in Fig. 10.14.

3. MEMBRANE BIOLOGICAL REACTORS FOR SOLID/LIQUID SEPARATION

3.1. Process Configurations

Depending on the mutual positions of the membrane and the biological process tank, two process configurations can be identified for the MBR. In the submerged membranes,

the membrane itself is completely immersed in the activated sludge, whereas in the side-stream configuration the filtration unit is located outside the main biological tank. Moreover, an external submerged configuration is possible, when the membrane is immersed in another tank, smaller than the biological process one (Fig. 10.15). Operationally, the most significant difference between submerged and side-stream schemes is a hydrodynamic difference. This

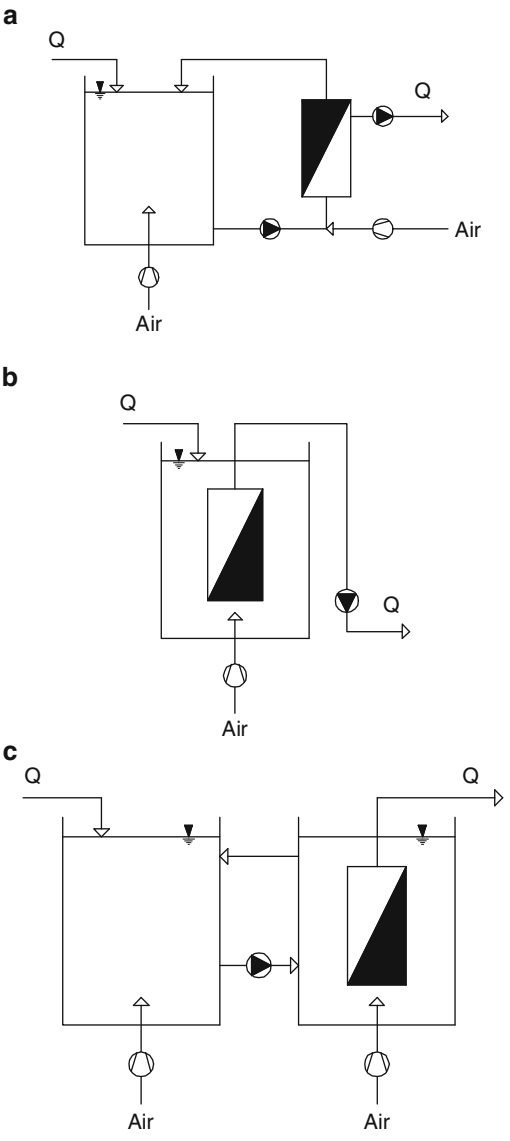


Fig. 10.15. Possible process configurations for membrane bioreactors: side-stream (**a**), submerged (**b**), external submerged (**c**).

can be seen as the way to perform the mechanical cleaning of the filtering surface. In both cases, a crossflow filtration is imposed; therefore, the feed flow is parallel to the membrane surface and perpendicular to the permeate flow through the membrane. However, while in side-stream configuration, the suitable hydrodynamic conditions are achieved by feed flow recirculation, in the submerged scheme coarse air bubbles are blown to obtain crossflow filtration. This difference in hydrodynamics brings with it relevant consequences in terms of power consumption costs. Therefore, the side-stream scheme is typically adopted for the industrial wastewater treatment (small and highly concentrated polluted flows) while submerged membranes are preferred for the municipal wastewater.

As in most membrane processes, MBRs can be operated under either constant flux or constant TMP. Usually, constant flux operation is preferred in order to easily handle influent hydraulic loading fluctuations.

3.2. Fouling in MBRs

Fouling represents a major limitation of membrane processes as well as MBR technology. In fact, because of cleaning operations, a filtration process needs to be stopped, thus reducing the net productivity of the membrane itself. Furthermore, frequent chemical backwashing can damage the constitutive material, therefore shortening the expected module life.

Fouling dynamics is clearly influenced by feed composition, hydrodynamic conditions, membrane material and module geometry. More generally, fouling can be defined as a consequence of all elements, which cause a drop in membrane performances. Some of the above-mentioned factors play a relevant role in MBR fouling, due to a very heterogeneous feed characterization. Moreover, a typical social approach in wastewater treatment is aimed to minimize all operational costs for treating "wastes." In MBRs, this means to reduce much of the power consumption to preserve optimal hydrodynamics, resulting in a higher fouling rate. This section aims to describe fouling causes and mechanisms in membrane bioreactors, as well as showing the most common strategies adopted to tackle the problem.

3.2.1. Impact Factors

3.2.1.1. Sludge Composition

Activated sludge is the conventional expression used to define a suspension in which microorganisms tend to aggregate and form flocs. Such flocs are involved in biological processes (aerobic, anaerobic and anoxic) where biodegradable pollutants are used as substrate by the microorganisms, resulting in the formation of new cells and simple compounds (organic and inorganic). The latter derives from more complex endogenous and exogenous compounds (catabolism products). Since its introduction at the beginning of the twentieth century, the activated sludge process has represented the most diffused alternative for the biological wastewater treatment. Actually, flocs feature an extremely heterogeneous structure, which makes fouling in MBRs more difficult to approach than in other membrane applications. Many studies have been carried out during the last 10 years in order to assess the fouling impact of each component of the biological suspension. However, the results obtained point out a strong discrepancy that can be interpreted as the consequence of differently configured experimental rigs and non-standardized pre-treatment techniques (Table 10.4).

Table 10.4
Impact on fouling by different activated sludge components

Fraction	Bouhabila et al. (6)	Defrance (7)	Wisniewski and Grasmick (8)
Suspended solids (%)	26	5	52
Colloids (%)	50	30	25
Dissolved macromolecules (%)	24	65	23

On the basis of above remarks, several factors have to be considered when biomass effect on membrane fouling is discussed, MLSS concentration being the most evident. Yamamoto and co-workers (9) observed a dramatic flux decrease in a submerged membrane plant when a solid concentration higher than 40 g/L was imposed. The direct effect of MLSS on fouling can be expressed in terms of cake resistance, as proposed by Chang et al. (10):

$$R_C = \alpha \cdot V^* \cdot \text{MLSS} \tag{20}$$

where R_C is the cake resistance due to the solids, 1/m; α is the specific cake resistance measured under a dead-end filtration test, m/kg; V^* is the permeate volume per unit area, m^3/m^2; MLSS is the suspended solids concentration in the bulk, kg/m^3. Magara and Itoh derived an experimental relationship between MLSS and J working on a constant TMP system (11):

$$J = -1.57 \log (\text{MLSS}) + 7.84 \tag{21}$$

with J as $m^3/m^2/day$ and MLSS as g/m^3. Other similar equations have been proposed to include the impact of dissolved organic carbon (DOC), turbulence, TMP and COD. Krauth and Staab, for example, proposed Eq. (22) for a side-stream MBR, considering both MLSS and turbulence, the latter represented by Reynolds number *Re* (12):

$$J = J_0 \, e^{\frac{c(\text{MLSS}-\text{VSS})Re}{\text{VSS}}} \tag{22}$$

where J_0 is the initial flux, $L/m^2/h$; VSS (volatile suspended solids in the mixed liquor) concentration, kg/m^3; c is a TMP-dependent empirical constant, dimensionless; *Re* is the Reynolds number. Other authors like Ishiguro et al. (13) obtained a relationship between flux and DOC:

$$J = A + B \log (\text{DOC}) \tag{23}$$

with A and B empirical parameters.

As well as influencing directly membrane performances, the MLSS concentration can also have an impact on permeability by varying sludge viscosity. For example, Ueda and Hata showed that an increase in MLSS induces higher viscosity, resulting in a lower permeability (14).

Particle size distribution is an additional biomass-associated factor in membrane fouling. For a given membrane, cake resistance has a quadratic dependence on particle size, as shown in Eq. (24):

$$R_C = \frac{180(1-\varepsilon)}{(\rho d_p^2 \varepsilon^3)} V^* \cdot \text{MLSS} \tag{24}$$

where ε is the cake porosity, dimensionless; ρ is the particle density, kg/m^3; d_p is the particle diameter, m. Equation (24) derives from Carman–Kozeny equation for filtration processes and according to it, the smaller the size, the higher the induced resistance. In MBRs, particle size is affected by operational conditions related to both hydrodynamics and biology. Shear stresses induced by pump recirculation as well as process temperature, pH and nutrient concentration can cause the floc break-up, resulting in a lower floc settling capacity and in a higher fouling propensity, as reported by Wisniewski and Grasmick (15). Shimizu and co-workers (16) evaluated the impact of particle size on the inertial lift, identifying a particle diameter range (8–15 μm) responsible in flux control level with which lower inertial lift velocity was observed. Particle shape affects the cake layer formation, since particles with a high circularity coefficient are able to form more compact layer than branched ones. In this sense, interesting results have been obtained by Connel et al. (17).

Bacteria yield some polymeric substances also referred to as extracellular polymeric substances (EPS) the production of which can be attributed to cell lysis, active secretion by microorganisms, adsorption from the environment and shedding of cell surface material (18). EPS represent the frame in which all floc components are embedded, and they can supply cells with protective barrier against toxic compounds, also providing an additional carbon source when lack of external carbon subsists. Most researchers – *inter alia* Zhang et al. (19) and Frølund et al. (20) – agree in defining EPS as the sum of different components as proteins, carbohydrates, DNA residual and humic substances. Typically, EPS measurement is based on a first extractive phase and then an analytical determination of all the extracted components. Up to now, no standardized protocol has been developed for EPS extraction, thus generating difficulties in comparing results. A comparative review of extraction methods has been published by Zhang et al. (19). Nagaoka and co-workers (21–24) have evaluated the impact of EPS on the membrane permeability in a submerged MBR; they developed a mechanistic mathematical model, which includes accumulation, consolidation and detachment of EPS on the membrane surface. Results obtained can be summarized as follows:

- Effects of EPS on sludge filterability mainly depends on an increase of the suspension viscosity
- Detachment rate from the membrane surface depends on the shear rate induced by air scouring rather than the density of polymeric compounds
- EPS production rate is affected by influent organic loading for total organic carbon (TOC), and such effect is mostly evident when daily load values lower than 2 kg TOC/m^3/day are applied
- Alternate aerobic–anoxic conditions permit EPS reduction through biological denitrification, thus ameliorating membrane permeability

Recently, Rosenberger and Kraume have compared the filterability of activated sludge sampled from several MBRs and one conventional activated sludge plant (25). The dewaterability has been evaluated considering the filterability index I_{40} (dimensionless) that corresponds to

the sludge permeate flux after 40 min related to clear water flux. All measurements have been carried out on a suitable test cell. According to the authors, suspended EPS (measured on the supernatant after sample centrifugation and washing) seems to affect filterability more than EPS attached to the microbiological flocs (determined after extraction with a cation-exchange resin). At the same time, longer sludge age (and consequently low food/microorganisms F/M ratio) promote suspended EPS removal, therefore improving the filterability. Cho and Fane (26) detected the role of EPS in fouling transients in MBR under the so-called "sub-critical flux" regime, which is described in detail afterwards in this section. The authors observed two different phases in the TMP trend, under flux values lower than a critical threshold were imposed. The initial and gradual TMP rise was ascribed to the EPS deposition with consequent distribution of local fluxes across the membrane surface. The subsequent sudden increase of TMP occurred when local fluxes exceeded the critical flux associated to dominant foulant (biomass).

Other microorganisms-derived substances, the so-called soluble microbial products (SMP), are moderate formula weight biodegradable compounds primarily springing from cell lysis and able to diffuse through cell membrane. SMP can be divided into two different fractions: utilization associated products (UAP), directly produced during substrate metabolism, and biomass associated products (BAP), derived from biomass. In Lapsidou and Rittman (18), a unified theory is proposed, in order to bridge "the EPS school of thought" and "the SMP school of thought"; such theory identifies soluble EPS as SMP, when substrate hydrolysis is negligible.

3.2.1.2. Module Geometry

Module shape plays a dual role in fouling dynamics, influencing both the pattern of foulants deposition and the effects of externally imposed hydrodynamic conditions. For hollow fibre membranes, the relevance of fibre orientation has been investigated by Chang and Fane and the flux distribution along the fibre was studied (27). A vertical fibre system allowed to achieve higher enhancements of flux with air bubbling than a horizontal one. In Equation (28), the same authors have modelled the filtration behaviour of submerged hollow fibres under two different conditions:

- Initial flux along the fibre lower than the critical flux
- Maximum initial flux along the fibre higher than the critical flux but, at the same time, average imposed flux smaller than the critical flux

In the former case, no particle deposition was observed and the flux distribution can be described by model developed for filtration of pure water. In the latter case, an initial deposition occurred, then a steady-state filtration was attained. According to the simulation results, the optimal fibre radius for fibre lengths from 0.5 to 3.0 m ranges between 0.2 and 0.35 mm.

Also, the channel diameter indirectly affects fouling by influencing aeration intensity when submerged tubular modules are used. Once the air flow rate is fixed, the smaller the lumen size, the greater the air flow rate per unit of cross-section area (i.e. the lower the fouling rate). Similar considerations can be done for flat sheet membranes were cartridges are assembled so that a feed channel is clearly identifiable.

3.2.1.3. Operational Conditions

The effect of the hydrodynamic conditions on fouling in MBRs is further discussed in Sect. 3.2.3. Here, the relevance of HRT and SRT are presented.

Visvanathan and co-workers (29) carried out experimental tests on a submerged hollow fibre module and observed that longer HRT can reduce fouling with respect to shorter ones, also as a consequence of the contextual decrease of MLSS concentration. Analogue indications have been provided by Dufresne et al. (30).

The impact of SRT on fouling is not clear. Under a fairly acceptable simplification, SRT can be defined as the average time that a microbial colony spends within the biological tank. Therefore, longer SRT values determine higher MLSS concentrations, which result in increased fouling as reported by Xing et al. (31). Similarly, a negative effect has been associated to higher viscosity when long sludge ages (i.e. high MLSS) are kept (32). Though, some researchers (Fan et al. (33), Chang et al. (34)) measured smaller EPS content under longer SRT and, on the whole, they noticed a reduced fouling rate for long SRTs.

3.2.2. Mechanisms

When microfiltration and ultrafiltration are coupled with biological process, like in MBRs, fouling develops through different and often simultaneous mechanisms. The term scaling is used to describe the precipitation of inorganic salt crystals on the membrane surface or in its pores. Such a phenomenon can reveal itself exclusively when the solubility index of a certain salt is exceeded, and it is emphasized by concentration polarization. Therefore, scaling is most commonly considered in nanofiltration and reverse osmosis. However, when an ultrafiltration module is used, some divalent salts can be rejected by the membrane, leading to partial scaling also in MBRs (mainly calcium and magnesium). Furthermore, if simultaneous phosphorus precipitation is operated, iron and aluminium salts can precipitate on the membrane surface.

Organic substances are usually adsorbed on the membrane surface because of the interactions between membrane material and sludge. This is particularly evident with new polymeric membranes, which are often derived from a combination of different polymers.

Pore blocking is a consequence of fine particles deposition on membrane pores, and it is influenced by particle size distribution and influent feed characteristics.

Biofouling is another typical fouling mechanism in MBRs, mainly due to the deposition of both bacteria and EPS on the membrane surface. The main negative effects of biofouling on membrane filtration processes can be summed up by the following points:

- Increase of hydraulic resistance
- Intensification of concentration polarization
- Reduction of the membrane life

As shown in Sect. 3.2.3, the lack of effective pre-treatment devices causes coarse matter build-up on the membrane surface, which can only be eliminated with manual inspections and cleaning or, if structural damages have occurred, with module substitution.

3.2.3. Control Strategies

3.2.3.1. Preliminary Treatment Facilities

Because of the high capital costs of membranes, an accurate evaluation of the pre-treatment devices should be carried out before utilizing MBR technology for both new installations and upgrading of existing plants. Suitable mechanical and chemical pre-treatment units allow to remove coarse debris, colloids, greases, oils and all other influent components directly involved in fouling. During the design phase, the choice of the preliminary treatment facilities is strongly influenced by the process scheme and the chosen membrane typology. Contextually, the characteristics of the pre-treatment units like screen mesh size, working principle, maintenance required, solid-waste production and disposal, power consumption are basilar.

When the MBR is supposed to be fed with raw sewage, a previous micro-screen (0.25–2.00 mm) should be installed on the feed-stream. If a primary settling tank is already available (i.e. when an upgrading through MBR is foreseen), a basket filter can be sufficient to protect the membrane, because the coarse solid matter has likely been removed in the former stage. An in-depth study has been developed by STOWA (35); the research compared various micro-screening installations on the feed-stream of four different membrane bioreactors, and the related removal efficiencies are shown in Table 10.5.

A major aspect in choosing a micro-screen is certainly represented by its working principle. For example, in some rotating drum screens, the development of a biofilm can lead to considerable COD and suspended solids (SS) removal efficiencies, therefore resulting in a lower concentration of biodegradable carbon for possible down-stream denitrification process.

The smaller the mesh size, the larger the power consumption. This can be explained by high energy demand for moving the screens and also by the significant head losses along the whole treatment scheme.

The solid-waste disposal should also be considered. Depending on its putrescibility and water content, "sludge" deriving from micro-screens can be merged with solids from coarse grits or sent to either digestion or thermal treatment.

3.2.3.2. Optimization of Hydrodynamics

As previously mentioned, keeping a fair turbulence regime near the membrane surface is the most common approach used to reduce both polarization and fouling. Such optimal conditions

Table 10.5
Comparative evaluation of removal efficiencies for different pre-treatment units (Adapted from STOWA (35))

	Wedge-wire screen	Vibrating static screen	Drum-filter	Rotating brush raked screen	Pre-sedimentation
Mesh size (mm)	0.25	0.75	0.5	0.75	–
COD (%)	13	12	29	9	39
SS (%)	28	44	63	20	69
Greases (%)	28	42	58	24	37
Mineral oils (%)	76	68	49	56	36

can be achieved, by means of recirculation pumps (side-stream configuration) and feed sided air pulsing (submerged configuration). In the former case, crossflow velocities up to 5–7 m/s can be achieved whereas in the latter, the superficial velocity ranges usually between 0.1 and 1 m/s when a specific air flow rate between 0.2 and 1.5 $Nm^3_{air}/m^2_{membrane}/h$ is supplied. Thomas et al. (36) identified an optimal crossflow velocity of 3 m/s for PVDF tubular membrane operating according a side-stream scheme. When this value was imposed, the minimum fouling rate was observed and the authors attributed this behaviour to an increased shear-induced back-diffusion. The increase in resistance above 3 m/s was considered to be brought about by a more compact fouling layer and increased pore blocking caused by the higher pressure deriving from the higher feed flow rate. Sometimes, feed recirculation is coupled with air bubbles, in order to optimize the energy costs. Ghosh and Cui (37) and Cui et al. (38) modelled the wall shear variation and distribution around a bubble injected in a tubular channel as slug flow and evaluated the consequences in terms of mass transfer coefficient. These results have been experimentally confirmed by Chang and Judd (39).

For immersed-membrane systems, Kishino et al. (40) applied the model originally proposed by Chisti et al. (41) to assess the velocity of a gas–liquid flow induced by air blowing in a column of liquid. When the system geometry can be classified as an airlift reactor (i.e. a column of liquid divided into a gas-sparged riser and an unaerated downcomer), the superficial liquid velocity in the riser (U_{Lr}; m/s) can be determined according to the following equation:

$$U_{Lr} = \left(\frac{2gh_D(\varepsilon_r - \varepsilon_d)}{\frac{K_T}{(1-\varepsilon_r)^2} + K_B \left(\frac{A_r}{A_d}\right)^2 \frac{1}{(1-\varepsilon_d)^2}} \right)^{0.5} \tag{25}$$

where g is the gravitational acceleration, m/s^2; h_D is the gas–liquid dispersion height, m; A_r and A_d are the cross-sectional areas of the riser and the downcomer, respectively, m^2; ε_r and ε_d are the frictional gas-holdup values in the riser and the downcomer, dimensionless; K_T and K_B are the frictional loss coefficients for the top and the bottom regions, dimensionless. The authors successfully compared calculated results with those experimentally determined with an electrokinetograph, pointing out the relevant role of the gas–liquid dispersion height in terms of obtainable crossflow velocities under a given air flow rate.

Ueda and co-workers (42) studied the effects of aeration on TMP in a large pilot scale MBR equipped with horizontally oriented hollow fibre submerged membranes. They found that the cake removing efficiency was improved by either augmenting the airflow rate or increasing the aeration intensity (expressed as the air flow rate per unit footprint area) by concentrating membrane modules over a smaller floor area. In (43), the authors determined a critical crossflow velocity (0.3 m/s) on a hollow fibre pilot scale module, below the critical value, a sudden and constant TMP increase was noticed. An exhaustive review about the use of gas bubbling to enhance membrane processes has been published by Cui et al. (44).

3.2.3.3. Optimization of Suction

A good suction regime can significantly improve the membrane performances. Discontinuing the permeate extraction, enhances the mechanical cleaning effect of turbulence induced by air blowing or feed recirculation. A major limitation in relaxation operation is certainly represented by the larger membrane surface required, once the influent flow and permeate flux are fixed. Periodical permeate backwashing can also ameliorate the behaviour of the membrane, reducing the gradual permeability loss due to pore blocking. The applicability of backwashes mainly depends on membrane structure and geometry.

3.2.3.4. Chemical Cleaning

Utilization of chemicals for cleaning membranes is the most common method to tackle fouling. Indeed, chemical oxidizing agents or some acids are able to remove both organic and inorganic compounds in pores. Typology of chemical, duration, frequency and concentration to use are strongly dependent on:

- Membrane features (geometry, structure, constitutive material)
- Foulants to be removed
- Required effluent quality
- Cleaning by-products disposal

Basically, chemical washing can restore quasi-virgin membrane performance, but it cannot be considered as a panacea for all fouling typologies. Particularly, when the biofouling is the main problem, using chemical reagents only causes bacterial inactivation, therefore leaving "dead biomass" resistance where there was a "live biomass" resistance.

Membrane manufacturers provide detailed information about the chemical cleaning procedure to adopt. However, it is necessary to assess the operation frequency according to the specific characteristics of the considered system, as influent composition, SRT, EPS and SMP production. A careful optimization of chemical cleanings allows to preserve membrane characteristics, as well as extending the modules life. Usually, organic foulants are removed by means of sodium hypochlorite (NaClO); the concentration is dependent on the membrane chosen. Some technologies (for example, Kubota, see Sect. 3.3.1) need rare cleaning operations with high concentration of NaClO (up to $5,000\,g/m^3$ as active chlorine); inorganic matter is removed with highly concentrated oxalic acid solution. If the membrane is directly immersed in the main biological tank, it may be better to have a separate tank for the chemical cleaning, in order to avoid microbial inhibition due to high chemicals concentrations. In other cases (for instance, GE Zenon and Siemens Memcor see Sects. 3.3.2 and 3.3.3), the chemical cleaning procedure is more frequent, and it relies on low concentrations (100–$200\,g/m^3$) of oxidants and/or acids.

3.2.4. Critical Flux Concept

Such concept was introduced in the mid 1990s by Field et al. (45) and Howell (46), and it is based on the hypothesis formulated by Field et al. (45) which states that on start-up of the filtration, there exists a flux below which a decline of flux with time does not occur. This flux is defined as critical flux, and it depends on the hydrodynamics and other variables. In its strongest form, this statement affirms that it is possible to determine a permeate flux, the

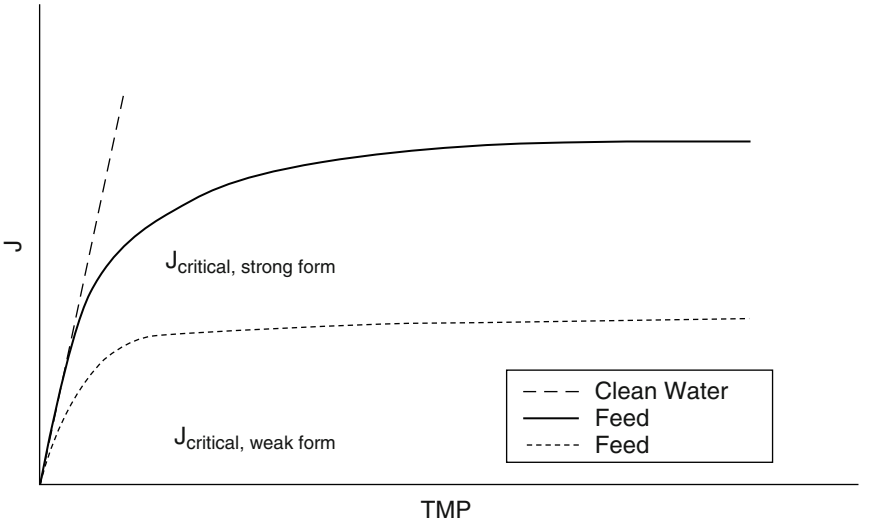


Fig. 10.16. Strong form and weak form of critical flux.

so-called critical flux J_{crit}, for which the fouling rate (expressed as dTMP/dt) is zero. At the same time, the critical flux hypothesis implies that for fluxes lower than J_{crit} the TMP vs. flux relationship is the same for clean water filtration. In reality, as proposed by Wu et al. (47), there is also a weak form of the critical flux concept according to which the critical flux corresponds to the point where the linear trend of J vs. TMP breaks off. This linearity is different than that obtainable during pure water filtration because of the initial adsorption and pore blocking phenomena (see Fig. 10.16). Similar observations have been reported for a bench scale hollow fibre MBR by Bouhabila et al. (48) who defined a "secondary critical flux" corresponding to the flux value at which the linear relationship J–TMP breaks off.

Several techniques are available for the experimental assessment of the critical flux; the simplest is based on the flux-stepping method. This method has been widely applied at both laboratory and large pilot scale, and it is based on imposing progressively increasing fluxes and monitoring TMP at the same time. The critical flux is therefore defined as the flux at which TMP starts to increase instead of remaining constant over time; an example of the stepwise approach is shown in Fig. 10.17.

The direct observation through microscope (DOTM) technique has been proposed by Li et al. (49) and developed at the UNESCO Centre for Membrane Science and Technology – University of New South Wales (Australia). It is based on a visual determination of the critical flux, obtainable thanks to a specific membrane which becomes transparent when wet. Critical flux J_{crit} is then defined as the flux at which particles start to deposit on the membrane surface.

Another method used for evaluating the critical flux consists in carrying out a particle mass balance. In this case, the critical flux is expressed as the flux value at which the particle concentration in the flowing fluid does not change. Basically, such method relies on the calculation of the deposition rate against flux; then the critical flux is back extrapolated to

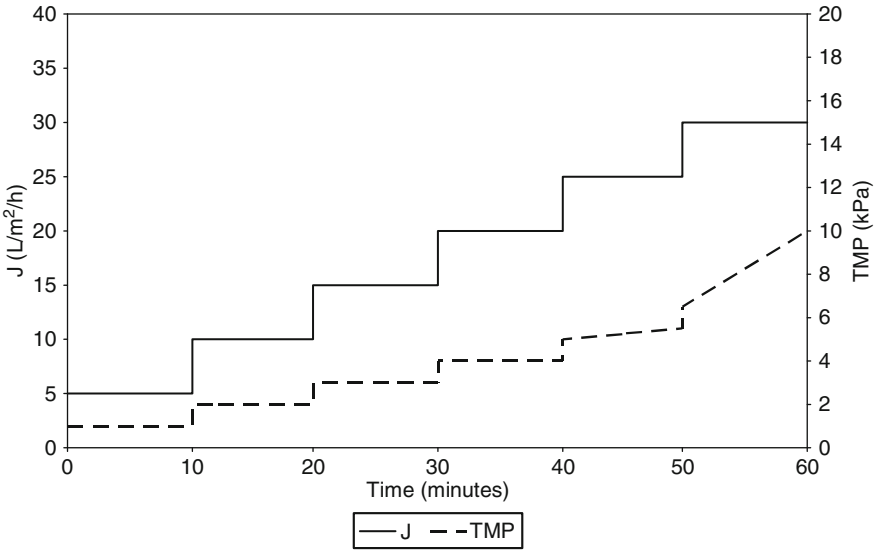


Fig. 10.17. Critical flux determination by flex-stepping method.

the point of zero deposition. Kwon and Vigneswaran showed the dependency of J_{crit} on the particle size; the smaller the particle diameter the lower J_{crit} (50).

Many studies on critical flux have been carried out on bench experimental rigs, fed with "ideal" suspensions with well-known particle size distribution (45–48) suggested that in complex matrixes there are different critical fluxes, each one associated to the components in the suspension and the actual critical flux is the lowest. For MBRs, the high heterogeneity of the suspension plays a relevant role in critical flux assessment. Many stepwise assessments have shown the inexistency of the pure critical flux (51, 52), pointing out a constant TMP increase even at very low fluxes. All the experimental determinations of J_{crit} for MBRs show a strong dependency on both influent composition and size of the plant and, as most of them have been performed on lab-scale rigs fed with synthetic sewage, their results cannot be directly applied to full scale plant operation. Due to the above-mentioned reasons, for membrane bioreactor it seems more reasonable to talk about a sustainable flux, intended as a repeatable flux pattern in a cycle of operating and cleaning such that there is no residual fouling. This concept strictly correlates the fouling control strategies to the operational costs aiming to assess the optimal flux capable of minimizing chemical cleaning and, therefore, to enhance the membrane lifetime. In this sense, for every full scale MBR plant, it would be desirable to install a separated module with its own suction pump, flow-meter and pressure transducer; this would allow to carry out some periodical stepwise tests and to check the degree of fouling for all installed modules.

3.3. Commercial Membrane

The objective of this section is to provide information about some of the most important membrane modules used in MBR applications.

3.3.1. Kubota

Kubota Corporation (Osaka, Japan) developed a polymeric (chloride polyethylene) plate and frame membrane with flat sheets packaged in modules. Each module consists of two parts which are laid one on top of the other. The top part is also referred to as membrane case and where between 50 and 400 flat sheet cartridges are located, according to the size of the plant. The lower part is called the diffuser case and contains a coarse air bubbles diffuser. Its functions is mainly to offer mechanical support to the top section and to channel the air flow and the activated sludge between the membrane panels. At earlier versions, both lower and top sections were built in a glass fibre reinforced plastic; the new modules are made in stainless steel. Following modules are available:

- FS 50 and FS 75 (water depth of 2.0–2.5 m)
- ES 50, ES 75, ES 100, ES 125, ES 150, ES 200 (water depth of 2.5–3.0 m)
- AS 100, AS 150 (water depth of 3.0–4.0 m)
- EK 300, EK 400 (water depth higher than 4.0 m)

The name of each module consists of two letters (which define the operational hydraulic head) and a number, which indicates the number of panels included in the module. An "out-to-in" filtration is achieved; each membrane panel has an effective filtering surface of 0.8 m^2; the nominal pore size is 0.4 μm. A 6 mm wide channel between every two cartridges allows the activated sludge to flow. The Kubota modules can be assembled according to a single deck or a double deck scheme; the latter configuration (Fig. 10.18) allows to minimize the required footprint also optimizing the energy consumption due to air blowing.

Figure 10.19 shows a Kubota process schematic for carbon and nitrogen removal (denitrification and nitrification) with modules located in a suitable separate tank. MLSS concentration up to 20–25 g/L can be applied, and the specific air flow rate for membranes cleaning usually ranges from 0.6 to 0.9 m^3/cartridge. Suction can be operated continuously, but sometimes the introduction of relaxation phases can improve the performance of membranes. The maximum flux at 20°C varies between 33 and 42 $L/m^2/h$, depending on the application; the operational flux is usually about 20–25 $L/m^2/h$, and the TMP should never be higher than 40 kPa. The required pre-treatment screen is 3 mm but, of course, narrower mesh can improve the membrane permeability.

Usually, maintenance operations are due to cleaning of coarse bubble air diffusers and chemical washing of the membranes. The former operation is very simple and completely automized. A valve at the end of the air pipe opens periodically allowing the sludge deposited in the air pipe to be removed by the air jet. Chemical cleaning of sheets is aimed to remove both organic and inorganic foulants; it is operated in situ. Aeration is switched off, and then a volume of 3 L/cartridge fed along the suction line, with a flow rate of 600–700 L/h. In this way, the membranes break off their own support, and they lean one against another, therefore reducing the leakage of the chemical solution through the filtering surface and resulting in

Fig. 10.18. Kubota double deck module.

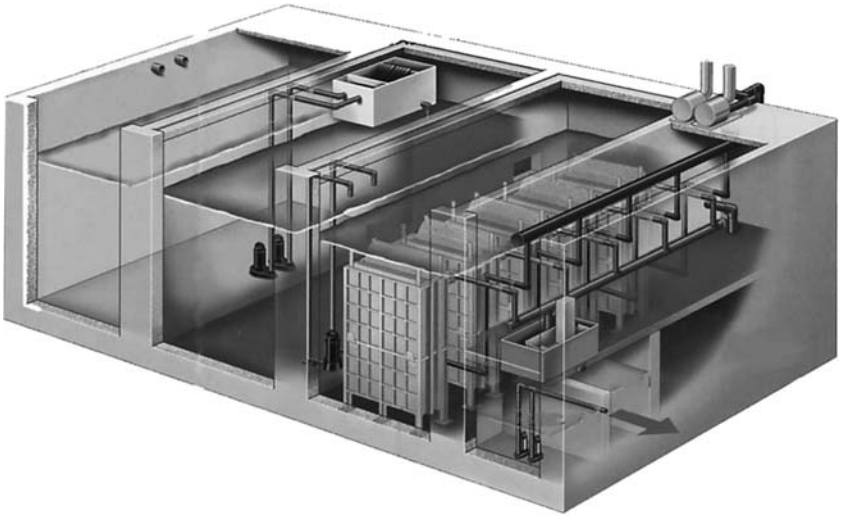


Fig. 10.19. Kubota flow scheme for nitrogen removal.

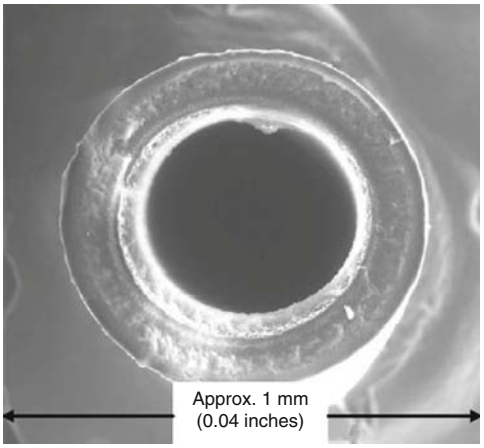


Fig. 10.20. Cross-section of Zenon hollow fibre membrane.

a complete soak. The overall duration of this phase is 2 or 3 h; then the suction is started, returning the early permeate back to the preliminary treatment units. The aeration is started a few seconds after and, finally, the extracted permeate is directed to the finale discharge. At first, a $5,000\,g/m^3$ sodium chloride solution is backwashed through the membrane (organic fouling removal). Then, if necessary, a $10,000\,g/m^3$ oxalic acid solution is slightly backwashed through the membrane (inorganic fouling removal). The operation frequency is about three to six times a year for the former and once a year for the latter. Air diffusers cleaning is completely automized and is executed once a week.

3.3.2. General Electric Zenon

Since 1991, Zenon Environmental Inc. (Oakville, ON, Canada) has continuously developed its immersed membrane called ZeeWeed®, which can be placed in the main biological tank or in a specific one. Zenon ZeeWeed® ZW 500 c is a PVDF asymmetric reinforced hollow fibre membrane (0.75 mm internal diameter). A special phase of the manufacturing process confers hydrophilic behaviour to the polymer (Fig. 10.20). The nominal pore size is 0.04 μm; modules are packaged in PVC-stainless steel cassettes; each module has an actual filtering surface of $23.3\,m^2$ and cassettes can include 10, 22 or 32 modules. An "out-to-in" filtration takes place under a gentle suction and the permeate extracted from each cassette is collected in a manifold linked to the pump.

The module ZeeWeed® ZW 500 d has been launched in 2002. It works exactly like ZW 500 c but the permeate is extracted from both top and bottom of the module. The module surface is $31.6\,m^2$, with 48 or 64 modules per cassette; Fig. 10.21 shows a Zenon ZW 500d (overall height 2,536 mm; overall width 1,745 mm, overall depth 2,112 mm) with 64 filtering units. Zenon membranes operate according to a relaxation scheme with a flux ranging between 20 and $30\,L/m^2/h$; maximum hourly flux is about $45–50\,L/m^2/h$) depending on hydrodynamics and feed characteristics. The maximum TMP value is about 70 kPa, but most commonly, the transmembrane pressures vary between 7 and 50 kPa. Typical pre-treatment

Fig. 10.21. Zenon cassette ZW 500 d®-64.

screen is 1 mm or less. Mechanical cleaning is achieved by means of coarse air bubbles supplied according to a pulsing operation (10 s on and 10 s off); the specific air flow rate is in the range 0.2–1 $Nm^3_{air}/m^2_{membrane}/h$, as a function of the suctioned permeate flux. Although some works have been published (53, 54) where MLSS concentration values up to 20 kg/m^3 have been tested, nowadays the solids concentration is rarely higher than 13 kg/m^3. Common values are between 8 and 11 kg/m^3. The reinforced structure allows the Zenon module to be backwashed with both permeate and chemical solutions where frequency of back-pulse cleaning is strongly dependent on the process considered. Typical backwash operations alternate 30 s at 20 $L/m^2/h$ of backwashing with 120 s of pause. This cycle is repeated for about ten times. Chemicals are chosen according to foulants to be removed. Often, sodium chloride is used (200–300 g/m^3 in terms of active chlorine) but potassium permanganate, chlorine dioxide and chloramines can be utilized.

3.3.3. Siemens Water Technologies - Memcor

Memcor Limited is a Siemens Water Technologies company. In 2003, Memcor Ltd. launched its MBR module, which is a PVDF hollow fibre membrane for microfiltration (nominal pore size 0.1 μm). Each single module has an actual filtering surface of 37.6 m^2 and more modules can be assembled as in Fig. 10.22; the membranes are then immersed in a tank outside the biological reactor ("out-to-in" filtration). Typically, a suction phase (720 s) and a relaxation phase (60 s) alternate are used and a backwash can also be operated, depending on the specific case. Standard service flux at 20°C ranges from 25 to 30 $L/m^2/h$,

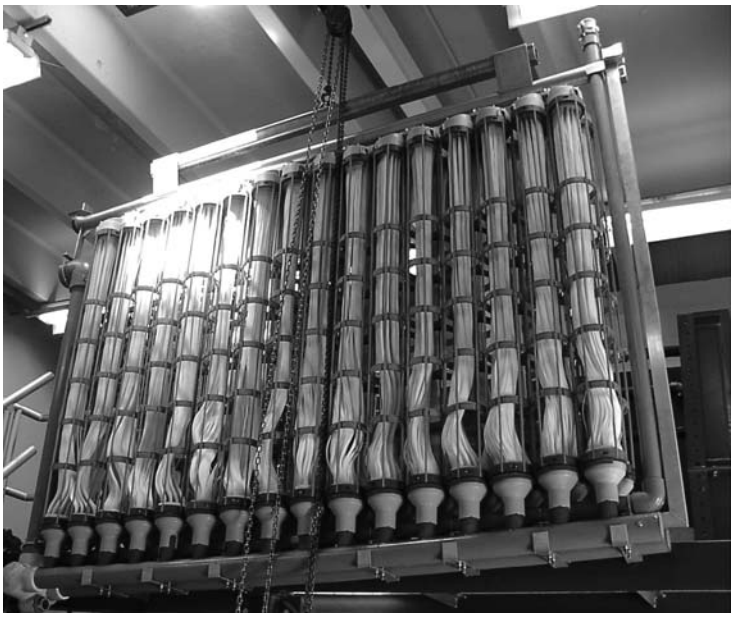


Fig. 10.22. US-Filter Memcor membranes.

with a maximum of 35–40 $L/m^2/h$. The MLSS concentration in the biological tank has to be lower than 12 kg/m^3, which means about 14–15 kg/m^3 in the membrane tank due to the concentrating effect exercised by the membrane. A 2 mm pre-screen should be placed on the influent feed stream.

A jet mixing device assures suitable hydrodynamic conditions. The mixed liquor is introduced into the membrane tank, near the bottom of a cluster of membranes. At the same location, a venturi is used to increase the velocity of the mixed liquor. Simultaneously, low pressure air is introduced into the mixed liquor and distributed within the membrane bundle. Usually, the mixed liquor recirculation flow is about five to six times the permeate flow. In a complete nitrogen removal scheme, the outlet of the membrane tank can be guided back to the pre-denitrification tank, in order to achieve the nitrate reduction to gaseous bi-atomic nitrogen. The specific low pressure air flow rate is 0.2 N $m^3_{air}/m^2_{membrane}/h$.

A chemical maintenance cleaning is performed weekly; sodium hypochlorite is used, 100 g/m^3. Every 4–6 months a Clean in Place (CIP) operation is recommended, to remove compounds built up in the pores.

3.3.4. X-Flow

X-Flow MBR process is based on a side-stream configuration. Tubular PVDF membranes with a nominal pore size of 0.03 μm are located externally and the bioreactor is fed with mixed liquor. The most applied module is called Compact 38 GRH and two different versions are available, depending on the internal diameter of each tube (5.2 or 8 mm). Tubes are fit in a reinforced glass fibre 3 m long housing, whose diameter is 0.22 m. Each module

Fig. 10.23. X-Flow module for MBR applications.

offers an actual filtering surface of 27 or 33 m^2, according to the internal diameter of the fibre. The activated sludge flows through each tube, resulting in a "in-to-out" filtration. This crossflow MBR system is used for industrial wastewater treatment, leachate treatment and municipal wastewater applications have also been installed. Maximum flux at 20°C is about 70 $L/m^2/h$, but more commonly, a 20–30 $L/m^2/h$ is imposed. The module can be used for MLSS concentration up to 25 kg/m^3. The maximum TMP applicable is about 500 kPa and the anti-fouling strategy can also involve an additional air supply of 0.2–0.5 N $m^3_{air}/m^2_{membrane}/h$, either pulsing or continuously. Chemical cleanings are carried out with NaClO, H_2O_2 or acids, depending on foulants to be removed (Fig. 10.23).

3.3.5. Mitsubishi

Mitsubishi Rayon Co. Ltd. (Tokyo, Japan) produces a polyethylene hollow fibre membrane called Sterapore™ Sur having a 540 μm outer diameter and a nominal pore size of 0.4 μm. Following units are available:

- SUR 5334 (53 m^2), whose dimensions are ($D \times W \times H$, in mm): $670 \times 832 \times 2,250$
- SUR 10534 (105 m^2), $670 \times 1,357 \times 2,250$
- SUR 21034 (210 m^2), $670 \times 1,357 \times 3,420$
- SUR 31534 (315 m^2), $670 \times 1,357 \times 4,380$

Unlike Zenon, the fibres are horizontally oriented (Fig. 10.24); however, similar to Zenon, Memcor and Kubota an "out-to-in" filtration takes place. Each module offers a 1.5 m^2 filtering surface and more modules can be put one on top of another to form a filtration unit. Usually, a relaxation process is adopted, alternating a 900 s suction phase and a 120 s pause period. The average permeate flux at 20°C is about 10–12 $L/m^2/h$, and it rarely exceeds 15 $L/m^2/h$. The maximum TMP is 80 kPa.

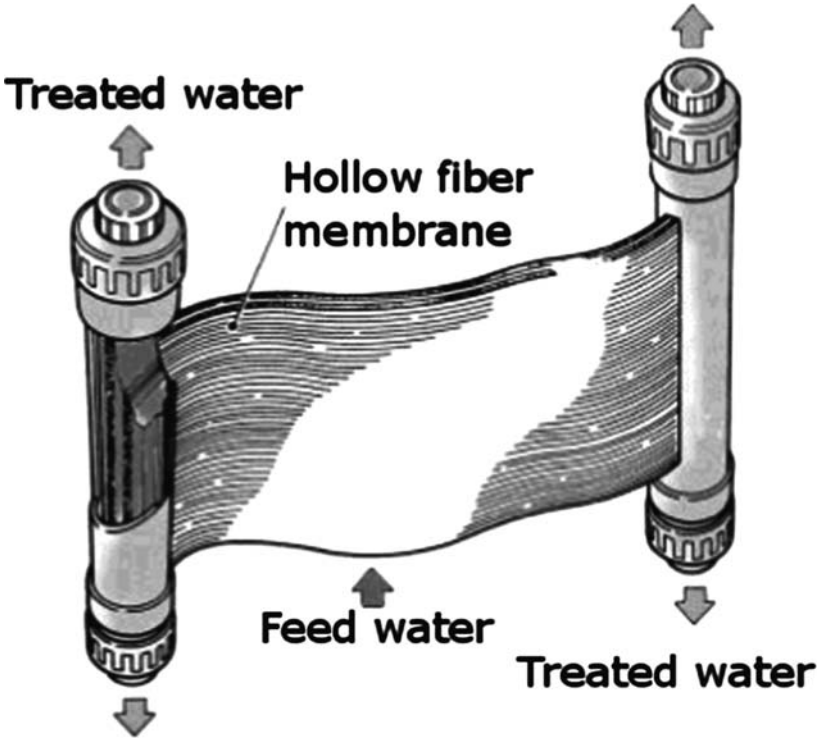


Fig. 10.24. Working principle of Mitsubishi Rayon Sterapore ™ membrane.

Such as other immersed membrane processes, Sterapore™ SUR membrane is mechanically cleaned by means of coarse air bubbles with a specific air flow rate of 0.2–0.5 $N\,m^3_{air}/m^2_{membrane}/h$. Two chemical cleaning procedures can also be adopted. The in situ protocol is operated every 4–6 months with sodium hypochlorite ($3,000\,g/m^3$) in a volume of about $2\,L/m^2_{membrane}$; the holding time is 2–3 h. The ex situ cleaning uses NaClO ($3,000\,g/m^3$) or a mixture of sodium hypochlorite and NaOH ($5,000\,g/m^3 + 4\%$); the volume of reagent has to be sufficient to immerse the unit and the holding time is about 15 h.

3.3.6. Huber

The German company Hans Huber AG (Berching, Germany) commercializes its submerged MBR named HUBER VRM® process consisting of a polymeric (polyacrilonitrile (PAN) or polyethersulphone (PES)) plate and frame ultrafiltration (3.7×10^{-2} μm, 150 kDa) system immersed in the activated sludge tank. The smallest filtering unit is the plate whose surface is $0.75\,m^2$. Four membrane plates form a module (Fig. 10.25) and six or eight modules circularly arranged around a rotating shaft form an element; a variable number of elements can be fitted on the same shaft to generate a unit. The smallest unit (VRM® 20/60, 60 modules circularly arranged with four per each element) has a total membrane surface of $180\,m^2$. The largest unit (VRM® 30/480, 480 modules circularly arranged with six per element) is equivalent to a total area of $2,880\,m^2$.

Fig. 10.25. Flat sheet module of Huber VRM®.

A schematic description of the process is shown in Fig. 10.26. An "out-to-in" filtration takes place, and the permeate suctioned by each module is suctioned by a pipe; sludge concentration ranges between 12 and 16 g/L of MLSS. The maximum flux is about 27 $L/m^2/h$ with a TMP typically lower than 20 kPa. The principle of membrane cleaning consists in the generation of directed air and sludge flows, using at the same time the radial acceleration along the rotating membranes. The specific air flow rate is about 0.25–0.35 $N\,m^3_{air}/m^2_{membrane}/h$. Soaking and slight backwashing can be operated depending on the membrane fouling conditions.

4. DESIGN OF THE BIOLOGICAL TANK FOR COD AND NITROGEN REMOVAL

4.1. Introduction

Typical approaches to designing wastewater treatment plants include calculations related to both short-term and long-term fluctuations of flowrates and mass loadings. A set of influent flows, which must be based on accurate measurements of present sources and on forecasting of future flows for residential, commercial, industrial contributions has to be determined. Once the actual equivalent population to be served is known, different flow values can be determined for different goals. For example, the average daily flow allows estimating the energy consumption costs, but the maximum daily flow is needed to size equalization basins or sludge pumping systems. Similarly, the peak hourly flowrate is necessary to choose suitable mechanical treatment units (grit chambers, sedimentation tank, filters) while the minimum hourly and the minimum daily flowrates are required to select flowmeters and influent channel cross section to avoid solid deposition. All these flowrate values are often assessed according to empirical equations, which consider per capita water supply/consumption and statistical determinations of population increase. Sometimes, the flowrates are dependent on local

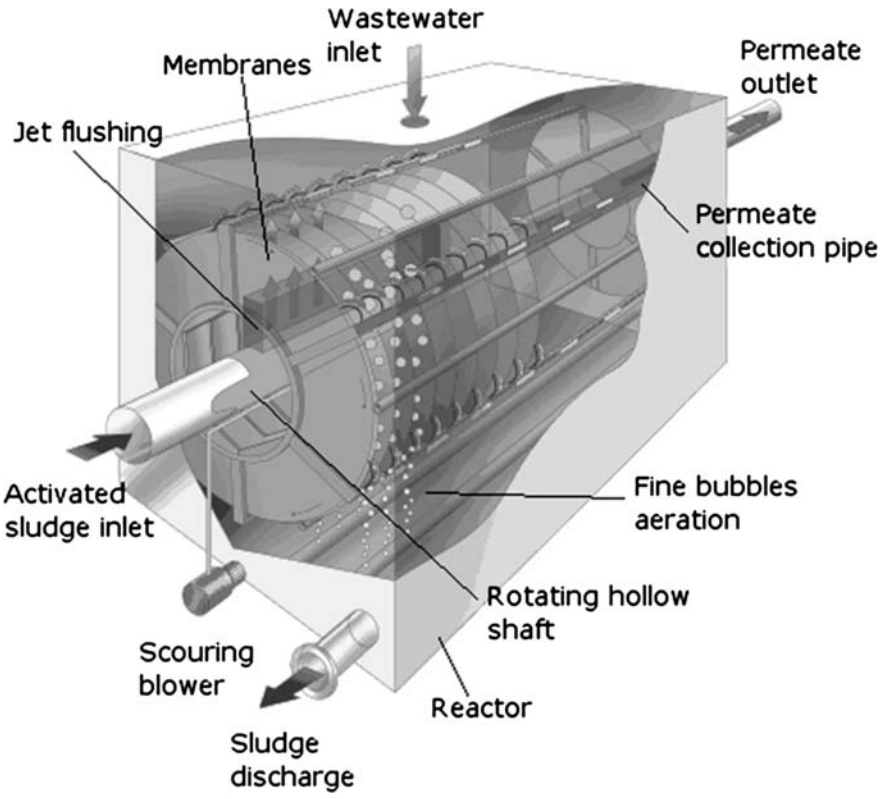


Fig. 10.26. Schematic of Huber VRM® process.

regulations or even on specific indications provided by the customer who awards a contract for the construction of the treatment facility. A description of the selection procedure of influent flowrate is available in (55).

Another factor to be considered is the typology of sewer system, which delivers wastewater to the treatment facility. When a unitary drainage system has to be served (i.e. rainfall water and black water are carried to the treatment units) and there is rainy weather, flows to be treated just with physical processes (Q_{wwmf}, mechanically treated flow during wet weather) have to be distinguished from flows which are subject to biological treatment (Q_{wwbf}, biologically treated flow during wet weather).

In designing a membrane bioreactor, the dual nature of the process should be considered; thus, both hydraulic and biological aspects have to be taken into account. This section is aimed to provide a procedure for the MBR unit design. Here, all calculations related to design flowrates, pre-treatment units (grits, screens and primary settling tanks) are not considered. As a general rule, the installation of screens according to manufacturer's recommendations is strongly advised, in order to avoid structural damages to membranes and to reduce fouling impact. If a unitary drainage system is considered, hydraulic sizing has to be carried out according to the maximum flowrate, which enters the biological tank during rainy periods

(Q_{wwbf}); for separate drainage systems, the maximum dry weather flowrate must be taken into consideration (Q_{dwmf}). Once chosen the design flowrate, it has to be divided by the maximum applicable flux J_{T} (related to a given minimum temperature) to determine the actual filtering surface (A_{membrane}) required. Therefore, the membrane surface for unitary or separate sewage is given by Eqs. (26) and (27), respectively:

$$A_{\text{membrane}} = \frac{Q_{\text{wwbf}}}{J_{\text{T}}} \tag{26}$$

$$A_{\text{membrane}} = \frac{Q_{\text{dwmf}}}{J_{\text{T}}} \tag{27}$$

In reality, the choice of the hydraulic design flow cannot neglect the fouling dynamics of the membrane, and thus the experimentally assessed sustainable flux, the duration of peak flows and possible backwash and/or relaxation operations should be taken into consideration. When an industrial wastewater MBR has to be designed, flow fluctuations can be evaluated more easily but both fouling propensity of the membrane and substrate biodegradability must be preliminarily measured with experiments on a suitable pilot scale plant.

Similar to conventional activated sludge processes, the biological process for MBRs has to be sized according to a certain design flow Q_{bpdf} (biological process design flow) corresponding to a given mass loading that must allow to face diurnal fluctuations of influent flow. The flowrate Q_{bpdf} is typically assumed to be n times higher than the average daily flow Q_{ave} with n usually ranging between 1 and 3, depending on the size of the plant and local regulations. The design approach presented in this section can be applied to both conventional and membrane separation activated sludge processes aimed to carbon and nitrogen removal according to a pre-denitrification configuration with an internal recycle of mixed liquor from the aerated tank to the anoxic one and an additional sludge recycle from the secondary settling unit to the anoxic one, the latter being null for membrane bioreactors. Obviously, the method can also be used for only nitrification scheme. From a conceptual standpoint, such method derives from the one proposed by Ekama et al. (56) and it is based on COD and nitrogen fractionation according to their biodegradability. Here, an explicit reference to effluent targets to be achieved is introduced, leading to the optimization of the most important design parameters. The method is valid for completely mixed reactors, i.e. those reactors for which the concentration values of each component in the system and in the effluent are the same. It allows to estimate only the biological volume; the bulk volume occupied by membranes has to be assessed according to the module chosen and information provided by the manufacturer.

Major factors to be estimated are

- SRT
- Anoxic fraction of the biological volume, f_{anoxic}
- Recirculation rate of aerated mixed liquor, a

If biokinetics for heterotrophic and autotrophic bacteria involved in C and N removal are known and a first trial value of f_{anoxic} has been fixed, the method is able to calculate SRT, f_{anoxic} and a, which allow to respect the pre-established effluent quality standards minimizing

the process volume. First, a COD and N fractioning approach is introduced; then, the impact of environmental conditions on biokinetics is described. Finally, the step by step procedure is shown with a design example in Sect. 4.5. All "influent" fractions or concentrations are related to the wastewater entering the biological process tank and not to the raw sewage.

4.2. Influent COD and TKN Fractioning

Biodegradable organic matter in wastewater is traditionally expressed in terms of BOD (biochemical oxygen demand) determined with 5-, 7- or 20-day-long tests. However, this is limited by the long analysis time, which is often inadequate to describe the actual dynamics of depurative processes. Although it can be measured very quickly, total COD does not allow to classify organic compounds according to their own biodegradability. Basically, in order to achieve a well-reasoned designing, influent total COD concentration (S_{ti}) can be divided into a biodegradable fraction (S_{bi}) and an unbiodegradable fraction (S_{nbi}). Both these fractions can also be split up into a soluble and a particulate fraction, giving S_{sbi} (soluble biodegradable influent COD), S_{pbi} (particulate biodegradable influent COD), S_{snbi} (soluble non-biodegradable influent COD) and S_{pnbi} (particulate non-biodegradable influent COD). Therefore, defining dimensionless parameters f_{sb} (soluble biodegradable fraction), f_{pb} (particulate biodegradable fraction), f_{snb} (soluble non-biodegradable fraction) and f_{pnb} (particulate non-biodegradable fraction), the following equations can be written:

$$S_{bi} = (1 - f_{snb} - f_{pnb}) S_{ti} \tag{28}$$

$$S_{sbi} = f_{sb} S_t \tag{29}$$

$$S_{pbi} = (f_p) S_t \tag{30}$$

$$S_{snbi} = f_{snb} S_{ti} \tag{31}$$

$$S_{pnbi} = f_{pnb} S_{ti} \tag{32}$$

The S terms in Eqs. (28)–(32) are related to COD concentrations and are expressed as g COD/m^3. An analogue classification can be made by distinguishing the particulate and soluble fractions of influent COD as well as for the biodegradable and unbiodegradable fraction. In this case, the soluble biodegradable COD concentration S_{sbi} is also referred to as readily biodegradable COD (RBCOD). It represents the substrate fraction that can be quickly assimilated by cells. The particulate biodegradable COD S_{pbi} is associated to the slowly biodegradable COD (SBCOD), which needs to be hydrolyzed before being used by cells. Depending on the specific compounds, hydrolysis develops with different rates. This leads to define a Rapidly Hydrolysable COD, which can be in both soluble and particulate fraction. A part of S_{pbi} corresponds to the active biomass entering the wastewater treatment plant. According to these categories, Table 10.6 shows some values of COD fractions for different raw and settled sewages. Table 10.7 provides typical values for some of the above-mentioned fractioning parameters.

Table 10.6
Percentages of influent COD fractions for different wastewater

	RBCOD	SBCOD	S_{snbi}	S_{pnbi}	Active biomass	References
Raw wastewater						
Italy	13	52	5	15	15	(57)
South Africa	20	62	5	13	–	(58)
Denmark 1	20	40	2	18	20	(59)
Settled wastewater						
Switzerland 1	32	45	11	11	–	(60)
Hungary	29	43	9	20	–	(60)
Denmark 2	24	49	8	19	–	(60)
Denmark 1	29	43	3	11	14	(59)
Switzerland 2	16	40	10	9	25	(61)
France	25	41	6	8	–	(62)
South Africa	28	60	8	4	–	(58)

Table 10.7
f_{cv} and f_i values (Adapted from Ekama et al. (56))

			Wastewater	
Parameter	Symbol	Unit	Raw	Settled
COD/VSS ratio	f_{cv}	g COD/g VSS	1.45–1.50	1.45–1.50
VSS/MLSS ratio	f_i	g VSS/g MLSS	0.60	0.83

Sometimes, particulate non-biodegradable COD S_{pnbi} is given as VSS (volatile suspended solids) concentration X_{ii}:

$$X_{ii} = \frac{S_{pnbi}}{f_{cv}} \tag{33}$$

Chemical oxygen demand (COD) fractioning can be easily carried out through respirometry. This is a well-established discipline based on measuring oxygen consumption by microbial activity for substrate degradation and removal. Respirometric measurements performed on activated sludge and raw or settled wastewater rely on dissolved oxygen variation in a controlled system (the so-called respirometer) and on the rate of such variation (oxygen uptake rate (OUR)). Depending on the specific method adopted, respirometry allows to evaluate the exact composition of COD in the considered wastewater. Thus, the combination of conventional chemical analysis as total COD measurement with respirometric tests that gives biodegradable COD fractions rapidly and slowly. Similarly, respirometry permits to detect toxic compounds in the wastewater. This chapter will not provide technical indications about respirometry since several methods have been published to assess the COD fractions percentages, and some examples are reported in different works (63–67).

Table 10.8
Influent TKN fractionation (Adapted from Ekama et al. (56))

Parameter	Symbol	Unit	Wastewater	
			Raw	Settled
Ammonia/TKN fraction	f_{na}	g N/g N	0.60–0.80	0.70–0.90
TKN/VSS ratio of particulate nonbiodegradable COD	f_n	g N/g VSS	0.09–0.12	0.09–0.12
Soluble nonbiodegradable fraction of TKN	f_{snbN}	g N/g N	< 0.04	< 0.05

Nitrogen in influent wastewater is measured as total Kjeldahl nitrogen (TKN), which is obtainable by summing free saline ammonia and organic nitrogen. Influent TKN (N_{ti}) fractioning generates the following components: ammonia nitrogen N_{ai}, soluble non-biodegradable organic nitrogen N_{snbi}, particulate non-biodegradable organic nitrogen N_{pnbi} and biodegradable organic nitrogen N_{oi}. All these components are expressed in g N/m^3, and they can be determined as a function of N_{ti}, simply introducing the following dimensionless fractions:

- Ammonia fraction f_{na}
- Soluble unbiodegradable organic nitrogen fraction f_{snbN}

The nitrogen fraction of the influent unbiodegradable COD as VSS is called f_n, and it is expressed as g N/g VSS. The following equations can be introduced:

$$N_{ai} = f_{na} N_{ti} \tag{34}$$

$$N_{snbi} = f_{snbN} N_{ti} \tag{35}$$

$$N_{pnbi} = \frac{f_n S_{pnbi}}{f_{cv}} = f_n X_{ii} \tag{36}$$

$$N_{oi} = N_{ti}(1 - f_{na} - f_{snbN}) - \frac{f_n f_{pnb} S_{ti}}{f_{cv}} \tag{37}$$

$$N_{ti} = N_{ai} + N_{snbi} + N_{pnbi} + N_{oi} \tag{38}$$

Typical values for such parameters are given in Table 10.8.

4.3. Impact of Environmental Conditions on the Bacterial Growth and the Substrate Removal

Reproduction by fission consists in the division of a single cell in two new organisms, and it is the main bacterial reproduction mechanism. The net biomass concentration variation dx in the time interval dt is expressed as:

$$\left(\frac{dx}{dt}\right)_{net} = \left(\frac{dx}{dt}\right)_{growth} - \left(\frac{dx}{dt}\right)_{decay} \tag{39}$$

Particularly, $(\mathrm{d}x/\mathrm{d}t)_{\text{growth}}$ represents the term due to the formation of new cellular material, and it is given by

$$\left(\frac{\mathrm{d}x}{\mathrm{d}t}\right)_{\text{growth}} = \mu x \tag{40}$$

where μ is the biomass growth rate, g VSS/(g VSS)/day and x corresponds to the biomass concentration at time t, kg VSS/m^3. The term $(\mathrm{d}x/\mathrm{d}t)_{\text{decay}}$ indicates the dead biomass in the time interval $\mathrm{d}t$, and it is given by

$$\left(\frac{\mathrm{d}x}{\mathrm{d}t}\right)_{\text{decay}} = bx \tag{41}$$

with b cellular death rate for the considered bacterial species, g VSS/(g VSS)/day.

Ratio between the net bacterial growth and the associated substrate consumption is defined as cellular yield coefficient Y (g VSS/g substrate), as in the following equation:

$$Y = \frac{(\mathrm{d}x)_{\text{growth}}}{\mathrm{d}S} \tag{42}$$

Deriving in time Eq. (42):

$$Y = \frac{(\mathrm{d}x/\mathrm{d}t)_{\text{growth}}}{\mathrm{d}S/\mathrm{d}t} = \frac{\mu}{\nu} \tag{43}$$

with ν substrate removal rate per biomass unit, g substrate/(g VSS)/day.

Kinetic parameters (i.e. growth and decay rates) for a given bacterial species depend mainly on the following factors: influent characteristics and substrate availability, temperature, pH, and availability of an electron acceptor. If the only bacterial growth limiting factor is substrate concentration, the bacterial growth rate μ can be expressed as:

$$\mu = \bar{\mu}\frac{S}{K_{\text{substrate}} + S} \tag{44}$$

where $\bar{\mu}$ is the maximum bacterial growth rate obtainable under unlimited substrate availability condition, g VSS/(g VSS)/day; S is substrate concentration, g substrate/m^3; $K_{\text{substrate}}$ is the half-saturation constant, that is, the concentration value for which bacterial growth rate is $1/2\bar{\mu}$, g substrate/m^3. Then, Eq. (39) becomes:

$$\left(\frac{\mathrm{d}x}{\mathrm{d}t}\right)_{\text{net}} = \left(\bar{\mu}\frac{S}{K_{\text{substrate}} + S} - b\right)x \tag{45}$$

which is the well-known Monod equation. In the same way, substrate removal rate changes into Eq. (46):

$$\frac{\mathrm{d}S}{\mathrm{d}t} = \frac{\bar{\mu}}{Y}\frac{S}{K_{\text{substrate}} + S}x = \bar{\nu}\frac{S}{K_{\text{substrate}} + S}x \tag{46}$$

$\bar{\nu}$ is the maximum substrate removal rate, g substrate/(g VSS)/day. Equation (46) is also referred to as Michaelis–Menten equation.

Table 10.9
Kinetic and stoichiometric parameters for autotrophs and heterotrophs (Adapted from Ekama et al. (56) and Henze et al. (60))

Parameter	Symbol	Unit	Value at 20°C
Yield coefficient for heterotrophic biomass	Y_h	g VSS/g COD	0.45
Maximum specific growth rate for heterotrophic biomass	μ_{hm}	1/day	6
Decay rate for heterotrophic biomass	b_h	1/day	0.24
Half-saturation constant for RBCOD	K_S	g/m^3	20
Yield coefficient for autotrophic biomass	Y_N	gnVSS/g N	0.1
Maximum specific growth rate for autotrophic biomass	μ_n	1/day	0.20–0.80
Decay rate for autotrophic biomass	b_n	1/day	0.04
Half-saturation constant for TKN	K_n	g/m^3	1
Denitrification rate associated to RBCOD consumption	K_1	g $N\text{-}NO_3$/(g aVSS)/day	0.72
Denitrification rate associated to SBCOD consumption	K_2	g $N\text{-}NO_3$/(g aVSS)/day	0.1008
Denitrification rate associated to endogenous substrate consumption	K_3	g $N\text{-}NO_3$/(gaVSS)/day	0.072

Kinetic ($\bar{\mu}, b$) and stoichiometric parameters (Y) of activated sludge can be easily determined by respirometry (66, 67). However, in case of an ex novo plant design, these values can be obtained from the literature; some examples are summarized in Table 10.9. On the other hand, in case of an existing plant extension, it is strongly advised to use kinetic parameters experimentally determined on the biomass taken from the plant itself. In the following sub-paragraphs, mathematical relations describing the influence of several factors on the biokinetics for both heterotrophic and autotrophic bacterial population are investigated.

4.3.1. Feed

4.3.1.1. NITRIFICATION

Concerning nitrification, the maximum value of the specific autotrophic biomass growing rate (μ_{nm}) is dependent on the influent composition. This effect is particularly evident if some aliquots of industrial water are contained in the influent. In such a case, it is possible to observe an inhibiting action due to the presence of some toxic compounds. Experimental studies have shown that an increase in the industrial water fraction causes a drop in the value of μ_{nm}, with a great influence on the process volume, as it is put in evidence in Sect. 4.4.

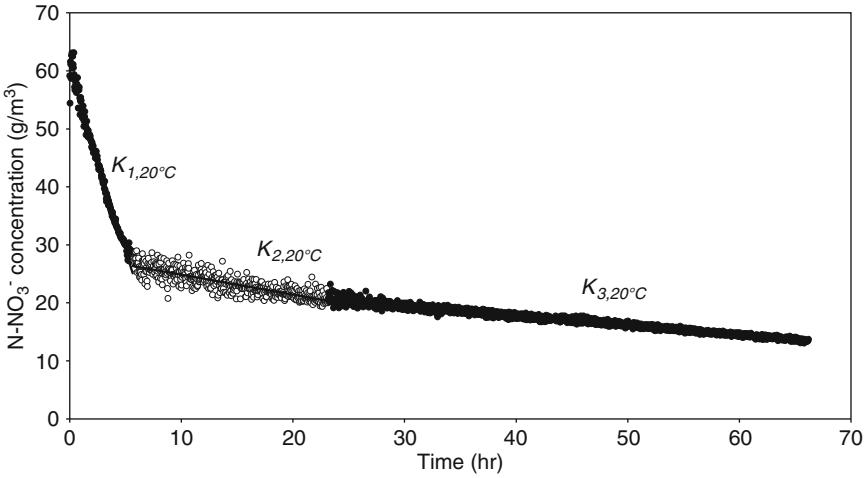


Fig. 10.27. NUR test for assessment of denitrification rates.

4.3.1.2. DENITRIFICATION

The denitrifying bacteria activity is strongly influenced by the composition of the organic substrate used as electron donor. The organic substance entering the biological denitrification stage can be composed of different quality substrate:

- Raw or settled sewage (internal carbon source)
- Biodegradable material deriving from cellular lysis (endogenous carbon source)
- Pure organic compounds as methanol, acetate, glucose, etc. (external carbon source)
- Industrial sewage with low nitrogen content (external carbon source)

It is possible to individuate experimentally three different denitrification phases. In the first, more rapid phase, carbon source is mainly formed by readily biodegradable COD fraction present within the sewage. In the second phase, denitrification shows a lower velocity, since the electron donor is the sewage slowly biodegradable COD fraction. Finally, the endogenous substrate is used as electron donor by heterotrophs. These three phases are clearly shown in Fig. 10.27 in which are reported the results obtained during a nitrate uptake rate (NUR) test under 20°C and anoxic conditions after adding a known $N\text{-}NO_3^-$ concentration to a sample of activated sludge.

4.3.2. Temperature

4.3.2.1. NITRIFICATION

Growth and death of autotroph nitrifying biomass kinetics depend on the process temperature. Ekama et al. (56) proposed the following expression for the nitrifying autotroph biomass:

$$\mu_{\mathrm{nmT}} = \mu_{\mathrm{nm20}} \times 1.123^{(T-20)} \tag{47}$$

$$K_{\mathrm{nT}} = K_{\mathrm{n20}} \times 1.123^{(T-20)} \tag{48}$$

$$b_{\mathrm{nT}} = b_{\mathrm{n20}} \times 1.029^{(T-20)} \tag{49}$$

where μ_{nmT} and b_{nT} are the bacterial growth and death rates for nitrifying biomass at the temperature T (°C), both expressed in g nVSS/(g nVSS)/day where nVSS represents nitrifying volatile suspended solids in the mixed liquor; K_{nT} is the half-saturation constant at the same temperature, g N/m^3. These values are determined on the basis of reference temperature (20°C) measurements.

The growth and decay rates of heterotrophs are functions of temperature according to following equations:

$$\mu_{\mathrm{hmT}} = \mu_{\mathrm{hm20}} \times 1.072^{(T-20)} \tag{50}$$

$$b_{\mathrm{hT}} = b_{\mathrm{h20}} \times 1.029^{(T-20)} \tag{51}$$

with μ_{hmT} and b_{hT} the bacterial growth and death rates for heterotrophic biomass at the temperature T (°C), both expressed in g aVSS/(g aVSS)/day where the acronym aVSS indicates the active volatile suspended solids in the mixed liquor. The half-saturation constant K_{ST}(g N/m^3) at temperature T can be calculated once determined its value at 20°C:

$$K_{\mathrm{ST}} = K_{\mathrm{S20}} \times 1.123^{(T-20)} \tag{52}$$

4.3.2.2. Denitrification

Denitrification rates associated to the consumption of readily and slowly biodegradable COD are defined as K_1 and K_2; their dependence on temperature is described by the following relationships:

$$K_{1\mathrm{T}} = 0.72 \times 1.2^{(T-20)} \tag{53}$$

$$K_{2\mathrm{T}} = 0.1008 \times 1.08^{(T-20)} \tag{54}$$

Both denitrification velocities are specific per active heterotrophic biomass unit and are expressed in g N-NO_3^-/(g aVSS)/day. If the carbon source is composed of biodegradable cellular lysis product, denitrifying velocity g N-NO_3^-/(g aVSS)/day is given by

$$K_{3\mathrm{T}} = 0.072 \times 1.03^{(T-20)} \tag{55}$$

When $T < 13$°C, $K_{2\mathrm{T}} = K_{3\mathrm{T}}$ can be assumed.

4.3.3. pH

4.3.3.1. Nitrification

A generic representation of nitrification process as a whole process (without distinguishing ammonia conversion to nitrite and nitrite oxidation to nitrate) is

$$a\,\mathrm{NH_4^+} + b\,\mathrm{O_2} + c\,\mathrm{HCO_3^-} \rightarrow d\,\mathrm{C_5H_7O_2N} + e\,\mathrm{NO_3^-} + f\,\mathrm{H_2CO_3}$$

where the term $C_5H_7O_2N$ indicates the new cellular matter synthesized during the biochemical reaction. The nitrification-associated alkalinity consumption (i.e. HCO_3^- utilization) can cause pH decrease, thus influencing maximum autotrophs growth rate and half-saturation

constant when pH is out of the range between 7.2 and 8.5. Particularly, the following pH-effect on μ_{nm} has been proposed by Ekama et al. (56):

$$\mu_{nmpH} = \mu_{nm7.2} \quad \text{if } 7.2 < \text{pH} < 8.5 \tag{56}$$

$$\mu_{nmpH} = \mu_{nm7.2} \times 2.35^{(\text{pH}-7.2)} \quad \text{if } 5 < \text{pH} < 7.2 \tag{57}$$

where 2.35 is the sensitivity coefficient to pH for μ_{nm}. Similarly, for half-saturation constant is

$$K_{npH} = K_{n7.2} \quad \text{if } 7.2 < \text{pH} < 8.5 \tag{58}$$

$$K_{npH} = K_{n7.2} \times 2.35^{(7.2-\text{pH})} \quad \text{if } 5 < \text{pH} < 7.2 \tag{59}$$

4.3.3.2. DENITRIFICATION

The overall denitrification reaction is

$$a\text{NO}_3^- + b \text{ carbonaceous substrate} + c\ \text{H}_2\text{CO}_3 \rightarrow d\ \text{N}_2 + e\ \text{H}_2\text{O} + f\ \text{HCO}_3^-$$

According to the previous reaction, bicarbonate is produced so balancing the alkalinity decrease which occurs during nitrification. Usually, in municipal wastewater treatment plants, alkalinity production does not affect the value of pH considerably. The situation could be different in case of industrial sewage treatment that produces a high nitric nitrogen load for the denitrification tank. In any case, pH influence on denitrifying bacteria activity is clear for pH values lower than 7 or higher than 9.1; the optimum is the range between 7.8 and 9.1. The final product of the reaction could be influenced by pH value: pH lower than 7.3 causes an increase in the effluent nitrite concentration.

4.3.4. Dissolved Oxygen Concentration

4.3.4.1. NITRIFICATION

Low dissolved oxygen concentration in the nitrification tank causes a reduction of the maximum autotrophic biomass growth rate that can be quantified with Eq. (60):

$$\mu_{nO} = \mu_{nmO} \frac{\text{DO}}{K_O + \text{DO}} \tag{60}$$

where μ_{nmO} is the maximum specific growth rate at concentration DO, g nVSS/(g nVSS)/day; K_O is the half-saturation constant, g/m^3; DO is the dissolved oxygen concentration in the tank, g/m^3.

K_{nO} is characterized by a wide variability; in fact, literature (56, 60) offers values in the range between 0.2 and 1.0 g/m^3. The causes of this variability can be found (a) in the intrinsic difference between the concentration within the sludge floc and the concentration measured in the reactor, (b) the mixing condition that can change the oxygen concentration within the tank itself. The typical dissolved oxygen concentration adopted in the wastewater treatment tank not to inhibit the nitrification is about 2 g/m^3; in any case a value not lower than 1–1.5 g/m^3 is suggested.

4.3.4.2. Denitrification

Concerning denitrification, it is known that the reduction of nitric nitrogen to gaseous nitrogen is inhibited by the presence of dissolved oxygen since the use of O_2 as electron acceptor is more advantageous than NO_3^- on the energetic point of view for heterotrophic bacterial biomass. For this reason, a slow mixing is suggested within the denitrification tank.

4.4. Design Procedure

After having determined the $Q_{\text{bpdf}}(\text{m}^3/\text{day})$, the design method consists of the following steps:

1. Influent wastewater characterization and experimental determination of biokinetics
2. Choice of a first trial value for f_{anoxic} (dimensionless), depending on required effluent quality in terms of N concentration
3. Calculation of the design sludge age ($\text{SRT}_{\text{design}}$, days) that permits to achieve steady nitrification and desired effluent ammonia concentration $\text{N}_{\text{a,out}}(\text{g N/m}^3)$ at process temperature T (°C)
4. Determination of maximum and minimum values of the un-aerated fraction of the process volume, $f_{\text{anoxic,max}}$ and $f_{\text{anoxic,min}}$, respectively (both dimensionless). The chosen value of f_{anoxic} has to be included in this range
5. Assessment of the overall volume $V_{\text{total}}(\text{m}^3)$, the nitrification volume $V_{\text{nitrification}}(\text{m}^3)$ and the denitrification volume $V_{\text{denitrification}}(\text{m}^3)$
6. Estimation of the daily sludge production from the biological process tank, X_{waste} (kg MLSS/day)
7. Calculation of the total effluent COD concentration, $S_{\text{t,out}}(\text{g COD/m}^3)$
8. Calculation of effluent TKN, $\text{TKN}_{\text{out}}(\text{g N/m}^3)$
9. Estimation of nitrogen used for cell synthesis, $\text{N}_{\text{synthesis}}(\text{g N/m}^3)$
10. Determination of the nitrification capacity of the system, $\text{N}_{\text{capacity}}(\text{g N/m}^3)$
11. Evaluation of the denitrification potential, $D_{\text{potential}}(\text{g N/m}^3)$
12. Assessment of the denitrification capacity, $D_{\text{capacity}}(\text{g N/m}^3)$
13. Choice of the sludge recirculation ratio from settling tank r_{s} (if needed)
14. Determination of the best aerated mixed liquor recirculation ratio, a_{optimum} (dimensionless)
15. Choice of a suitable aerated mixed liquor recirculation ratio, a (dimensionless)
16. Calculation of effluent nitrate concentration, $\text{N} - \text{NO}_{3\ \text{out}}{}^-(\text{g N/m}^3)$
17. Calculation of the effluent total nitrogen concentration, $\text{N}_{\text{t,out}}(\text{g N/m}^3)$
18. Estimation of the daily required oxygen mass $\text{O}_{\text{total}}(\text{kg O}_2/\text{day})$
19. Iteration process to optimize f_{anoxic} and a

Detailed description of each step is provided in the following pages.

4.4.1. Design Sludge Age

The design SRT has to be able to ensure nitrification efficiency compatible with discharge limits, also under most critical temperature conditions. In other terms, the SRT imposed must allow nitrifiers to grow so that the required ammonia removal could be achieved. Equation (43) can be written for nitrifying biomass, giving Eq. (61):

$$\left(\frac{\text{d}x_{\text{nitrifiers}}}{\text{d}t}\right)_{\text{growth}} = Y_{\text{N}}\frac{\text{dN}_{\text{a,effluent}}}{\text{d}t} \tag{61}$$

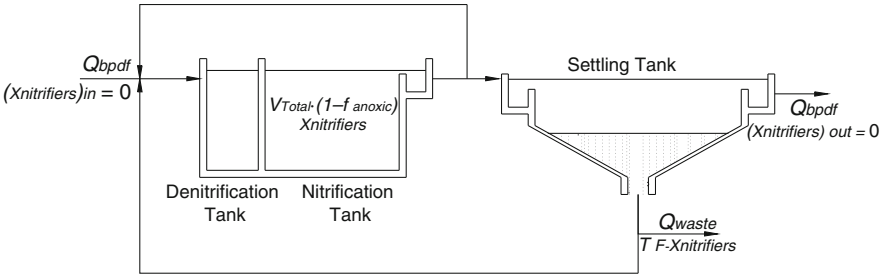


Fig. 10.28. Modified pre-denitrification Ludzack–Ettinger activated sludge process for nitrogen removal.

where $x_{\text{nitrifiers}}$ concentration of nitrifying bacteria in the system (kg nVSS/m^3) and $\text{N}_{\text{a,effluent}}$ effluent ammonia concentration (g N/m^3), equal to ammonia concentration in the process volume (complete mixing hypothesis).

Assuming dissolved oxygen not to be a limiting factor and pH > 7.2, the growth rate for nitrifiers at temperature T is

$$\mu_{\text{nT}} = \mu_{\text{nmT}} \frac{\text{N}_{\text{a,effluent}}}{K_{\text{nT}} + \text{N}_{\text{a,effluent}}} \tag{62}$$

Consider Fig. 10.28. The mass balance for nitrifying microorganisms in the whole system can be written as follows:

$$\begin{pmatrix}\text{Accumulation}\\ \text{in time } \Delta t\end{pmatrix} = \begin{pmatrix}\text{Nitrifying biomass}\\ \text{entering the system}\\ \text{from sewage}\end{pmatrix} + \begin{pmatrix}\text{New nitrifying biomass}\\ \text{generated in the}\\ \text{nitrification tank}\end{pmatrix}$$
$$- \begin{pmatrix}\text{Dead nitrifying biomass}\\ \text{in the entire system}\end{pmatrix} - \begin{pmatrix}\text{Biomass removed}\\ \text{with sludge waste}\end{pmatrix} - \begin{pmatrix}\text{Biomass lost}\\ \text{with final effluent}\end{pmatrix}$$

By neglecting both nitrifying biomass entering from sewage and nitrifying biomass outgoing from the sedimentation unit (which is a fairly reasonable hypothesis), the mathematical expression of the balance is

$$\frac{\Delta X_{\text{nitrifiers}}}{\Delta t} = \mu_{\text{nmT}}(1-f_{\text{anoxic}}) \frac{\text{N}_{\text{a,effluent}}}{K_{\text{nT}}+\text{N}_{\text{a,effluent}}} x_{\text{nitrifiers}} V_{\text{total}} - b_{\text{nT}} x_{\text{nitrifiers}} V_{\text{total}} - T_{\text{F}} x_{\text{nitrifiers}} Q_{\text{waste}} \tag{63}$$

where $\Delta X_{\text{nitrifiers}}$ is the variation of the nitrifying biomass in time Δt (kg nVSS/day), Q_{waste} is the daily volumetric withdrawal rate assumed to be extracted from the biological tank (m^3/day). T_{F} (thickening factor) is a dimensionless factor indicating the concentration of waste sludge. Its value depends on where the waste sludge extraction is operated and, for a given SRT, it influences directly Q_{waste}. It can be assumed:

- $T_{\text{F}} = 1$, if the sludge is extracted from the biological tank
- $T_{\text{F}} = 2$–2.5 if the sludge is removed from the secondary settling tank

- $T_F = 1.1–1.5$ when the sludge is removed from the membrane tank in external submerged membrane bioreactors

By assuming steady-state conditions ($\Delta X_{\text{nitrifiers}}/\Delta t = 0$) and dividing by the term $x_{\text{nitrifiers}} V_{\text{total}}$, Eq. (63) becomes:

$$\mu_{\text{nmT}}(1 - f_{\text{anoxic}}) \frac{\text{N}_{\text{a,effluent}}}{K_{\text{nT}} + \text{N}_{\text{a,effluent}}} - b_{\text{nT}} - \frac{T_F x_{\text{nitrifiers}} Q_{\text{waste}}}{x_{\text{nitrifiers}} V_{\text{total}}} = 0 \tag{64}$$

The term $x_{\text{nitrifiers}} V_{\text{total}} / T_F x_{\text{nitrifiers}} Q_{\text{waste}}$ is the mathematical form of the sludge age concept (SRT), given by the ratio between the mass of microorganisms within the system and the mass of microorganisms which is daily removed from the system itself. Hence, the effluent ammonia concentration is obtained by rearranging Eq. (64):

$$\text{N}_{\text{a,effluent}} = \frac{K_{\text{nT}} \left(b_{\text{nT}} + \frac{1}{\text{SRT}}\right)}{\mu_{\text{nmT}}(1 - f_{\text{anoxic}}) - \left(b_{\text{nT}} + \frac{1}{\text{SRT}}\right)} \tag{65}$$

Now, once fixed the design temperature T and the required effluent ammonia concentration ($\text{N}_{\text{a,out}}$), $\text{SRT}_{\text{design}}$ is given by setting $\text{N}_{\text{a,effluent}}$ equal to $\text{N}_{\text{a,out}}$:

$$\text{SRT}_{\text{design}} = \frac{1}{\left(\text{SF}\mu_{\text{n,T,pH,O}}(1 - f_{\text{anoxic}}) \frac{\text{N}_{\text{a,out}}}{\text{N}_{\text{a,out}} + K_{\text{n,T,pH}}} - b_{\text{nT}}\right)} \tag{66}$$

Equation (66) is derived from the mass balance of Eq. (64), and it points out that the design SRT is the reciprocal ratio of the net increase rate for nitrifying biomass, the growth rate term being dependent on the anoxic fraction and environmental conditions (pH, T, dissolved oxygen, substrate availability). SF is a dimensionless safety factor (lower than 1), which is applied in order to avoid underestimated evaluations of the process volume because of possible errors in the experimental determination of μ. Thus, $\mu_{\text{n,T,pH,O}}$ is the actual nitrifiers growth rate under design conditions and can be determined with Eq. (67):

$$\mu_{\text{nT,pH,O}} = \mu_{\text{nm20}} \times 1.123^{(T-20)} \times 2.35^{(\text{pH}-7.2)} \frac{\text{DO}_{\text{a}}}{K_{\text{O}} + \text{DO}_{\text{a}}} \tag{67}$$

where the term related to pH influence for both μ and K_{n} has to be taken into account only when pH is lower than 7.2, as shown in Sect. 4.3.

4.4.2. Anoxic Fraction

The anoxic fraction of the process volume (f_{anoxic}) has to be included between a maximum value $f_{\text{anoxic,max}}$, above which non nitrification occurs and a minimum value $f_{\text{anoxic,min}}$, which allows the readily biodegradable COD (S_{sbi}) to be removed in the denitrification tank. Hence Eq. (68) determines the maximum anoxic fraction:

$$f_{\text{anoxic,max}} = 1 - \frac{\left(b_{\text{nT}} + \frac{1}{\text{SRT}_{\text{design}}}\right)}{\mu_{\text{n,T,pH,O}}} \tag{68}$$

During denitrification, nitrate serves as electron acceptor, because of oxygen lack. Therefore, the conversion coefficient 2.86 g O_2/g N-NO_3^- is introduced to assess the minimum anoxic fraction, representing the stoichiometrically equivalent oxygen content of nitrate:

$$f_{\text{anoxic,min}} = \frac{f_{\text{sb}}(1 - f_{\text{cv}}Y_{\text{h}})(1 + b_{\text{hT}}\text{SRT}_{\text{design}})}{2.86K_{1\text{T}}Y_{\text{h}}\text{SRT}_{\text{design}}} \tag{69}$$

4.4.3. Overall Volume, Nitrification Volume, Denitrification Volume

The total volume is carried out through a preliminary determination of the overall volatile biomass, this being considered as the sum of following components:

- Active biomass, X_{active} (g VSS)
- Inert volatile suspended solids, $X_{\text{i,VSS}}$ (g VSS)
- Endogenous residue VSS, $X_{\text{endogenous}}$ (g VSS)

X_{active} represents the biomass actively involved in substrate removal mechanisms and can be calculated as a function of biodegradable substrate and SRT, according to Eq. (70):

$$X_{\text{active}} = Q_{\text{bpdf}}S_{\text{bi}}\frac{Y_{\text{h}}\text{SRT}_{\text{design}}}{1 + b_{\text{hT}}\text{SRT}_{\text{design}}} \tag{70}$$

$X_{\text{i,VSS}}$ is the mass of inert volatile suspended solids and can be calculated with:

$$X_{\text{i,VSS}} = Q_{\text{bpdf}}S_{\text{ti}}\frac{f_{\text{pnb}}\text{SRT}_{\text{design}}}{f_{\text{cv}}} \tag{71}$$

$X_{\text{endogenous}}$ indicates the unbiodegradable residual fraction of biomass, derived from cell lysis:

$$X_{\text{endogenous}} = f b_{\text{hT}}\text{SRT}_{\text{design}}X_{\text{active}} \tag{72}$$

where f is a dimensionless parameter called endogenous residue that represents the non-biodegradable fraction of dead cellular matter. Its value ranges can be assumed equal to 0.2 (56).

Once the design value x_{volatile}(kg VSS/m^3) of the VSS concentration in the mixed liquor is fixed, the required process volume is

$$V_{\text{total}} = \frac{X_{\text{volatile}}}{x_{\text{volatile}}} = \frac{X_{\text{active}} + X_{\text{i,VSS}} + X_{\text{endogenous}}}{x_{\text{volatile}}} \tag{73}$$

Then, the denitrification and nitrification/carbon oxidation volumes are

$$V_{\text{denitrification}} = f_{\text{anoxic}}V_{\text{total}} \tag{74}$$

$$V_{\text{nitrification}} = (1 - f_{\text{anoxic}})V_{\text{total}} \tag{75}$$

4.4.4. Daily Sludge Production

Assuming a certain VSS/MLSS ratio (f_{VSS}, g VSS/g MLSS), the total mass of MLSS in the system is given by

$$X_{\text{total}} = \frac{X_{\text{volatile}}}{f_{\text{VSS}}} \tag{76}$$

f_{VSS} gives indication about the sludge content of organic matter. It depends on the sludge age; long SRT sludge tends to be better stabilized, and therefore to have a lower volatile fraction of total suspended solids. Usually, VSS/MLSS ratio for suspended growth processes can vary between 0.65 and 0.80, 0.70–0.75 being the most common range of values. The daily biological sludge production (i.e. not including sludge coming from primary settling tank or preliminary treatment units) is named X_{waste} (kg VSS/day) and is estimated by

$$X_{waste} = \frac{X_{total}}{SRT_{design}} \tag{77}$$

4.4.5. Effluent COD

The effluent COD is determined as the sum of three different components:

- Soluble unbiodegradable COD entering the treatment plant, S_{snbi}
- COD associated to effluent VSS
- Residual soluble biodegradable COD, which is not removed by the biological process

Therefore, effluent COD $S_{t,out}$ is

$$S_{t,out} = S_{snbi} + f_{cv} f_v x_{t,out} + \frac{K_{ST}(1 + b_{hT} SRT_{design})}{SRT_{design}(Y_h \bar{v}_{ST} - b_{hT}) - 1} \tag{78}$$

where f_v is the volatile fraction of effluent solids, g VSS/g MLSS; $x_{t,out}$ is the effluent total solids concentration, g MLSS/m^3; $\bar{v}_{ST}$ is the maximum specific removal rate for readily biodegradable substrate, g COD/(g VSS)/day at the design temperature T. Commonly, the residual soluble biodegradable COD term is neglected. S_{sbi} is utilized in a time, which is largely shorter than the HRT of the system. Besides, in MBRs, the term due to effluent solid concentration can also be omitted as all solids are completely retained by the membrane.

Therefore, by negelecting for sake of simplicity the soluble microbial products generated in the biological process, Eq. (78) changes into:

$$S_{t,out} = S_{snbi} \tag{79}$$

4.4.6. Effluent TKN

Assuming that all organic biodegradable particulate nitrogen is completely hydrolyzed during the process (which is a fairly acceptable hypothesis), effluent TKN is given by

- Un-nitrified ammonia nitrogen, which corresponds to the design pre-fixed effluent N-NH_4^+ concentration
- Soluble un-biodegradable organic nitrogen $N_{snb,out}$
- Particulate organic nitrogen $N_{p,out}$ associated to effluent VSS

In particular:

– $N_{snb,out}$ is easily calculated, being the same that N_{snbi}:

$$N_{snb,out} = N_{snbi} = f_{snbN} N_{ti} \tag{80}$$

– Particulate organic nitrogen value derives from total suspended solids effluent concentration:

$$N_{p,out} = x_{t,out} f_{cv} f_n \tag{81}$$

– Hence Eq. (82) is obtained:

$$\mathrm{TKN_{out}} = \mathrm{N_{a,out}} + \mathrm{N_{snb,out}} + \mathrm{N_{p,out}} \tag{82}$$

which, in the case of MBRs (no effluent suspended solids), leads to:

$$\mathrm{TKN_{out}} = \mathrm{N_{a,out}} + \mathrm{N_{snb,out}} \tag{83}$$

4.4.7. Aerated Mixed Liquor Recirculation Optimization

Nitrification capacity $\mathrm{N_{capacity}}$ physically represents the nitrate mass per unit of influent flow produced in the aerated tank under fixed design conditions. It can be determined through a simple nitrogen mass balance in which nitrite and nitrate concentrations entering the system with influent wastewater are neglected, being this largely acceptable:

$$\mathrm{N_{capacity}} = \mathrm{N_{ti}} - \mathrm{TKN_{out}} - \mathrm{N_{sysnthesis}} \tag{84}$$

$\mathrm{N_{synthesis}}$ is the nitrogen which is removed from the system through sludge wastage:

$$\mathrm{N_{synthesis}} = f_{\mathrm{n}} \frac{X_{\mathrm{volatile}}}{\mathrm{SRT_{design}} Q_{\mathrm{bpdf}}} \tag{85}$$

The denitrification potential $D_{\mathrm{potential}}$ represents the maximum nitrate mass per unit of influent flow, which can be denitrified to gaseous bi-atomic nitrogen $\mathrm{N_2}$ in the volume $V_{\mathrm{denitrification}}$ determined with Eq. (74). According to the denitrification overall reaction, the $D_{\mathrm{potential}}$ is a function of the available biodegradable organic matter, which is used as an electron donor by denitrifying heterotrophs. Hence, assuming that a complete removal of RBCOD is achieved in the anoxic tank (i.e. $f_{\mathrm{anoxic}} > f_{\mathrm{anoxic,min}}$), $D_{\mathrm{potential}}$ is given by

$$D_{\mathrm{potential}} = S_{\mathrm{bi}} \left\{ \frac{f_{\mathrm{sb}}(1 - f_{\mathrm{cv}} Y_{\mathrm{h}})}{2.86} + \frac{K_{2\mathrm{T}} f_{\mathrm{anoxic}} Y_{\mathrm{h}} \mathrm{SRT_{design}}}{1 + b_{\mathrm{hT}} \mathrm{SRT_{design}}} \right\} \tag{86}$$

Denitrification capacity expresses the overall nitrate loading (per unit of influent flow) mass, which is recirculated to the anoxic reactor from both nitrification/oxidation tank and secondary settler. Considering the scheme in Fig. 10.29 with a zero influent nitrate concentration $\mathrm{N_{ni}}$, following equation can be written:

$$D_{\mathrm{capacity}} = \frac{\mathrm{N_{capacity}}(a + r_{\mathrm{s}})}{1 + a + r_{\mathrm{s}}} + \frac{\mathrm{DO_a} a}{2.86} + \frac{\mathrm{DO_s} r_{\mathrm{s}}}{2.86} \tag{87}$$

with $\mathrm{DO_a}$ and $\mathrm{DO_s}$ dissolved oxygen concentrations in aerated mixed liquor and settled recirculated sludge, respectively (both in g $\mathrm{O_2/m^3}$). Second and third terms in Eq. (87) represent the nitrate equivalent loading expressed as oxygen generated in aerobic tank and secondary sedimentation and entering the anoxic tank. In particular, the third term is zero for MBRs, as no settling units are used.

To optimize the nitrate removal efficiency in the anoxic stage, the mixed liquor recirculation ratio should be chosen, so that the denitrification capacity is equal to the denitrification potential. Thus:

$$D_{\mathrm{capacity}} = D_{\mathrm{potential}} \tag{88}$$

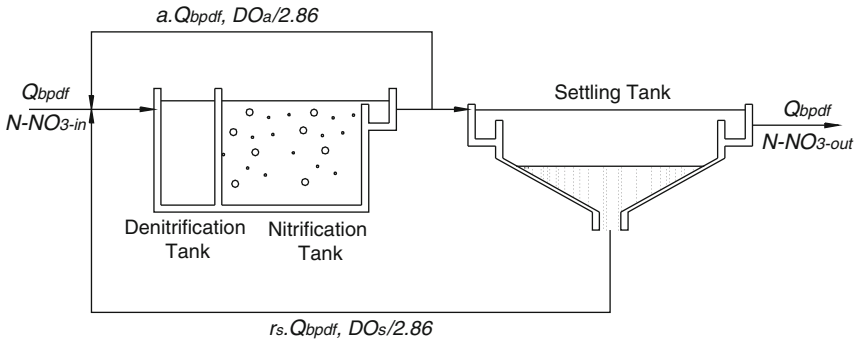


Fig. 10.29. Scheme for nitrogen removal with internal mixed liquor recirculation.

Equation (88) can be solved as a second degree polynomial to determine the best recirculation ratio a_{optimum}, so giving typical solution:

$$a_{\text{optimum}} = \frac{-B + \sqrt{B^2 - 4AC}}{2A} \tag{89}$$

where A, B and C are calculated according to following equations

$$A = \frac{\text{DO}_\text{a}}{2.86} \tag{90}$$

$$B = \text{N}_{\text{capacity}} - D_{\text{potential}} + \frac{(r_\text{s} + 1)\text{DO}_\text{a} + r_\text{s}\text{DO}_\text{s}}{2.86} \tag{91}$$

$$C = r_\text{s}\text{N}_{\text{capacity}} - (r_\text{s} + 1)\left(D_{\text{potential}} - \frac{r_\text{s}\text{DO}_\text{s}}{2.86}\right) \tag{92}$$

The actual design value for a has to be chosen according to economical considerations (the higher the a, the greater the power consumption for pumps) and also considering the effluent nitrate concentration to be achieved. a values higher than a_{optimum} ensure a lower nitrate effluent concentration due to dilution of nitrate, but it is not recommended to operate in this way because of larger energy costs.

4.4.8. Effluent Total Nitrogen

Effluent nitrate concentration is given by

$$\text{N-NO}_{3\ \text{out}}^{-} = \frac{\text{N}_{\text{capacity}}}{1 + a + r_\text{s}} \tag{93}$$

Consequently, total effluent nitrogen is

$$\text{N}_{\text{t,out}} = \text{N-NO}_{3\ \text{out}}^{-} + \text{TKN}_{\text{out}} \tag{94}$$

Equation (94) assumes that nitrite concentration in the effluent is negligible, as usual in well-operated wastewater treatment plant under normal conditions.

4.4.9. Daily Oxygen Consumption and Hourly Air Flowrate

Terms to be considered in calculating the overall oxygen mass for the biological process (O_{total}, kg O_2/day) can be summarized as follows:

- Oxygen consumption for cell synthesis and endogenous respiration of heterotrophic bacteria, O_{s+e}(kg O_2/day), depending on influent organic biodegradable matter and active biomass decay
- Oxygen consumption for nitrification, O_n(kg O_2/day), direct function of nitrification capacity
- Oxygen recover due to denitrification, O_d(kg O_2/day), function of denitrification efficiency. This term physically represents the oxygen saving due to using nitrate as electron acceptor in the f_{anoxic} fraction of the overall volume

Such three fractions are estimated with Eqs. (95)–(97):

$$O_{s+e} = (1 - f_{cv}Y_h)Q_{bpdf}S_{bi} + f_{cv}(1 - f)b_{hT}X_{active} \tag{95}$$

$$O_n = 4.57 \times Q_{bpdf}N_{capacity} \tag{96}$$

$$O_d = 2.86 \times Q_{bpdf}(N_{capacity}\text{-N-}NO_{3\ out}^{-}) \tag{97}$$

where 4.57 g O_2/g N-NH_4^+ is the conversion factor representing the stoichiometric amount of oxygen needed to oxidize a unit mass of ammonia. Finally, the daily total oxygen mass to provide to the system is

$$O_{total} = O_{s+e} + O_n - O_d \tag{98}$$

Once determined O_{total}, the air flow rate $Q_{air,biol}$(Nm^3/h) for the biological process can be determined according to Eq. (99):

$$Q_{air,biol} = \frac{SO}{C_O E} \tag{99}$$

where SO (kg/day) is the oxygen transfer rate under standard conditions (20°C, initial DO = 0.01 g/m^3, tap water), C_O is the oxygen fraction in air, 284 g O_2/m^3_{air}, E is the oxygen transfer efficiency at standard conditions of the aeration system. SO derives from Eq. (100):

$$SO = \frac{O_{20}}{\alpha_F(\beta O_{ave,T,H} - DO_a)1.024^{(T\text{-}20)}}O_{total} \tag{100}$$

with O_{20}, oxygen saturation concentration in water at 20°C at sea level, g/m^3. β is a correction factor used to keep into account the differences in oxygen solubility between wastewater and tap water, due to some components in water as salts and surface-active substances. As shown by Tchobanoglous et al. (55), commonly the β value of 0.95 is adopted for wastewater, the range between 0.7 and 0.98 being the most typical.

$O_{ave,T,H}$ is the average oxygen saturation concentration in clean water at temperature T and altitude H (m), g/m^3. According to Tchobanoglous et al. (55), it can be calculated with:

$$O_{ave,T,H} = O_{T,H} \times \frac{1}{2}\left(\frac{P_H + P_{w,depth}}{P_H} + \frac{O_t}{21}\right) \tag{101}$$

Table 10.10
Saturation concentration of dissolved oxygen for different values of temperature (at sea level and for 0 g/L of salinity) (Adapted from Tchobanoglous et al. (55))

Temperature (°C)	Saturation concentration for dissolved oxygen (g/m^3)
0	14.6
2	13.81
4	13.09
6	12.44
8	11.83
10	11.28
12	10.77
14	10.29
16	9.86
18	9.45
20	9.08
22	8.73
24	8.40
26	8.09
28	7.81
30	7.54
40	6.41

where $O_{T,H}$ is the saturation oxygen concentration in clean water at temperature T and altitude H, g/m^3; P_H and $P_{w,depth}$ are the atmospheric pressure and the pressure at the air release point due to water column (both expressed in m H_2O), O_t is the percentage of oxygen leaving the aeration tank, usually assumed to be 18–20. The change in atmospheric pressure with elevation can be expressed with:

$$P_H = P_{\text{sea level}}\, e^{\left(-\frac{gMH}{RT}\right)} \tag{102}$$

where $P_{\text{sea level}}$ is the atmospheric pressure at sea level (101.325 kPa), g is the gravity acceleration (m/s^2), M is the molecular weight of air (28.97 kg/kmol), R is the universal gas constant 8,314 J/(kmol)/K and Temperature T must be expressed in K. To convert P_H from Pa to metres of water, the value obtained from Eq. (102) has to be divided by water specific weight, 9.802 kN/m^3. Then, $O_{T,H}$ is given by

$$O_{T,H} = O_{T,\text{sea level}}\, e^{\left(-\frac{gMH}{RT}\right)} \tag{103}$$

Some values for $O_{T,\text{sea level}}$ at different temperatures are shown in Table 10.10.

The α_F factor (dimensionless) in Eq. (100) is physically given by the following expression:

$$\alpha_F = \frac{K_L a(\text{wastewater})}{K_L a(\text{tap water})} \tag{104}$$

where $K_L a$ (wastewater) and $K_L a$ (tap water) are the oxygen transfer coefficients measured in the sludge and in clean water, respectively. Therefore, α_F depends on the aeration device, the geometry of the system and, once such conditions are fixed, it depends on the MLSS concentration. For conventional activated sludge processes, a 0.7–0.8 α_F is usually assumed; for MBRs, according to the pilot scale tests performed by Günder and Krauth (68) on different bioreactors, the α_F dependency on MLSS is given by Eq. (105):

$$\alpha_F = e^{-0.082 \cdot \text{MLSS}} \tag{105}$$

with MLSS concentration expressed in kg MLSS/m^3. Equation (105) provides very low α_F values when MLSS is higher than 7 kg/m^3 (e.g. $\alpha_F = 0.35$ at 12 kg/m^3). Other studies (69) found less affecting values on full scale MBRs, with $\alpha_F = 0.6$ at 12 kg/m^3.

4.4.10. Design Parameters Optimization

The final step of the calculation procedure consists in optimizing f_{anoxic} and a, and it can be easily carried out by implementing the procedure on a spreadsheet.

4.5. Design Example

A 100,000 PE wastewater treatment plant located at 400 m above sea level and fed by a separate drainage system has to be upgraded to 170,000 PE, because of the new sewage connections and foreseen population increase. The present plant is configured according to a pre-denitrification scheme (modified Ludzack–Ettinger, see Fig. 10.28), structured with two parallel identical treatment lines. Because of the lack of surface availability, the upgrading plant cannot be entirely designed as a conventional activated sludge, and MBR technology has been chosen to treat 57% (40,000 PE) of additional flow rate being the remaining 43% (30,000 PE) addressed to a new conventional activated sludge line. A pre-treatment scheme with coarse grit and a micro-screen (1 mm) will be installed upstream both new CAS and MBR, so resulting in an influent total COD of 560 g COD/m^3 and an influent TKN of 50 g N/m^3. COD fractionation, carried out by respirometric methods, gave the following composition:

- Soluble biodegradable fraction (f_{sb}): 0.2
- Particulate biodegradable fraction (f_{pb}): 0.6
- Soluble non-biodegradable fraction (f_{snb}): 0.05
- Particulate non-biodegradable fraction (f_{pnb}): 0.15

Ammonia nitrogen is 80% of influent TKN.

According to preliminary evaluations, the daily average flowrate is supposed to be treated by the MBR 16, 000 m^3/day, with a peak value of 1, 340 m^3/h; the daily average flow to treat with the new CASP is 12, 000 m^3/day.

Respirometric determination of biokinetics and stoichiometric parameters on sludge sampled from the existing aerobic reactor and from a pilot scale MBR fed with the same wastewater gave results shown in Table 10.11.

Table 10.11
Experimental values of the kinetic and stoichiometric parameters for conventional activated sludge biomass and MBR biomass

	CAS		MBR	
	Aut.	Het.	Aut.	Het.
Maximum growth, μ_{m20}(1/day)	0.6	6	0.45	4
Decay, b_{20} (1/day)	0.04	0.24	0.04	0.27
Yield, Y (g VSS/g substrate)	0.24	0.45	0.24	0.45
Half-saturation constant, K (g susbtrate/m^3)	0.71	100	0.90	50

Assuming an operational MLSS concentration of 3.5 kg MLSS/m^3 for CAS and 12 kg MLSS/m^3 for MBR and a submerged side stream-configuration for membrane filtration calculate the following values:

- Required MBR surface, considering a $J_{\max 20}$ of 45 L/m^2/h and a relaxation operation with a 9-min suction and a 1-min pause
- Required air flowrate for membrane cleaning
- Minimum needed volumes (anoxic and aerobic) for both CAS and MBR processes
- Daily sludge production in new CAS and MBR
- Daily oxygen consumption in new CAS and MBR

The effluent quality required for both lines is

- COD: 125 g COD/m^3
- N-NH_4^+: 3 g N/m^3($N_{a,out}$)
- N total: 10 g N/m^3

4.5.1. Solution

4.5.1.1. Membrane Surface and Cleaning Air Flowrate

The required membrane surface has to be calculated considering the most critical conditions, i.e. peak influent flow and lowest temperature (highest permeate viscosity). The maximum permeate flux, referred to the design temperature (13°C), can be determined according to Eqs. (8) and (9):

$$\eta_T = e^{\left(-24.71+\frac{4,209}{286.15}+4.527\times10^{-2}\times286.15-3.4\times10^{-5}(286.15)^2\right)} = 1.34 \times 10^{-3}\ \text{Pa s}$$

$$J_{\max,13} = \frac{\eta_{20} J_{\max,20}}{\eta_{13}} = \frac{1.1\ \text{m Pa s} \times 45\ \text{L/m}^2\text{/h}}{1.34\ \text{m Pa s}} = 36.9\ \text{L/m}^2\text{/h}$$

Such flux corresponds to the maximum gross flux while the filtration unit is supposed to work according to periodical relaxation phases, even during peak flows. Therefore, the net flux has to be estimated by Eq. (5):

$$J_{\text{net},\max,13} = 36.9\ \text{L/m}^2\text{/h}\frac{540\ \text{s}}{(540+60)\ \text{s}} = 33.2\ \text{L/m}^2\text{/h}$$

The required membrane area is calculated with Eq. (27):

$$A_{\text{membrane}} = \frac{1,340\,\text{m}^3/\text{h} \times 1,000\,\text{L/m}^3}{33.2\,\text{L/m}^2/\text{h}} = 40,631.45\,\text{m}^2$$

Assuming to install hollow fibre Zenon modules ZW 500d-64, the number of required cassettes is

$$n_{\text{ZW500d}-64}\text{cassettes} = \frac{40,361.45\,\text{m}^2}{64\,\text{module/cassette} \times 31.6\,\text{m}^2/\text{module}} = 19.9 \approx 20\,\text{cassettes}$$

This results in an actual filtering surface of $40,448\,\text{m}^2$ which allows to better face possible temperature reductions or influent flow increases. Assuming a $0.5\,\text{N}\,\text{m}^3_{\text{air}}/\text{m}^2_{\text{membrane}}/\text{h}$ with a 10 s ON–10 s OFF-cycle the air flowrate for turbulence creation is

$$Q_{\text{air,Zenon}} = 40,448\,\text{m}^2 \times 0.5\,\text{N}\,\text{m}^3/\text{m}^2_{\text{membrane}}/\text{h}\frac{10\,\text{s}}{(10\,\text{s} + 10\,\text{s})} = 10,112\,\text{N}\,\text{m}^3/\text{h}$$

Then, suitable blowers must be chosen, considering hydraulic head loss, losses in the pipes, and the aerator pressure loss at the maximum air flowrate.

Similarly, if a flat sheet membrane is adopted (i.e. Kubota EK 400), the modules to be installed are

$$n_{\text{Kubota EK 400}} = \frac{40,361.45\,\text{m}^2}{400\,\text{cartridge/module} \times 0.8\,\text{m}^2/\text{cartridge}} = 126.1 \approx 128$$

modules (assuming that a double-deck scheme is adopted).

The actual installed surface is $40,960\,\text{m}^2$. The suggested specific air flowrate is $0.75\,\text{N}\,\text{m}^3_{\text{air}}/\text{m}^2_{\text{membrane}}/\text{h}$, but it can be reduced to a half having assumed the double deck configuration (Fig. 10.18), so obtaining:

$$Q_{\text{air,Kubota}} = \frac{40,960\,\text{m}^2 \times 0.75\,\text{N}\,\text{m}^3/\text{m}^2/\text{h}}{2} = 15,360\,\text{N}\,\text{m}^3/\text{h}$$

In choosing blowers, a hydraulic head loss of at least 4.0 m has to be taken into account, being the module itself 3.5 m high.

4.5.1.2. COD AND N FRACTIONS

Using Eqs. (29)–(32), the following COD fraction concentrations are determined:

$$S_{\text{sbi}} = 0.2 \times 560\,\text{g COD/m}^3 = 112\,\text{g COD/m}^3$$

$$S_{\text{pbi}} = 0.6 \times 560\,\text{g COD/m}^3 = 336\,\text{g COD/m}^3$$

$$S_{\text{bi}} = (112 + 336)\,\text{g COD/m}^3 = 448\,\text{g COD/m}^3$$

$$S_{\text{snbi}} = 0.05 \times 560\,\text{g COD/m}^3 = 28\,\text{g COD/m}^3$$

$$S_{\text{pnbi}} = 0.15 \times 560\,\text{g COD/m}^3 = 84\,\text{g COD/m}^3$$

Assuming a $f_{na} = 0.8$ and values from Table 10.8, the N fractions are determined utilizing from Eqs. (34) to (37):

$$N_{ai} = 0.8 \times 50\,g\,N/m^3 = 40\,g\,N/m^3$$

$$N_{snbi} = 0.01 \times 50\,g\,N/m^3 = 0.5\,g\,N/m^3$$

$$N_{pnbi} = \frac{0.1\,g\,N/g\,VSS \times 84\,g\,COD/m^3}{1.48\,g\,COD/g\,VSS} = 5.68\,g\,N/m^3$$

$$N_{oi} = 50\,g\,N/m^3(1–0.8–0.01) - \frac{0.1\,g\,N/g\,VSS \times 0.15 \times 560\,g\,COD/m^3}{1.48\,g\,COD/g\,VSS} = 3.82\,g\,N/m^3$$

4.5.1.3. KINETIC AND STOICHIOMETRIC PARAMETERS UNDER DESIGN CONDITIONS

Consider equations introduced in Sect. 4.3, values of Table 10.10, pH 7.1 and $K_O = 0.2\,g\,O_2/m^3$. Under these conditions, the actual nitrifiers growth rate at temperature T is given by Eq. (67). Therefore, design nitrifying biomass growth rate for CAS and MBR are, respectively:

$$\mu_{nT=13°C,pH=7.1,DO_a=2,N=2,CAS} = 0.6/day \times 1.123^{(13-20)} \times 2.35^{(7.1-7.2)} \times \frac{2\,g\,O_2/m^3}{(0.2+2)g\,O_2/m^3} = 0.222/day$$

$$\mu_{nT=13°C,pH=7.1,DO_a=2,N=2,MBR} = 0.45/day \times 1.123^{(13-20)} \times 2.35^{(7.1-7.2)} \times \frac{2\,g\,O_2/m^3}{(0.2+2)\,g\,O_2/m^3} = 0.167/day$$

According to Eq. (49), autotrophic decay rate at design temperature for both CAS and MBR is

$$b_{n13} = 0.04/day \times 1.029^{(13-20)} = 0.033/day$$

Analogously, by Eq. (51), it is possible to determine the decay of heterotrophs:

$$b_{h13,CAS} = 0.24/day \times 1.029^{(13-20)} = 0.20/day$$

$$b_{h13,MBR} = 0.27/day \times 1.029^{(13-20)} = 0.22/day$$

The denitrification rates considering RBCOD and SBCOD as carbonaceous source are (see Eqs. (53) and (54)):

$$K_{1,13} = 0.72\,g\,N\text{-}NO_3^-/(g\,aVSS)/day \times 1.2^{(13-20)} = 0.2\,g\,N\text{-}NO_3^-/(g\,aVSS)/day$$

$$K_{2,13} = 0.1008\,g\,N\text{-}NO_3^-/(g\,aVSS)/day \times 1.08^{(13-20)} = 0.06\,g\,N\text{-}NO_3^-/(g\,aVSS)/day$$

4.5.1.4. BIOLOGICAL VOLUME FOR MBR LINE

$K_{nT,pH}$ can be determined by Eqs. (48) and (59):

$$K_{nT,pH} = 0.9\,g\,N/m^3 \times 1.123^{(13-20)} \times 2.35^{(7.2-pH)} = 0.435\,g\,N/m^3$$

Assuming anoxic fraction $f_{\text{anoxic}} = 0.35$, safety factor on nitrifiers growth SF = 0.9 and considering Eq. (66) the $\text{SRT}_{\text{design}}$, which ensures stable nitrification under design conditions is

$$\text{SRT}_{\text{design}} = \frac{1}{\left(0.9 \times 0.167/\text{day}(1-0.35) \times \dfrac{3\,\text{g N/m}^3}{(3+0.435)\,\text{g N/m}^3} - 0.033/\text{day}\right)} = 19.068\,\text{days}$$

Then, according to Eqs. (70)–(72):

$$X_{\text{active}} = 16,000\,\text{m}^3/\text{day} \times 448\,\text{g COD/m}^3 \times \frac{1}{1,000\,\text{g COD/kg COD}} \times \frac{0.45\,\text{g VSS/g COD} \times 19.068\,\text{days}}{1 + 0.22/\text{day} \times 19.068\,\text{days}} = 11,839.5\,\text{kg VSS}$$

$$X_{\text{i,VSS}} = 16,000\,\text{m}^3/\text{day} \times 560\,\text{g COD/m}^3 \times \frac{1}{1,000\,\text{g COD/kg COD}} \times \frac{0.15 \times 19.068\,\text{days}}{1.48\,\text{g COD/g VSS}} = 17,315.8\,\text{kg VSS}$$

$$X_{\text{endogenous}} = 0.2 \times 0.22/\text{day} \times 19.068\,\text{days} \times 11,839.5\,\text{kg VSS} = 9,933.3\,\text{kg VSS}$$

Thus, total volatile mass in the system is

$$X_{\text{volatile}} = (11,839.5 + 17,315.8 + 9,933.3) = 39,088.6\,\text{kg VSS}$$

The hypothesized volatile biomass concentration is 12 kg MLSS/m^3, therefore the total required volume is

$$V_{\text{total}} = \frac{X_{\text{volatile}}}{x_{\text{volatile}}} = \frac{39,088.6\,\text{kg VSS}}{0.7\,\text{kg VSS/kg MLSS} \times 12\,\text{kg MLSS/m}^3} = 4,635.4\,\text{m}^3$$

that is divided into an anoxic volume of 0.35 V_{total} (1,628.6 m^3) and an aerated volume of 0.65 V_{total} (3,024.4 m^3).

4.5.1.5. Effluent COD and TKN from MBR Line

Assuming an effluent SS concentration of 0 g SS/m^3 (fairly acceptable for the permeate passing through the membrane), Eq. (79) allows calculating the total effluent COD, which corresponds to the influent soluble unbiodegradable COD:

$$S_{\text{t,out}} = 28\,\text{g COD/m}^3$$

Considering Eqs. (80) and (83), TKN_{out} is given by

$$\text{TKN}_{\text{out}} = (3 + 0.5)\,\text{g N/m}^3 = 3.5\,\text{g N/m}^3$$

4.5.1.6. Aerated Mixed Liquor Recirculation Optimization for MBR Line

In order to determine the nitrification capacity $N_{capacity}$ of the so sized system, nitrogen consumption due to synthesis has to be evaluated, by Eq. (85):

$$N_{synthesis} = 0.1\,\text{g N/g VSS} \times \frac{39,088.6\,\text{kg VSS}}{16,000\,\text{m}^3/\text{day} \times 19.068\,\text{days}} \times 1,000\,\text{g VSS/kg VSS} = 12.81\,\text{g N/m}^3$$

Thus, according to Eq. (84):

$$N_{capacity} = (50 - 3.5 - 12.81)\,\text{g N/m}^3 = 33.69\,\text{g N/m}^3$$

Therefore, assuming a mixed liquor recirculation ratio of 3, the effluent nitrate and total nitrogen concentrations are given by Eqs. (93) and (94):

$$\text{N-NO}_{3\,\text{out}}^{-} = \frac{33.69\,\text{g N/m}^3}{1+3+0} = 8.42\,\text{g N/m}^3$$

Consequently, total effluent nitrogen is

$$N_{t,out} = (8.42 + 3.5)\,\text{g N/m}^3 = 11.92\,\text{g N/m}^3$$

This concentration is not compatible with required effluent standards; therefore, an optimization of f_{anoxic} and a should be carried out to assess the minimum required volume, which allows the effluent targets to be reached. During the iterative calculation (performed through solving function of common spreadsheet), the target cell is the overall volume to be minimized while f_{anoxic} and a are varied, both being bound by some reference values. In fact, the anoxic fraction must be included in the range between $f_{anoxic,min}$ and $f_{anoxic,max}$. Similarly, the recirculation ratio has to be determined after having evaluated the $a_{optimum}$, by imposing denitrification potential Eq. (86) to be equal to denitrification capacity Eq. (87). The final results obtained are

- $f_{anoxic} = 0.448$
- $\text{SRT} = 25.24\,\text{days}$
- $f_{anoxic,min} = 0.067$
- $f_{anoxic,max} = 0.638$
- $a = 4.28$
- $X_{active} = 12,375\,\text{kg VSS}$
- $X_{i,VSS} = 22,920\,\text{kg VSS}$
- $X_{endogenous} = 13,807\,\text{kg VSS}$
- $X_{volatile} = 49,102.5\,\text{kg VSS}$
- $V_{nitrification} = 3,227.7\,\text{m}^3$
- $V_{denitrification} = 2,617.8\,\text{m}^3$
- $V_{total} = 5,845.5\,\text{m}^3$
- $\text{HRT} = \frac{5,845.5\,\text{m}^3}{16,000\,\text{m}^3/day} \times 24\,\text{h/day} = 8.77\,\text{h}$
- $\text{F/M} = \frac{Q_{ave} S_{ti}}{X_{volatile}} = \frac{16,000\,\text{m}^3/\text{day} \times 560\,\text{g COD/m}^3}{49,102.5\,\text{kg VSS} \times 1,000\,\text{g COD/kg COD}} = 0.182\,\text{kg COD/(kg MLSS)/day}$
- $N_{capacity} = 34.34\,\text{g N/m}^3$

- $D_{\text{potential}} = 30.83\,\text{g N/m}^3$
- Effluent total COD $S_{\text{t,out}} = 28\,\text{g COD/m}^3$
- Effluent ammonia nitrogen $\text{N-NH}_{4\ \text{out}}^{+} = 3\,\text{g N/m}^3$
- Effluent TKN $= 3.5\,\text{g N/m}^3$
- Effluent nitrate $\text{N-NO}_{3\ \text{out}}^{-} = 6.5\,\text{g N/m}^3$
- Effluent total nitrogen $\text{N}_{\text{t,out}} = 10\,\text{g N/m}^3$

The daily sludge production is computed by combining Eqs. (76) and (77):

$$X_{\text{waste}} = \frac{49,102.5\,\text{kg VSS}}{0.7\,\text{kg VSS/kg MLSS} \times 25.24\,\text{days}} = 2,779.2\,\text{kg MLSS/day}$$

The specific sludge production (with respect to removed COD) is

$$X_{\text{waste,spec}} = \frac{X_{\text{waste}}}{Q_{\text{bpdf}}\,(\text{COD}_{\text{in}} - \text{COD}_{\text{out}})} = \frac{2,779.2\,\text{kg MLSS/day}}{16,000\,\text{m}^3\text{/day}(560 - 28)\text{g COD/m}^3} \times 1,000\,\text{g COD/kg COD}$$

$$X_{\text{waste,spec}} = 0.326\,\text{kg MLSS/kg COD}_{\text{removed}}$$

Assuming a thickening factor T_{F} of 1.1 (external submerged MBR), the daily volumetric sludge withdrawal is

$$Q_{\text{waste}} = \frac{2,779.2\,\text{kg MLSS/day}}{12\,\text{kg MLSS/m}^3 \times 1.1} = 210.5\,\text{m}^3\text{/day}$$

If the sludge wasting operation is supposed to last 6 h, the pumps have to be able to pump $35.09\,\text{m}^3\text{/h}$.

Equations (95)–(98) allow to calculate the overall daily oxygen mass required for the biological process. For sake of simplicity the mass of oxygen supplied to the system by means of membrane aeration is neglected in the following calculations. Thus:

$$\text{O}_{\text{s+e}} = (1 - 1.48 \times 0.45) \times 16,000\,\text{m}^3\text{/day} \times 448\,\text{g COD/m}^3 + \frac{1.48(1 - 0.2) \times 0.22\text{/day} \times 12,375\,\text{kg MLSS}}{1,000\,\text{g O}_2\text{/kg O}_2} = 5,632.7\,\text{kg O}_2\text{/day}$$

$$\text{O}_{\text{n}} = \frac{4.57\,\text{g O}_2\text{/g N} \times 16,000\,\text{m}^3\text{/day} \times 34.34\,\text{g N/m}^3}{1,000\,\text{g O}_2\text{/kg O}_2} = 2,511\,\text{kg O}_2\text{/day}$$

$$\text{O}_{\text{d}} = \frac{2.86\,\text{g O}_2\text{/g N} \times 16,000\,\text{m}^3\text{/day}\,(34.34 - 6.5)\,\text{g N/m}^3}{1,000\,\text{g O}_2\text{/kg O}_2} = 1,274\,\text{kg O}_2\text{/day}$$

$$\text{O}_{\text{total}} = 6,869.7\,\text{kg O}_2\text{/day}$$

The air flow rate required for the biological process is estimated by Eq. (99). Particularly, from Eq. (102) the atmospheric pressure at 400 m P_{400} can be evaluated:

$$P_{400} = \frac{1}{9.802} \times 101.325 \times \text{e}^{\left(-\frac{9.81 \times 28.97 \times 400}{8,314\,(273.15+13)}\right)} = 9.85\,\text{m}$$

From Table 10.10, the saturation concentration of dissolved oxygen at 13°C and at sea level can be assumed to be 10.53 g/m^3. Hence, the saturation concentration at 13°C and 400 m is Eq. (104):

$$O_{13,400} = 10.5\,\text{g O}_2/\text{m}^3 \times e^{\left(-\frac{9.81\times 28.97\times 400}{8,314(273.15+13)}\right)} = 10.04\,\text{g/m}^3$$

Assuming a tank depth of 5 m with diffusers at 4.7 m, $O_{ave,13,400}$ is given by Eq. (101)

$$O_{ave,13,400} = 10.04\,\text{g O}_2/\text{m}^3 \times \frac{1}{2}\left(\frac{9.85\,\text{m} + 4.7\,\text{m}}{9.85\,\text{m}} + \frac{19}{21}\right) = 11.96\,\text{g/m}^3$$

α_F can be set equal to 0.5, β is assumed to be 0.95; therefore, according to Eq. (100), SO is

$$\text{SO} = \frac{9.08\,\text{g O}_2/\text{m}^3}{0.5(0.95 \times 11.96\,\text{g O}_2/\text{day} - 2\,\text{g O}_2/\text{day}) \times 1.024^{(13-20)}} \times \frac{6,869.7\,\text{kg O}_2/\text{day}}{24\,\text{h/day}}$$
$$= 470.6\,\text{kg O}_2/\text{h}$$

Finally, considering the installation of air diffuser with 0.2 transfer efficiency, the air flowrate is

$$Q_{\text{air,biol}} = \frac{470.6\,\text{kg O}_2/\text{h}}{0.284\,\text{kg O}_2/\text{N m}^3 \times 0.2} = 138\,\text{N m}^3/\text{h}$$

If sludge wasting reduction is the main aim of the operator, the SRT can be increased, compatibly with the membrane behaviour at higher MLSS concentration. The trends of X_{waste} and MLSS content for different units of SRT are reported in Fig. 10.30; similarly, Fig. 10.31 shows the effluent nitrogen concentration for longer sludge ages, keeping constant a.

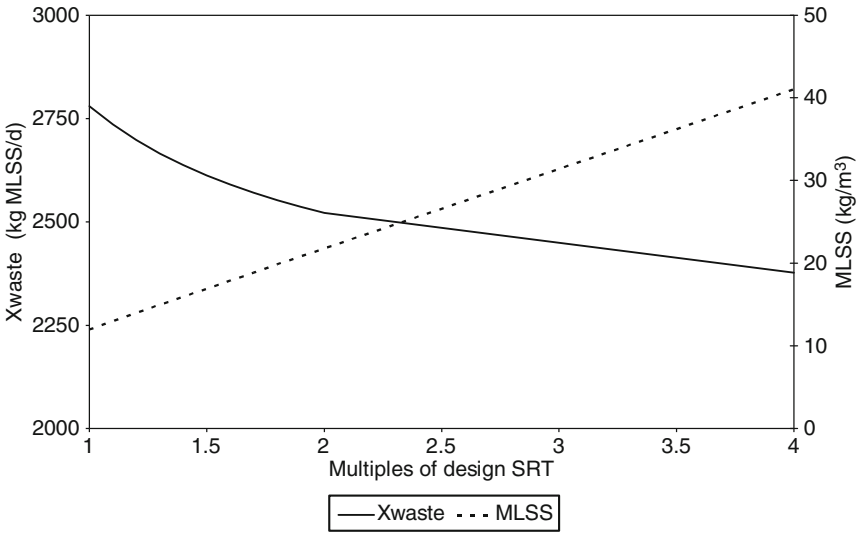


Fig. 10.30. Estimated sludge production and MLSS concentration for different SRTs.

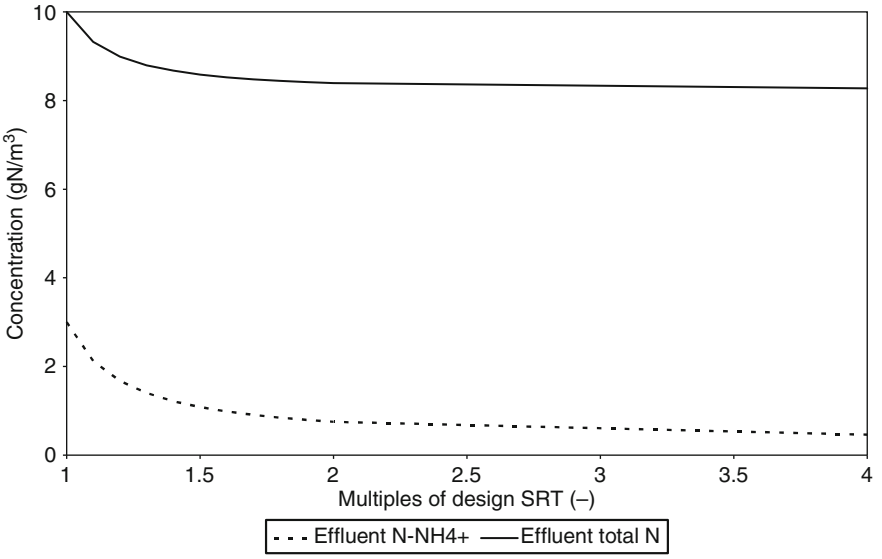


Fig. 10.31. Effluent ammonia and total nitrogen concentration for the membrane bioreactors sized in Sect. 4.5.1 under different sludge age values.

The behaviour of the so sized plant can be easily verified in terms of effluent quality during summer conditions (20°C), by modifying kinetics of heterotrophs and autotrophs. The effluent ammonia is given by rearranging Eq. (65):

$$\mathrm{N_{a,effluent}} = \frac{K_{\mathrm{nT}}\left(b_{\mathrm{nT}} + \frac{1}{\mathrm{SRT}}\right)}{\mathrm{SF}\mu_{\mathrm{nT,pH,DO_a}}(1 - f_{\mathrm{anoxic}}) - \left(b_{\mathrm{nT}} + \frac{1}{\mathrm{SRT}}\right)}$$

$$= \frac{0.98\,\mathrm{g\,N/m^3}\left(0.04/\mathrm{day} + \frac{1}{25.24\,\mathrm{days}}\right)}{0.9 \times 0.376/\mathrm{day}\,(1 - 0.448) - \left(0.04 + \frac{1}{25.24\,\mathrm{days}}\right)} = 0.73\,\mathrm{g\,N/m^3}$$

Nitrification capacity of the design volume at 20°C is $37\,\mathrm{g\,N/m^3}$ (Eq. (84), where all the involved terms influenced by temperature have been determined at 20°C). Therefore, assuming $a = 4.28$, effluent nitrate concentration is $7.0\,\mathrm{g\,N/m^3}$, which gives a total effluent nitrogen of $8.23\,\mathrm{g\,N/m^3}$. Hence, both ammonia and total nitrogen are largely compatible with the standards required. An optimization can be carried out with a conventional spreadsheet in order to reduce a (i.e. reduce energy consumption) and obtain the required effluent total N concentration, at 25.24 days sludge age; then, the optimal recirculation ratio is 3.22, as shown in Fig. 10.32.

Obviously, ammonia nitrogen is steadily lower than $3\,\mathrm{g\,N/m^3}$ and equal to $0.72\,\mathrm{g\,N/m^3}$, depending only on SRT, biokinetics and f_{anoxic} (all fixed) and not depending on a.

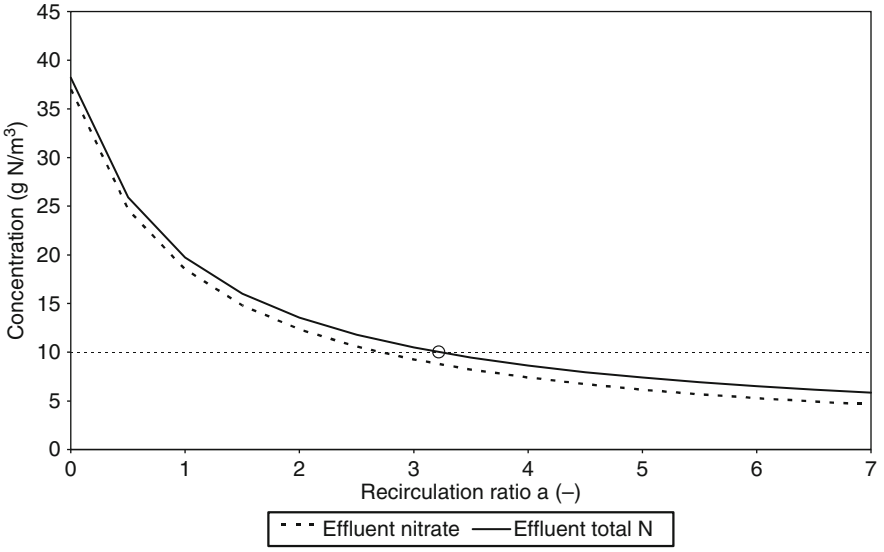


Fig. 10.32. Optimization of the recirculation ratio for the MBR plant sized in Sect. 4.5.1 under summer conditions.

4.5.1.7. Design of CAS Line

The same calculations can be done for CAS line, considering different kinetic parameters, a starting anoxic fraction of 0.35, a recycle ratio from secondary settling tank $r_s = 1$ (with $DO_s = 0\,g\,O_2/m^3$), $T_F = 2.2$ and a first trial a value of 3. The presence of a settler tank (non sized here) able to give a $35\,g\,SS/m^3$ concentration can be assumed. Results obtained after optimization are

- $f_{anoxic} = 0.432$
- $SRT = 16.44\,days$
- $f_{anoxic,min} = 0.066$
- $f_{anoxic,max} = 0.617$
- $a = 5.53$
- $X_{active} = 9,403\,kg\,VSS$
- $X_{i,VSS} = 11,200\,kg\,VSS$
- $X_{endogenous} = 6,076\,kg\,VSS$
- $X_{volatile} = 26,679\,kg\,VSS$
- $V_{nitrification} = 6,189.9\,m^3$
- $V_{denitrification} = 4,699.6\,m^3$
- $V_{total} = 10,889.5\,m^3$
- $HRT = \frac{10,889.5\,m^3}{12,000\,m^3/day} \times 24\,h/day = 21.78\,h$
- $F/M = \frac{Q_{ave} S_{ti}}{X_{volatile}} = \frac{12,000\,m^3/day \times 560\,g\,COD/m^3}{26,679\,kg\,VSS \times 1,000\,g\,COD/kg\,COD} = 0.251\,kg\,COD/(kg\,MLSS\,day)$
- $N_{capacity} = 30.53\,g\,N/m^3$
- $D_{potential} = 30.35\,g\,N/m^3$

- Effluent total COD $S_{t,out} = 64.3\ g\ COD/m^3$
- Effluent ammonia nitrogen $N\text{-}NH_{4\ out}^{+} = 3\ g\ N/m^3$
- Effluent TKN $= 5.95\ g\ N/m^3$
- Effluent nitrate $N\text{-}NO_{3\ out}^{-} = 4.05\ g\ N/m^3$
- Effluent total nitrogen $N_{t,out} = 10\ g\ N/m^3$
- Daily oxygen consumption $O_{total} = 4,748.4\ kg\ O_2/day$
- $Q_{air,biol} = 59.65\ N\ m^3/h$ (assuming $\alpha_F = 0.8$)
- Daily sludge production $X_{waste} = 2,317.63\ kg\ MLSS/day$
- Specific daily sludge production $X_{waste,spec} = 0.39\ kg\ MLSS/kg\ COD_{removed}$

Assuming an α_F of 0.8, the air flowrate can be estimated with the above-described method. The final value is $54.7\ N\ m^3/h$.

4.5.2. Some Design Evaluations

As shown in the design example, although higher operational MLSS concentration, the MBR volume is not proportionally reduced with respect to CAS one, because of the different kinetic parameters assumed for nitrification. In fact, MBR process seems to have a negative effect on nitrifiers activity, as found in the literature (70–72), because of various reasons ranging from lower F/M ratio to predation phenomena by protozoa and metazoa on dispersed nitrifying bacteria. This suggests to adopt more cautious values for μ_{nm}, and if it is possible, to carry out an accurate experimental determination of nitrifying biokinetics before sizing the biological process, using a pilot plant fed with the wastewater to be treated.

Moreover, the higher VSS concentration (i.e. the lower F/M ratio) leads to significant changes in terms of overall microbial activity, as shown by several authors (70, 73–76). Such effect is well pointed out by considering Eqs. (70) and (71); the impact of solid retention time on both active biomass fraction and inert VSS content is graphed in Fig. 10.33. The longer the sludge age, the bigger the inert matter accumulation and the smaller the active VSS fraction.

For a given system, such considerations can be easily applied also to nitrifiers, for which a mathematical expression analogue to Eq. (70) is

$$X_{nitrifiers} = \frac{Q_{bpdf}(N\text{-}NO_3^-)_{out} Y_N SRT_{op}}{(1 + b_{nT})} \tag{106}$$

where $X_{nitrifiers}$ is the mass of nitrifying microorganisms (kg nVSS) and SRT_{op} (days) is the operational sludge age, calculated according to the daily volumetric sludge withdrawal Q_{waste} chosen by the operator. Then, two nitrifying fractions can be determined: the apparent nitrifying fraction, expressed as the ratio between $X_{nitrifiers}$ and $X_{volatile}$ and the actual nitrifying fraction, given by the ratio of $X_{nitrifiers}$ and the active heterotrophic biomass X_{active}. It is

$$f_{nit,app} = \frac{X_{nitrifiers}}{X_{volatile}} \tag{107}$$

$$f_{nit,actual} = \frac{X_{nitrifiers}}{X_{active}} \tag{108}$$

The trend of such two fractions vs. SRT permits to observe the impact of sludge age on nitrification performances. Long sludge age allow a gradual increase of $f_{nit,actual}$ while, in

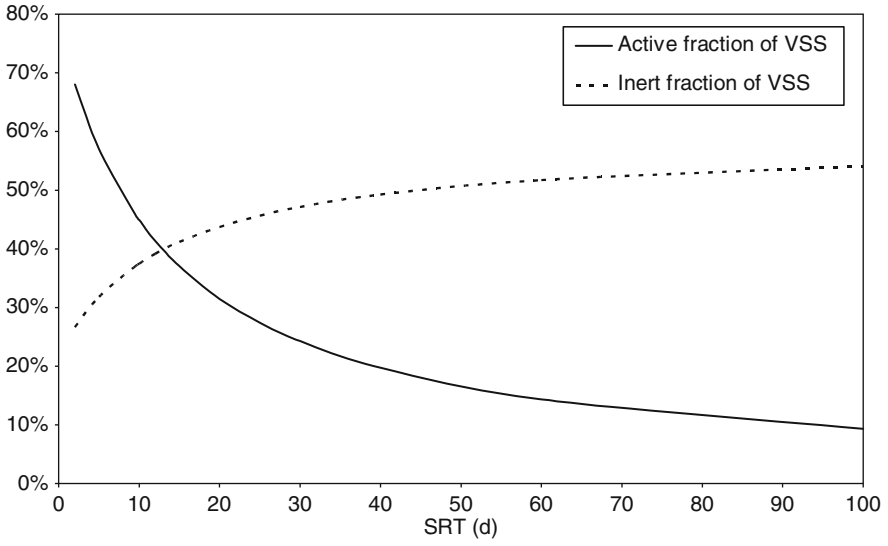


Fig. 10.33. Active and inert fractions of VSS for different sludge ages.

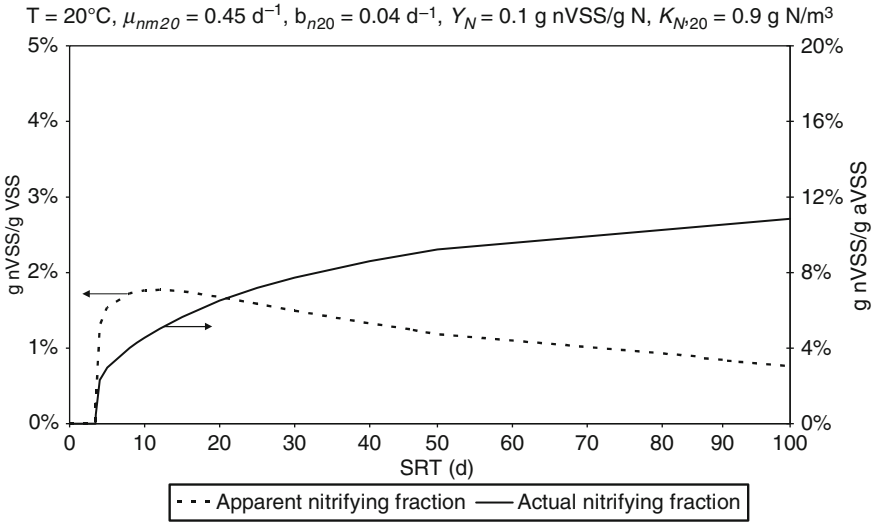


Fig. 10.34. Actual and apparent nitrifying fractions for various values of SRT.

terms of apparent nitrifying fraction, an optimum sludge age can be identified such that the accumulation of inert matter does not affect too much nitrification (Fig. 10.34).

The model described also permits to evaluate effluent ammonia and nitrate concentrations, under different values of sludge age, using Eqs. (65) and (93). For instance, in a only nitrifying MBR plant ($f_{\text{anoxic}} = 0$, $a = 0$, $r_s = 0$) operating at 20°C, N-NH_4^+ and N-NO_3^- in effluent are

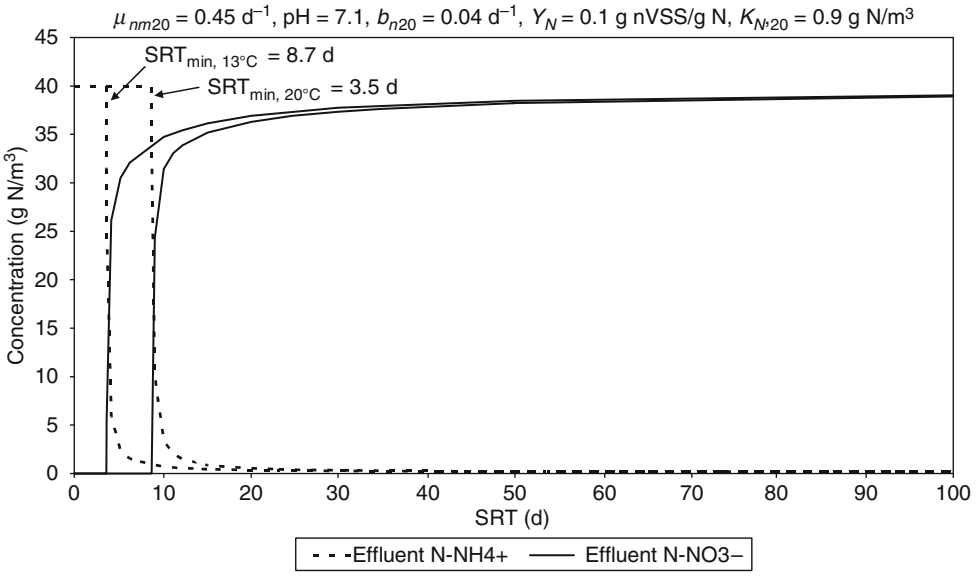


Fig. 10.35. Impact of SRT on the nitrogen removal efficiency of a nitrification scheme, under winter and summer conditions.

described in Fig. 10.32. If a lower value of temperature is considered, a longer sludge age has to be ensured in order to achieve nitrification (Fig. 10.35).

NOMENCLATURE

a = Aerated mixed liquor recirculation ratio
A = Empirical parameter (($m^3/m^2/s$) or ($m^3/m^2/day$) or ($L/m^2/h$))
A_d = Cross-sectional areas of downcomer (m^2)
$A_{membrane}$ = Filtering surface area (m^2)
$a_{optimum}$ = Optimal recirculation ratio for aerated mixed liquor
A_r = Cross-sectional areas of riser (m^2)
aVSS = Active volatile suspended solids (kg VSS/m^3)
b = Biomass decay rate (1/day)
B = Empirical parameter (($m^3/m^2/s$) or ($m^3/m^2/day$) or ($L/m^2/h$))
b_h = Heterotrophic decay rate (1/day) b_{hT} = Heterotrophic decay rate at the process temperature T (1/day)
b_n = Autotrophic decay rate (1/day)
b_{nT} = Autotrophic decay rate at the process temperature T (1/day)
BOD = Biochemical oxygen demand (g BOD/m^3)
c = Empirical constant dependent on TMP
C_B = Solute concentration in the bulk (kg/m^3)
C_G = Solute concentration in the gel layer (kg/m^3)

C_O = Oxygen fraction in air (kg/m^3_{air})
COD = Chemical oxygen demand (g COD/m^3)
D = Brownian diffusion coefficient (m^2/s)
$D_{capacity}$ = Denitrification capacity (g N/m^3)
d_p = Mean pore diameter (m)
$D_{potential}$ = Denitrification potential (g N/m^3)
DO = Dissolved oxygen concentration (g O_2/m^3)
DO_a = Dissolved oxygen concentration in the aeration tank (g O_2/m^3)
DO_s = Dissolved oxygen concentration in the settled recirculated sludge (g O_2/m^3)
DOC = Dissolved organic carbon, g/m^3
E = Aeration device efficiency
f = Endogenous residue
f_{anoxic} = Unaerated fraction of the process volume
$f_{anoxic,max}$ = Maximum anoxic fraction of the process volume
$f_{anoxic,min}$ = Minimum anoxic fraction of the process volume
f_{cv} = COD/VSS ratio (g COD/g VSS)
f_i = VSS/MLSS ratio in the influent (g VSS/g MLSS)
f_{VSS} = VSS/MLSS ratio in the sludge (g VSS/g MLSS)
f_n = Nitrogen fraction in influent non-biodegradable COD expressed as VSS (g N/g VSS)
f_{na} = Free ammonia fraction in influent TKN
$f_{nit,actual}$ = Actual fraction of nitrifying microorganisms (kg nVSS/kg aVSS)
$f_{nit,app}$ = Apparent fraction of nitrifying microorganisms (kg nVSS/kg VSS)
f_{pb} = Particulate biodegradable fraction of influent COD
f_{pnb} = Particulate non-biodegradable fraction of influent COD
f_{sb} = Soluble biodegradable fraction of influent COD
f_{snb} = Soluble non-biodegradable fraction of influent COD
f_{snbN} = Soluble non-biodegradable fraction of influent nitrogen
f_v = VSS/MLSS ratio in the effluent (g VSS/g MLSS)
g = Gravity acceleration (m/s^2)
H = Altitude (m)
h_1 = Depth at the top of the module (m)
h_2 = Depth at the bottom of the module (m)
h_D = Gas–liquid dispersion height (m)
HRT = Hydraulic retention time (h) or (day)
I_{40} = Filterability index
J = Permeate flux (($m^3/m^2/s$) or ($m^3/m^2/day$) or ($L/m^2/h$))
J_0 = Permeate flux at the beginning of the filtration process (($m^3/m^2/s$) or ($m^3/m^2/day$) or ($L/m^2/h$))
J_{20} = Permeate flux at 20°C (($m^3/m^2/s$) or ($m^3/m^2/day$) or ($L/m^2/h$))
J_{bd} = Back-diffusive flow from the membrane towards the bulk (($m^3/m^2/s$) or ($m^3/m^2/day$) or ($L/m^2/h$))
J_{conv} = Convective flux towards the membrane (($m^3/m^2/s$) or ($m^3/m^2/day$) or ($L/m^2/h$))

J_{crit} = Critical flux (($m^3/m^2/s$) or ($m^3/m^2/day$) or ($L/m^2/h$))
J_{gross} = Gross permeate flux (($m^3/m^2/s$) or ($m^3/m^2/day$) or ($L/m^2/h$))
$J_{max\,20}$ = Maximum permeate flux at 20°C (($m^3/m^2/s$) or ($m^3/m^2/day$) or ($L/m^2/h$))
J_{net} = Net permeate flux (($m^3/m^2/s$) or ($m^3/m^2/day$) or ($L/m^2/h$))
J_T = Permeate flux at the process temperature T (($m^3/m^2/s$) or ($m^3/m^2/day$) or ($L/m^2/h$))
k = Mass transfer coefficient (m/s)
K_1 = Denitrification rate associated to RBCOD consumption (g $N\text{-}NO_3^-$/g VSS/day)
K_{1T} = Denitrification rate associated to RBCOD consumption at the process temperature T (g $N\text{-}NO_3^-$/g VSS/day)
K_2 = Denitrification rate associated to SBCOD consumption (g $N\text{-}NO_3^-$/g VSS/day)
K_{2T} = Denitrification rate associated to SBCOD consumption at the process temperature T (g $N\text{-}NO_3^-$/g VSS/day)
K_3 = Denitrification rate associated to endogenous substrate consumption (g $N\text{-}NO_3^-$/g VSS/day)
K_{3T} = Denitrification rate associated to endogenous substrate consumption at the process temperature T (g $N\text{-}NO_3^-$/g VSS/day)
K_B = Coefficient of frictional loss in the bottom region
K_g = Geometric coefficient
K_La = Oxygen transfer coefficient (1/h)
K_n = Half-saturation constant for TKN (g N/m^3)
K_{nO} = Half-saturation constant for TKN at the dissolved oxygen concentration DO (g N/m^3)
K_{npH} = Half-saturation constant for TKN at the process pH (g N/m^3)
K_{nT} = Half-saturation constant for TKN at the process temperature T (g N/m^3)
$K_{n,T,pH}$ = Half-saturation constant for TKN at the process temperature T and the process pH (g N/m^3)
K_O = Oxygen half-saturation constant for autotrophs (g O_2/m^3)
K_S = Half-saturation constant for RBCOD (g COD/m^3)
K_{ST} = Half-saturation constant for RBCOD at the process temperature T (g COD/m^3)
$K_{substrate}$ = Half-saturation constant for the considered substrate (g/m^3)
K_T = Coefficient of frictional loss in the top region
M = Molecular weight of air (kg/kmol)
MLSS = Mixed liquor suspended solids concentration (kg $MLSS/m^3$)
$N_{a,effluent}$ = Ammonia concentration in effluent wastewater (g N/m^3)
$N_{a,out}$ = Required (by law limits) effluent ammonia concentration (g N/m^3)
N_{ai} = Influent free ammonia concentration (g N/m^3)
$N_{capacity}$ = Nitrification capacity (g N/m^3)
$N\text{-}NO_{3\ out}^-$ = Effluent nitrate concentration (g N/m^3)
N_{ni} = Influent nitric nitrogen (g N/m^3)
N_{oi} = Influent biodegradable organic nitrogen (g N/m^3)
N_{pnbi} = Influent particulate non-biodegradable organic nitrogen (g N/m^3)
$N_{p,out}$ = Effluent particulate non-biodegradable organic nitrogen associated to effluent VSS (g N/m^3)
N_{snbi} = Influent soluble non-biodegradable organic nitrogen (g N/m^3)

$N_{snb,out}$ = Effluent soluble non-biodegradable organic nitrogen (g N/m^3)
$N_{synthesis}$ = Nitrogen consumption for cell synthesis (g N/m^3)
N_{ti} = Influent TKN (g N/m^3)
$N_{t,out}$ = Effluent total nitrogen (g N/m^3)
nVSS = Nitrifying volatile suspended solids (kg VSS/m^3)
O_{20} = Saturation oxygen concentration in clean water at 20°C and at the sea level (g O_2/m^3)
$O_{ave,T,H}$ = Average oxygen transfer in clean water at temperature T and altitude H (g O_2/m^3)
O_d = Daily oxygen recover due to denitrification (kg O_2/day)
O_n = Daily required oxygen for nitrification (kg O_2/day)
O_{s+e} = Daily required oxygen for cell synthesis and endogenous respiration (kg O_2/day)
O_t = Percentage of oxygen leaving the aeration tank
$O_{T,H}$ = Saturation oxygen concentration in clean water at temperature T and altitude H (g O_2/m^3)
$O_{T,sea\ level}$ = Saturation oxygen concentration in clean water at temperature T and at the sea level (g O_2/m^3)
O_{total} = Daily required oxygen mass (kg O_2/day)
P_{feed} = Pressure on the feed side (Pa)
P_H = Atmospheric pressure at altitude H (Pa)
P_{in} = Inlet feed pressure (Pa)
P_{out} = Outlet feed pressure (Pa)
$P_{permeate}$ = Pressure on the permeate side (Pa)
$P_{w,depth}$ = Pressure at the air release point due to hydraulic head above the diffusers (Pa)
$P_{sea\ level}$ = Atmospheric pressure at sea level (Pa)
Q = Permeate flow (m^3/s)
$Q_{air,biol}$ = Air flow rate required for the biological process (N m^3/h)
Q_{ave} = Daily average flow (m^3/day)
Q_{BP} = Permeate back-pulse flow ((m^3/s) or (L/s))
Q_{bpdf} = Biological process design flow ((m^3/h) or (m^3/day))
Q_{dwmf} = Maximum dry weather flow (m^3/h)
$Q_{suction}$ = Permeate flow during the suction phase ((m^3/s) or (L/s))
Q_{waste} = Daily flow of waste sludge (m^3/day)
Q_{wwbf} = Biologically treated flow during wet weather (m^3/h)
Q_{wwmf} = Mechanically treated flow during wet weather (m^3/h)
R = Universal gas constant (J/kmol/K)
RBCOD = Readily biodegradable COD (g COD/m^3)
R_C = Cake resistance (1/m)
R_{CP} = Resistance due to concentration polarization (1/m)
R_F = Resistance due to fouling (1/m)
R_M = Intrinsic membrane resistance to filtration (1/m)
r_s = Sludge recirculation ratio from the settling tank
R_T = Overall resistance to filtration (1/m)
Re = Reynolds number
S = Substrate concentration (g substrate/m^3)

SBCOD = Slowly biodegradable COD (g COD/m^3)
S_{bi} = Biodegradable influent COD concentration (g COD/m^3)
SF = Safety factor for maximum growth rate of nitrifiers
S_m = Specific surface area/volume ratio (1/m)
S_{nbi} = Non-biodegradable influent COD concentration (g COD/m^3)
SO = Oxygen transfer rate under standard conditions in tap water ($T = 20°C$, $P = 1$ atm.) (kg O_2/day)
S_{pbi} = Particulate biodegradable influent COD concentration (g COD/m^3)
S_{pnbi} = Particulate non-biodegradable influent COD concentration (g COD/m^3)
SRT = Solids retention time (sludge age) (day)
SRT_{design} = Design sludge age (day)
SRT_{op} = Operational sludge age (day)
SS = Suspended solids concentration (g SS/m^3)
S_{sbi} = Soluble biodegradable influent COD concentration (g COD/m^3)
S_{snbi} = Soluble non-biodegradable influent COD concentration (g COD/m^3)
$S_{t,out}$ = Effluent total COD concentration (g COD/m^3)
S_{ti} = Total influent COD concentration (g COD/m^3)
T = Temperature ((K) or (°C) according to the specific indications)
t_{BP} = Duration of backwashing (s)
T_F = Thickening factor
t_{pause} = Duration of the relaxation phase (s)
$t_{suction}$ = Duration of the suction phase (s)
TKN_{out} = Effluent TKN (g N/m^3)
TMP = Transmembrane pressure (Pa)
TOC = Total organic carbon (g/m^3)
U_{Lr} = Superficial liquid velocity in the riser zone of a given airlift reactor (m/s)
v = Substrate removal rate per biomass unit (g substrate/g VSS/day)
$\bar{v}$ = Maximum substrate removal rate per biomass unit (g substrate/g VSS/day)
V^* = Permeate volume per unit area (m^3/m^2)
$V_{denitrification}$ = Denitrification process volume (m^3)
$V_{nitrification}$ = Nitrification process volume (m^3)
VSS = Volatile suspended solids concentration (kg VSS/m^3)
$\bar{v}_{ST}$ = Maximum removal rate for readily biodegradable substrate specific per biomass unit (g substrate/g VSS/day)
V_{total} = Overall process volume (m^3)
x = Biomass concentration (kg VSS/m^3)
X_{active} = Mass of active biomass (kg VSS)
$X_{endogenous}$ = Mass of endogenous VSS (kg VSS)
X_{ii} = Particulate non-biodegradable COD expressed as VSS (g VSS/m^3)
$X_{i,VSS}$ = Mass of inert VSS (kg VSS)
$x_{nitrifiers}$ = Concentration of nitrifying microorganisms (kg VSS/m^3)
$X_{nitrifiers}$ = Mass of nitrifying microorganisms (kg VSS)
$x_{t,out}$ = Effluent total solids concentration (g MLSS/m^3)

X_{total} = Mass of MLSS (kg VSS)
$x_{volatile}$ = Concentration of VSS in the biological process tank (kg VSS/m^3)
$X_{volatile}$ = Mass of VSS in the biological process tank (kg VSS)
X_{waste} = Daily sludge production (kg MLSS/day)
Y = Cellular yield coefficient (g VSS/g substrate)
Y_h = Heterotrophic cells yield coefficient (g VSS/g COD)
Y_N = Autotrophic cells yield coefficient (g nVSS/g N)
Δx = Membrane thickness (m)
Φ = Coefficient for R_C determination (s^2/kg)
α = Specific cake resistance (m/kg)
α_F = Alpha factor for oxygen transfer
β = Salinity coefficient
δ = Thickness of the concentration boundary layer (m)
ε = Cake porosity
ε_d = Frictional gas-holdup in the downcomer
ε_m = Membrane porosity
ε_r = Frictional gas-holdup in the riser
η = Permeate viscosity (Pa s)
η_T = Permeate viscosity at the process temperature T (Pa s)
μ = Biomass growth rate (1/day)
$\bar{\mu}$ = Maximum bacterial growth rate under unlimited substrate availability conditions (1/day)
μ_{hm} = Maximum heterotrophic growth rate (1/day)
μ_{hmT} = Maximum heterotrophic growth rate at the process temperature T (1/day)
μ_{nm} = Maximum autotrophic growth rate (1/day)
μ_{nmO} = Maximum autotrophic growth rate at the dissolved oxygen concentration DO (1/day)
μ_{nmpH} = Maximum autotrophic growth rate at the process pH (1/day)
μ_{nmT} = Maximum autotrophic growth rate at the process temperature T (1/day)
μ_{nO} = Actual autotrophic growth rate at the dissolved oxygen concentration DO (1/day)
μ_{nT} = Autotrophic growth rate at the process temperature T (1/day)
$\mu_{n,T,pH,O}$ = Autotrophic growth rate at the process temperature T under process dissolved oxygen concentration DO and the process pH (1/day)
ρ = Density of feed suspension (kg/m^3)

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11
Closed Ecological Systems, Space Life Support and Biospherics

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CONTENTS

Abstract This chapter explores the development of a new type of scientific tool – man-made closed ecological systems. These systems have had a number of applications within the past 50 years. They are unique tools for investigating fundamental processes and interactions of ecosystems. They also hold the potentiality for creating life support systems for space exploration and habitation outside of Earth's biosphere. Finally, they are an experimental method of working with small "biospheric systems" to gain insight into the functioning of Earth's biosphere. The chapter reviews the terminology of the field, the history and current work on closed ecological systems, bioregenerative space life support and biospherics in Japan, Europe, Russia, and the United States where they have been most developed. These projects include the Bios experiments in Russia, the Closed Ecological Experiment Facility in Japan, the Biosphere 2 project in Arizona, the MELiSSA program of the European Space Agency as well as fundamental work in the field by NASA and other space agencies. The challenges of achieving full closure, and of recycling air and water and producing high-production crops for such systems are discussed, with examples of different approaches being used to solve these problems. The implications for creating sustainable technologies for our Earth's environment are also illustrated.

Key Words Life support • biospherics • bioregenerative • food • air • water recycling • microcosm • closed ecological systems • Bios • NASA • CEEF • Biosphere 2 • BIO-Plex.

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1. INTRODUCTION

In the past few decades, it has become clear that our increasingly technological civilization is coming into greater conflict with the world of life, the biosphere of planet Earth. The modern technosphere is impacting adversely both vast areas of regional ecosystems and the reservoirs of the global environment, which we now appreciate are the life support systems for humans and all living creatures. The necessity to apprehend the laws of development of the biosphere (as a single whole) and for the human civilization to join it harmoniously is becoming more and more obvious and pressing.

It should also be clear that while we must study the biosphere, we have no right to conduct experiments that will endanger it. However, these researches can be done with small models, i.e., with artificial ecological systems. A new type of scientific object of study: Materially-closed ecological systems (CES) with different degrees of complexity and closure have emerged in the past half century coinciding with the advent of spaceflight. On such model ecosystems we can (and must) study both the particular laws of development of individual elements and components of the ecosystems, and the general regularities in the development of the entire biotic turnover. A new scientific discipline, Biospherics (biospherology), is being formed (1, 2). It is an integrative discipline, drawing on a number of fields of study, from biology, physiology, ecology, microbiology to engineering and social sciences. It is allied with ecological engineering in that engineering and ecology are brought together with the intent of maximizing natural ecological functions for achieving goals and solving environmental problems. Biospherics has the potential to develop the scientific basis for harmonizing the relationship of humanity, technology, and nature, and to open the path to the noosphere (the sphere of intelligence). Artificial ecological systems, from simple laboratory microsystems to more sophisticated human life-support systems (LSS) under extreme conditions on Earth and in Space, are one of its principal objectives. Biospherics is international in its scope and must be multi-disciplinary, using the achievements of many individual sciences. To design, construct, and study artificial "biospheres," it is necessary to intelligently design and manage the biogeochemical flows of matter and energy, to use sophisticated technologies and computer/information systems, to incorporate the achievements of genetics, biotechnology, and bioengineering and to make use of time-tested and reliable natural ecological mechanisms.

The needs of the current stage of development of civilization in the field of biospherics:

1. To create working models of the Earth's biosphere and its ecosystems and thus to better understand the regularities and laws that control its life. This is especially important because the Earth's biosphere is presently under ecological stress on a global scale.
2. To create artificial biospheres for human life support beyond the limits of the Earth's biosphere. These are essential for permanent human presence in space.
3. To create ground-based life-support systems that provide high quality of life in extreme conditions of the Earth's biosphere, such as polar latitudes, deserts, mountains, underwater, etc.
4. The use of artificial ecological systems offers the prospect of developing technologies for the solution of pollution problem in our urban areas and for developing high yield sustainable agriculture.

One of the principal motivations behind the creation of systems which are isolated from the general environment of Earth is to learn how to make life support systems and artificial biospheres that can regenerate, reuse, and recycle the air, water, and food normally provided by the Earth's biosphere. This is essential if we are to live outside the support of Earth's biosphere, rather than just travel in space.

Research and technological developments of these biological life support systems have enormous interest and relevance to sustainability in the environment on Earth. The conversion of natural ecosystems to agricultural areas, the loss of biodiversity, and the depletion of resources worldwide have raised questions concerning the increasing loss of life support capability for the biosphere as a whole and the impacts of the loss of ecosystems and species. The increased awareness of the ecological challenges facing humanity has led to a dramatically changed perspective of how we should regard our global biosphere. These perspectives and the focus on sustainable ways of living on the Earth have direct parallels with the challenges of developing closed ecological systems and bioregenerative life support technologies for space applications. In closed ecological systems, the emphasis is on recycle and reuse and not on the supply of new life support essentials. Research with materially closed ecosystems can thus help with the paradigm change from the destructive behaviors associated with the mindset of "unlimited resources" to that of conserving, recycling, and sustainably operating (1).

Calculation of the amount of material (air, water, food) needed for human life support is essential for cost benefit analysis, trade-off studies and the determination of when bioregenerative systems for spacecraft and space stations are advantageous compared with the approach used to date in space: Physiochemical technical systems and water, air, and food from stored supplies and resupply from Earth.

Such calculations are difficult and have yielded quite differing results. Requirements for food will vary depending on the weight and metabolism of different individuals; and water usage also depends on many factors. Furthermore, calculations based on terrestrial experience may differ from those encountered in the reduced gravity of space. Such studies have concluded that "in the course of a year, the average person is calculated to consume food three times his body weight oxygen, four times his weight, and drinking water eight times his weight. Over the course of lifetime, these materials would amount to exceed 1,000 times an adult's weight" (3).

The implications of these calculations are clear: Extended, and certainly, permanent human presence in space makes necessary "closing the loop" in the regeneration of air, food, and water involved in human life support (Table 11.1).

2. TERMINOLOGY OF CLOSED ECOLOGICAL SYSTEMS: FROM LABORATORY ECOSPHERES TO MANMADE BIOSPHERES

The emerging science of biospherics deals with the functioning of a variety of ecological systems which vary in size, degree of material closure and complexity as measured by size and complexity of their internal ecosystems. The following review of terminology for constructed (synthetic) ecosystems was reached among some of the leading researchers in the field at the

Table 11.1
Inputs required to support a person in space (1)

Inputs	1 day (kg/person)	1 year (kg/person)	Lifetime (kg/person)
Food (dry)	0.6	219	15,300
Oxygen	0.9	329	23,000
Drinking water	1.8	657	46,000
Sanitary water	2.3	840	58,800
Subtotal	5.6	2,045	143,100
Domestic water	16.8		
Total	22.4		

Second International Workshop on Closed Ecological Systems held at Krasnoyarsk, Siberia, in September 1989 (4, 5).

2.1. Materially-Closed Ecospheres

Folsome and colleagues pioneered small laboratory-sized systems (generally 100 ml to 5 l flasks), which differ from ecological microcosms and mesocosms is that they are essentially materially-closed (less the leak rate) (6–8). By contrast, ecological microcosms/mesocosms (the miniaturized ecosystems that ecologists use) are not completely isolated and were developed to permit study in the laboratory of small ecosystems taken from or imitating natural ecosystems (9). These ecological micro- and mesocosms are open to interchange with the surrounding air, and generally require inputs of nutrients and water to replace those lost by evaporation. Folsome, therefore, saw his laboratory flasks as heralding a new type of object – the materially-closed ecosystem. To differentiate these laboratory-sized systems from systems large enough to provide human life support, we can call them "materially-closed ecospheres." They are open to energetic input (indirect sunlight or artificial lighting) and information exchange (monitoring, sensors, and observation).

2.2. Bioregenerative Technology

Technology capable of providing life support resources (food, air, water) that use biological mechanisms, even if enhanced and supported by other technology, may be termed "bioregenerative technology." Examples are plant growth chambers in which a particular crop is grown that regenerates part of its atmosphere, purifies some quantity of water through transpiration, and produces food; or a wastewater processing unit in which aquatic plants and microbes digest sewage or graywater, producing biomass/edible crops as well as air and water regeneration. Bioregenerative technologies are crucial components of both CELSS and closed ecological life support systems.

2.3. Controlled Environmental Life Support Systems

All systems designed for space life support will rely on technology as well as biology – for controlling temperature, pumping air and water, processing food, etc. Such life support systems, only partially bioregenerative, use physiochemical means of handling wastes and

producing required food, air, and water. Hence, for short-duration missions and early phases of developing space life support systems when CELSS-type systems are used, some food, air, and water will be carried from Earth or stored as a backup for emergencies or failure of other regenerative systems. CELSS provide the desired range of temperatures, humidity, carbon dioxide, pH, nutrient solution (most CELSS systems have used hydroponics as the plant-growing technology), and a high intensity of artificial lights for maximal crop performance. However, a portion of the necessary life support materials may be provided by stored supplies and/or physiochemical methods of recycling or cleanup (e.g., lithium hydroxide canisters for CO_2 removal, catalytic oxidizers for trace gas metabolism, or vapor compression distillers and membrane technology for water revitalization) rather than using only biological methods for their uptake and regeneration.

2.4. Closed Ecological Systems for Life Support

A life support system that approaches complete internal sustainability and which is biologically-based is termed a closed ecological system, meaning that it is essentially materially closed, energetically and informationally open, and recycling its major elements and nutrients. Both the CELSS and Closed Ecological Systems have generally included just a few species of plants and/or algae as their biological component, in addition to the crew compartments and associated mechanical/computer operational technologies. Energetically, such a system must be open or it would decline due to increasing entropy. The light needed for photosynthesis is supplied by artificial lights or by sunlight, direct or delivered through light pipes. A heat sink on the outside receives surplus heat from the system. Usually, it is safer to house the energy-generating unit outside the sealed life support zone. This will also lessen the amount of air-scrubbing that is required if the energy production method produces pollutants. But, while the definition of a closed ecological life support system does not require energy production within its sealed boundary, it is certainly true that lessening energetic requirements and the accomplishment of energy generation in space via solar arrays, nuclear energy, use of extra-terrestrial energy resources, etc.) are important considerations in reducing logistical dependence on resupply from Earth.

2.5. Biospheric Systems

Since both CELSS and closed ecological systems contain essentially only one type of ecosystem – an agricultural one – for human life support, they differ from "biospheric systems," such as the Biosphere 2 project in Arizona, and the Japanese CEEF (Closed Ecology Experimental Facilities) which include a number of internal ecosystems. Biospheric systems are essentially materially closed, energetically and informationally open like a closed ecological life support system, and their internal complexity provides additional buffering capacity for air and water regeneration, and increases the long-term prospects of a system resistant to catastrophic decline, and to enhance the "live-ability" for its human inhabitants. These systems also offer new opportunities for research into the complexity of ecological mechanisms operating in our Earth's biosphere.

Currently, there are some nine experimental ecosystems that can be used to conduct investigations with material cycling closed to a greater or lesser extent. They are: The Ground

Experimental Complex at the Institute of Biomedical Problems in Moscow, Russia; Bios-3 in Krasnoyarsk, Siberia at the Institute of Biophysics; BIO-Plex (Advanced Life support System Test Bed) at the NASA Johnson Space Research Center in Houston, Texas; the NASA Kennedy Space Center "Breadboard" Plant Growth Facility in Florida, Biosphere 2 in Oracle, Arizona; the "Laboratory Biosphere" in Santa Fe, New Mexico; the C.E.B.A.S. aquatic ecosystem at the Ruhr University of Bochum in Germany; the CEEF complex at the Institute of Environmental Sciences in Japan; and the Pilot Plant that is being constructed in the framework of the European program MELiSSA at Universidad Autonoma de Barcelona, Spain.

In this chapter, we will discuss some problems of bioengineering and biotechnology of these closed ecological systems which are of importance for an understanding of their operation and use.

3. DIFFERENT TYPES OF CLOSED ECOLOGICAL SYSTEMS

3.1. Research Programs in the United States

3.1.1. CELSS Program of NASA

The Controlled Environmental Life Support System (CELSS) program was initiated in 1978 by NASA. Three NASA centers were primarily involved: The Kennedy Space Center where the "Breadboard" provided a test bed for plant cultivation experiments in a closed ecological system; the Johnson Space Center focused on food processing and human diets in space, and the Ames Research Center connected with basic research in system controls. Earlier, laboratory experiments with biological regenerative systems were based on monocultures of unicellular organisms, either photosynthetic (*Chlorella*) or chemosynthetic ones (*Hydrogenomonas*). They were not successful in that the systems used did not attain a stable, steady state and could not provide a significant portion of human nutritional needs. That is why NASA and its associated university researchers decided to include traditional agricultural crops, higher plants, as the core element in their bioregenerative life support systems.

The motivations for the use of higher plants include:

1. Crop plants have the capability of fulfilling the basic autotrophic (primary producer of complex organic molecules) link in a closed system, and thereby closing the regenerative loops for CO_2, O_2, and water. This basic equation **Eq. (1)**:

$$6CO_2 + 12H_2O \xrightarrow[\text{Chlorophyll}]{\text{Light(672 Kcal)}} 6(CH_2O) + 6O_2 + 6H_2O \qquad (1)$$

   is complemented by the action of heterotrophs such as humans reversing the equation in their oxidation of complex hydrocarbons (food) and in respiration, producing carbon dioxide, water, and minerals).
2. Unlike unicellular organisms (algae or bacteria), higher plants are easily digested and are customary sources of human food. Extensive literature on terrestrial (i.e., not in a closed environment or in microgravity) human nutritional needs and higher plant composition exists and forms a starting point for designing such systems.
3. Higher plants can purify water through the process of transpiration. Transpiration is the method whereby plants utilize the passage of water to achieve evaporative cooling. This has been

estimated at about 300 g of water evaporated for every gram of CO_2 fixed in photosynthesis. Such water can be condensed from the atmosphere of a closed system.

4. Higher plants also have the capability of processing waste materials from the crew members and other heterotrophs in the system.

The major CELSS plant crop studies included soybeans (by Raper at the North Carolina State University), sweet potatoes by a group (Hill, Mortley et al.) at Tuskegee Institute, white potatoes (by Tibbitts at the University of Wisconsin) and semi-dwarf wheat (by Salisbury and Bugbee at Utah State University). Later, the cultivar called Super-Dwarf wheat was grown under the conditions of space flight aboard the Mir space station and the NASA Space Shuttle in 1995 and 1996/97 (10).

It was shown (Utah State University experiments) that a plant growth area of 13 m^2 of high productivity dwarf wheat can provide the entire caloric requirements (but not all of the nutritional essentials) for one human, can absorb the metabolic carbon dioxide produced by this human and produce enough oxygen to allow the human to oxidize the calories contained in the wheat biomass. The excess oxygen would be equivalent to the amount of photosynthesis required to produce the non-edible biomass of the wheat plants (stems, roots, leaves). Further, production of the non-edible biomass would require more carbon dioxide than is generated by a human in the same period. This discrepancy could be resolved by oxidation of the non-edible biomass, either by a physical chemical process, or by a biological waste digestion system (11).

In 1986, the Breadboard Project (Fig. 11.1) was begun at Kennedy Space Center and active experimentation continued for over a decade. The Breadboard Project had as its goal the demonstration of the scaling-up from previous laboratory-sized research study into the production of food for human life support, water recycling, and atmospheric gas control in its biomass production chamber. Support laboratories investigated associated questions of waste recycling, food preparation, and overall data management. The Biomass Production Chamber (BPC) used is a renovated cylindrical steel hyperbaric facility approximately 3.5 m diameter by 7.5 m high. Originally used in the Mercury program, it has been modified for plant growth by the creation of two floors with eight plant racks and the installation of high pressure sodium lamps. Ventilation of the chamber is accomplished by ducts, which lead into an external air-handling system including filters. Temperature and humidity are controlled by a chilled water system and through atomized water injection. A compressed gas delivery system is used in the manipulation of atmospheric carbon dioxide and oxygen. The best leak rate achieved in the Breadboard BPC was loss of 5% of its volume per day. The configuration of growing areas inside yields a total plant area of 20 m^2. Air turnover in the BPC is about three times a minute, with ventilation air being ducted at the rate of 0.5 m^3/s into the chamber between lights and growing trays. Many years of experimentation involved many of the prime candidate food crops for space life support, along with analysis of atmospheric dynamics inside the closed system (12–15). Table 11.2 presents summary data on some of the candidate space crops grown and the amounts of life support materials produced.

The current NASA CELSS program covers different areas of tasks and purposes: from "Salad Machine" and CELSS Test Facility for using on Space Station to large-scale plant growth chambers, human habitats, and recycling equipment. Recently, a series of experiments

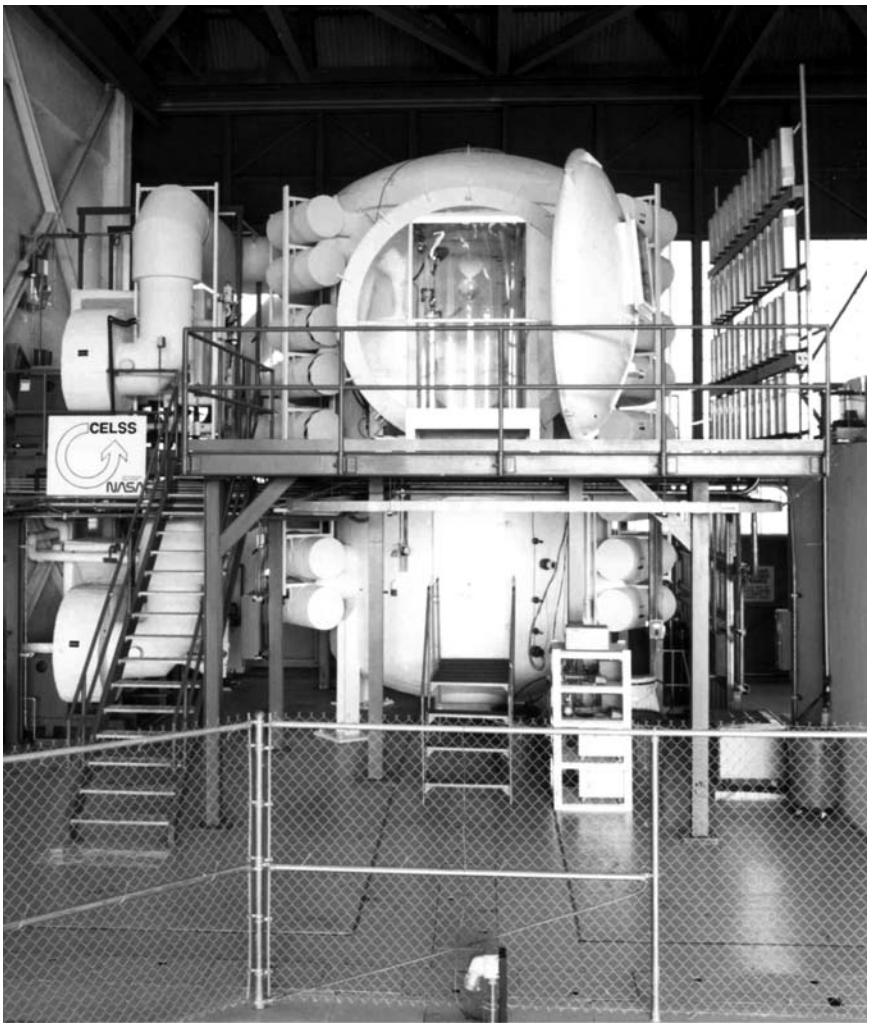

Fig. 11.1. Breadboard Plant Chamber at Hangar L at KSC, FL (front view, 1986). The chamber provided a closed atmospheric volume of about 113 m^3 (including air ducting) with 20 m^2 of crop growing area. External nutrient solution tanks were not in place at the time of this photo (12).

were conducted with the Advanced Life Support System Test Bed (ALSSTB) at the Johnson Space Center. The system is the largest of the NASA life support test systems, and the first in the US to involve humans in a system based on technology using both bioregenerative and physicochemical methods. The system can be considered as an integrative test bed developed from the experience of past NASA life support system development approaches. The three phases of the experiment with a crew were conducted (The principal references to this work: Lunar–Mars Life Support Test Project, Phase II Final Report, 1997; Lunar–Mars Life Support Test Project Phase III Final Report, 1998 (16)).

Table 11.2
Life support outputs of crops grown in the Kennedy Space Center Biomass Production Chamber (BPC) (12)

Crop/date	Days of operation (d)	Total biomass (kg)	Edible biomass (kg)	CO_2^a fixed (kg)	O_2^a produced (kg)	Water collected (kg)
Wheat 881[b]	77	23.06	9.24	35.5	25.8	3,615
Wheat 882[c]	64	26.14	Early harvest	40.3	29.3	5,700[d]
Wheat 891	86	37.76	11.01	58.2	42.3	6,903
Wheat 892	85	44.24	13.12	68.1	50.7	7,809
Wheat 931	85	64.11	18.25	98.7	71.8	7,500[d]
Wheat 941[e,f]	84	66.68	19.07	102.7	74.7	7,600
Soybean 891	90	26.62	8.58	45.0	32.7	7,758
Soybean 901	97	18.94	6.34	32.0	23.3	8,211
Soybean 902	97	20.80	7.79	32.5	25.6	8,450
Soybean 951[f,g]	90	13.51	5.18	22.8	16.6	2,594
Lettuce 901	28	–	Sequential	Harvest	Study	–
Lettuce 902	28	2.84	2.60	4.2	3.1	976
Lettuce 911	28	3.54	3.24	5.2	3.8	998
Lettuce 921	28	3.57	3.36	5.2	3.8	1,000[d]
Lettuce 931[f]	30	3.99	3.71	5.9	4.3	1,074
Potato 911	105	45.58	14.89	68.4	49.7	8,778
Potato 912	90	50.67	22.03	76.2	55.4	9,361
Potato 921	105	55.42	37.64	83.1	60.5	7,954
Potato 931	105	55.88	34.12	83.8	61.0	8,546
Potato 941[f]	418	272	167	409	296	28,446
Tomato 951[f,g]	84	11.03	5.15	16.6	12.1	3,426
Tomato 961	87[h]	33.87	17.06	50.9	37.0	12,700
Total	1991	880	409	1,344	980	149,390

[a] Estimated from total biomass and the percentage of carbon in tissue.
[b] Only the upper half of the chamber used.
[c] 3/4 of available growing area used; plant harvest prior to maturity.
[d] Some missing data; totals estimated by interpolation of water use trend.
[e] Data collected from level four only; water estimated until final data compiled.
[f] Studies where half the plants were grown on recycled nutrients from an aerobic bioreactor.
[g] Simultaneous test with tomato ($10\,m^2$) in half of the chamber and soybean ($10\,m^2$) in the other half.
[h] Upper chamber harvested at 84 days; lower chamber harvested at 91 days.

One purpose of the ALSTB Program is to validate regenerative life support technologies (e.g., air revitalization, liquid and solid waste recycling, active thermal control) through long term testing of integrated biological and physicochemical life support systems with human test subjects. The Lunar–Mars Life Support Test Project (LMLSTP) Phase I Test was performed in August of 1995. The purpose was to obtain engineering and scientific data to demonstrate the ability of a crop of wheat to provide air revitalization for a human test subject for a 15-day period. The test chamber was divided into two sections, the plant growth chamber, and the

airlock that was used as the human habitation chamber. It was successfully demonstrated that an 11.2 m^2 crop of wheat could continuously provide the CO_2 removal and O_2 production functions for the air revitalization needs of a single human test subject for 15 days. But over the short duration of this test, the populations of microorganisms in the internal atmosphere of the chamber increased (This was consistent with experience in Shuttle flights and previous closed chamber tests). No microorganism that would be of concern to human or plant health at the levels measured was identified. Next two (Phase II and Phase II A) experiments with four crew members were aimed to test physicochemical systems of water and air recycling. Both were successful.

A feature of the Phase III 90-days experiment (Fig. 11.2) was integration of biological and physicochemical regenerative processes. The physicochemical systems provided approximately 75% of the air revitalization. The remainder of the air revitalization was provided by a crop of wheat. The test results demonstrated that physicochemical and biological systems can be integrated to provide air revitalization. An integrated water recovery system was operated for 91 days in support of the Lunar–Mars Life Support Test Project Phase III test. The system combined both biological and physicochemical processes to treat a combined wastewater stream consisting of waste hygiene water, urine, and humidity condensate. Biological processes were used for primary degradation of organic materials as well as for nitrification of ammonium in the wastewater. Physicochemical systems removed inorganic salts from the

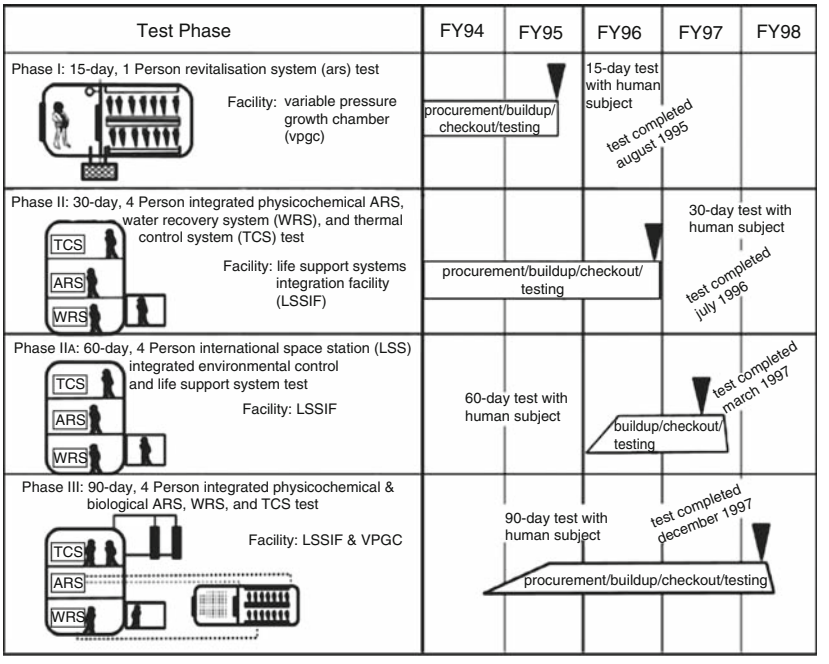

Fig. 11.2. NASA Johnson Space Center, Houston, Texas. LMLSTP 90-day three phase progression of steps for development of life support systems (17).

water and provided post-treatment. The integrated system provided potable water to the crew throughout the test. Overall positive results were obtained during Phase III test, and some difficulties were thoroughly analyzed (17).

Phase III test was the final test in a series of tests conducted to evaluate regenerative life support systems performance over increasingly longer durations. The Phase III test broke new ground for the U.S. Space Program by being the first test to look at the integration of biological and physicochemical systems for air, water, and solid waste recovery for a crew of four for 91 days. Microbial bioreactors were used as the first step in the water recovery system. This biologically based system continuously recovered 100% of the water used by the crew consistent with NASA's strict potable standards. The air revitalization system was a combination of physicochemical hardware and wheat plants which worked together to remove and reduce the crew's metabolically produced carbon dioxide and provide oxygen. In addition, for the first time, the crew's fecal matter was used as a source of carbon to produce carbon dioxide in an incineration system. The carbon dioxide was then used to support the plants for a portion of the test. After harvesting, the wheat was provided to the crew in the form of flour to use in baking bread. Overall, the test successfully demonstrated that biological systems can be integrated as part of a regenerative life support system. The use of plants to provide air revitalization while providing food for the crew and use of microbes to purify the wastewater were successfully demonstrated.

Some difficulties that emerged included better management of nutrient management of staged crops to prevent plant stress. Control systems must be developed to respond to events when the plants are maturing faster or slower than expected and harvests need to occur at different times than predicted. Controls need to be improved to prevent operation in uncontrolled conditions. The Water Recovery System suffers from two major technical problems. Conversion of raw food products to edible material was shown to be critical for using plants for human consumption. While the lettuce was eaten as is, the problems with processing wheat indicate that this is perhaps the tip of the iceberg in the development of food processing systems. Finally, integrated control systems that take into account the overall operation of the whole life support system and make adjustments as necessary without human intervention would have a huge payoff in crew and ground personnel time (17).

A future test complex at NASA Johnson Space Center referred to as ALSSIT (Advanced Life Support Systems Integration Test Bed (formerly known as BIO-Plex) will be the basis for future long-duration human missions on lunar and planetary surfaces. The overall objective of the ALSSIT Project is to support large-scale, long-duration testing of integrated, high fidelity, and biological and physicochemical regenerative life support systems with human test subjects under closed, controlled conditions. Human accommodations will be provided in the habitat to provide for the needs of four crew members and up to eight crew members during 48 h crew changeovers. ALSSIT will be comprised of a series of interconnected chambers with a sealed internal environment outfitted with a system of internally distributed utilities capable of supporting a test crew of four for periods exceeding 1 year. The full configuration calls for a habitat chamber, a life support systems chamber, two biomass production chambers, and a laboratory chamber, all of which will be linked by an interconnecting tunnel with access through an airlock. The multichamber test complex will be monitored and controlled from a nearby

control center. The life support system will perform air revitalization, waste recovery, solid waste processing, thermal management, food production, and integrated command and control functions. ALSSIT will serve as the focal point for other disciplines to conduct research and to develop supporting technologies, techniques, and procedures pertinent to future planetary missions via cooperative and collaborative experimentation and testing. Different human tests are planned for the future.

NASA considers that for expansion of the human experience into the far reaches of space, it becomes imperative to minimize consumables and increase the autonomy of the life support system. Two basic classes of life support systems must be developed, those directed toward applications on transportation/habitation vehicles (e.g., space shuttle, international space station (ISS), next generation launch vehicles, crew-tended stations/observatories, planetary transit spacecraft, etc.) and those for lunar or planetary surfaces. The Advanced Life Support Project Plan was developed to define the Project objectives, Project-level requirements, the management organizations responsible for the Project throughout its life cycle, and Project-level resources, schedules, and controls. This Plan is the top-level document for the Project and provides guidance and direction for its implementation by the participating NASA field centers, namely Ames Research Center (ARC), Kennedy Space Center (KSC), Marshall Space Flight Center (MSFC), and the Johnson Space Center (JSC) serving as the lead center. The Project Plan will be reviewed and updated annually to ensure that the Project remains properly focused and responsive to the goals of the Agency and Biological and Physical Research Enterprise (18). The goal of the Advanced Life Support Project is to provide life support self-sufficiency for human beings to carry out research and exploration safely and productively in space for benefits on Earth and to open the door for extended on-orbit stays and planetary exploration. The five major technical objectives of the Advanced Life Support Project are as follows:

1. Provide Advanced Life Support technologies that significantly reduce life cycle costs, improve operational performance, promote self-sufficiency, and minimize expenditure of resources for long-duration missions.
2. Develop and apply methods of systems analysis and engineering to guide investments in technology, resolve and integrate competing needs, and guide evolution of technologies.
3. Resolve issues of microgravity performance through space flight research and evaluation.
4. Ensure timely transfer of new life support technologies to missions.
5. Transfer technologies to industrial and residential sectors for national benefit.

To accomplish these objectives, the Advanced Life Support Project will conduct a focused Research and Technology Development (R&TD) effort to advance technology readiness of regenerative life support and thermal control components, validate regenerative life support technologies integration through long-term testing with humans, and identify terrestrial applications for life support technologies. JSC, as designated lead center, has delegated the authority and overall Advanced Life Support Project management responsibility to the Engineering Directorate, Crew and Thermal Systems Division (CTSD). CTSD also is responsible for the development of biological and physicochemical subsystem/component (Technical Research Levels 3–6) technologies and flight experiments; the integration of

physicochemical/biological systems technologies, including systems-level testing with humans; and the lead for systems modeling and analysis activities.

As summarized in the Project Technical Summary, advanced life support technologies must:

- Regenerate air, water, and food in a manner that minimizes overall logistical burdens, minimizes demands on space habitat resources, ensures habitability, and promotes self-sufficiency.
- Manage wastes to maintain a safe environment within the habitat and minimize waste storage and buildup, and process wastes to achieve optimum resource recovery, when required.
- Minimize involvement of the crew in life support system operation while assuring proper monitoring and control of essential systems.
- Provide effective environmental monitoring to preclude hazardous conditions (e.g., fire, buildup of toxic contaminants).
- Provide thermal control without the use of expendable heat sinks and without imposing a hazard to the crew.
- Assure prolonged reliability of components and systems.
- Provide for in situ maintenance.
- Minimize the impact of life support on planetary environments.

Figure 11.3 depicts the research and technology development (R&TD) phases for the Project, emphasizing that as the technology and research level (TRL) of candidate technologies increases, the number of options decrease. Thus, the Project will continue to develop only those technologies that show the most promise in terms of meeting mission requirements. Major schedule milestones for the Advanced Life Support Project are based on experiments

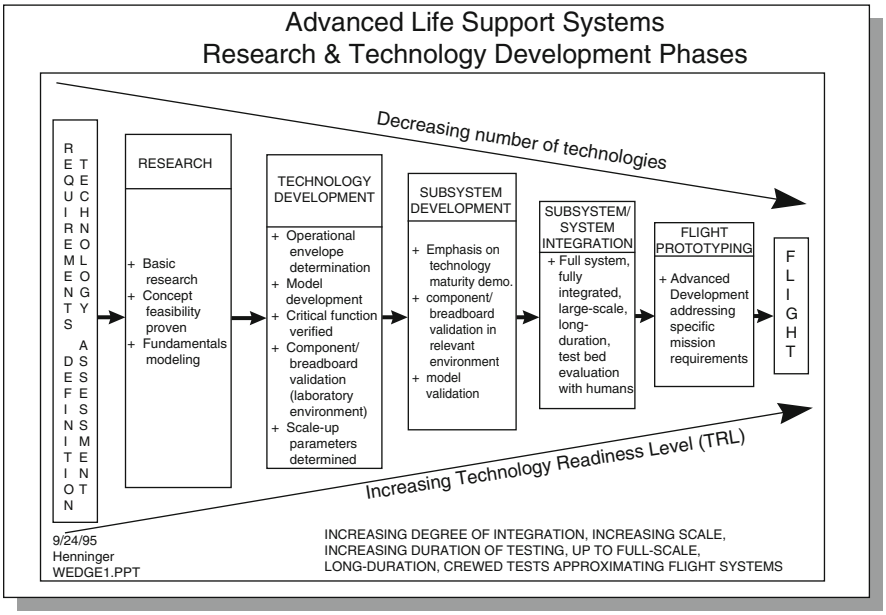

Fig. 11.3. Advanced life support research and technology development phases, NASA JSC (17).

with a modified BIO-Plex. The BIO-Plex facility, located in the Johnson Space Center Building 29 rotunda, is an atmospherically closed multi-chamber test bed large enough to house a crew of four for test durations in excess of 400 days. The multi-chamber facility shall provide sufficient volume within which all life support system test articles for air revitalization, water recovery, biomass production, food processing, solids processing, and thermal control can be located, with the exception of external thermal control system hardware. The multi-chamber facility shall also provide sufficient internal volume to accommodate power, lighting, communications, data network, thermal system, air distribution, water distribution (potable and fire protection), and drainage utility runs throughout the complex. These energy, information, and safety utilities shall be interfaced to the external facility systems via a localized penetration interface for the purposes of simplifying leak detection, increasing aesthetics and maintaining high facility fidelity.

3.1.2. Biosphere Design: Lessons from the Biosphere 2 Experiment

3.1.2.1. Overview of Biosphere 2 Facility

Biosphere 2 is a pioneering $150,000,000, research, and development laboratory designed to study global ecology and to test bioregenerative life support on a biospheric scale. The facility, built in Oracle, Arizona, was designed by Biospheric Design, Inc. to operate on a long-term basis (50–100 years) and to study viability and dynamics of life cycles within a complex seven biome system that included a coral reef, marsh, agriculture, rainforest, savannah, desert, and human habitat. From the years of 1991–1994, the Biosphere 2 facility was essentially materially closed (with an annual air leakage rate under 10%), energetically open to electricity and sunlight, and covered some 1.2 hectares (3.15 acres) in its airtight footprint, including over 200, 000 m^3 (seven million cubic feet) of atmospheric volume (Fig. 11.4). The name Biosphere 2 was chosen to emphasize that the Earth's biosphere (Biosphere 1) was the only biosphere known to science. A detailed list of scientific papers for the first 3 years of its operation (September 21, 1991 to September 6, 1994) can be viewed at www.biospherics.org and in (19).

Biosphere 2 was materially isolated by a skin of steel spaceframe and double laminated glass panels above ground, and by a stainless steel liner underground. Energetically, it was open to sunlight, thermal and electrical energy produced for the operation of its heating, cooling, and other mechanical systems. It was also informationally open to communications media: electronic, radio and visual. The structure of Biosphere 2 included two variable volume chambers ("lungs") permitting expansion/contraction of the internal atmosphere without incurring leakage by keeping the pressure carefully adjusted to the outside pressure. The research and development for Biosphere 2 spun off a number of other technologies of potential application for environmental protection and monitoring and for potential space applications in smaller life support systems. These technologies include soil beds for air purification, aquatic plant wastewater recyclers, non-polluting analytic and monitoring labs, multilevel cybernetic systems for system operation and analysis, and high yield sustainable soil-based agricultural systems. The visible structure was underlain by the complex technosphere (4, 20–22).

Fig. 11.4. Biosphere 2, Oracle, Arizona, constructed 1985–1991, operated as a closed ecological system and biospheric laboratory, 1991–1994 in two closure experiments with crews.

Mission One, a 2 year experiment, was conducted from 1991 to 1993 with a crew of eight biospherians who operated the intensive agricultural system, managed and monitored the other biomes, and maintained the equipment and computers inside the facility. After a Transition Mission to carefully measure results of Mission One and improve some technical details, a second closure experiment ran from March 10, 1994 to September 6, 1994 with a crew of seven people. The new owner then shut down all further closed system research.

Biosphere 2 achieved a flourishing complex life-support system by the designed integration of seven biomes: rainforest, savannah, desert, marsh, ocean, intensive agriculture, and human habitat. The tallest structure of the rainforest rose 27.7 m high. The ocean contained a coral reef system, including a shallow lagoon area and sandy beach. Its waves were generated by a vacuum pump wave generator. An ecosystem modeled on the estuarine Everglades ecology adjoined the coral reef with a series of communities that graded from freshwater marsh to oligohaline Spartina grass marsh, through areas dominated by white mangrove (*Laguncolaria racemosa*) and black mangrove (*Avicenna germinans*) to the more highly saline waters that support oyster beds and red mangrove (*Rhizophera mangle*) (4, 23).

3.1.2.2. The Scale of Biospheric Design

Designing a biospheric scale materially closed, informationally and energetically open life system requires co-coordinating an unusual number of sciences and disciplines. Biospheric scale means that there will be three or more "biomes" or relatively independent ecosystems. For life support, a human habitat (living and working area), agricultural ecosystem, including means for reusing and recycling inedible crop material, and a wastewater recycling system are required. The system shares a common atmosphere, water, and waste recycling systems,

Fig. 11.5. Mark van Thillo, co-captain of the eight-person biospherian crew, in the technosphere of Biosphere 2 during the closure experiment, 1991–1993.

genetic pool, temperature/weather regimes and overall psychological and economic dimensions. To create such things during the closure experiment, a biospheric system requires three new integrative disciplines: Biospherics (how to create and study complex ecological entities) technospherics (the world of technologies, especially from the viewpoint of ecotechnics) (Fig. 11.5), and ethnospherics (designing for the human culture which will emerge with a wide range of behavior and values) (4, 24, 25). The key factor in biosphere design is deciding how many humans will be supported. Certain parameters governing minimum size can then be calculated for given human requirements for food, temperature, hygiene, safety, beauty, fulfillment, and socializing that must be met (26).

Biosphere 2 was designed to determine the technical, biological, and cultural vectors of a total biospheric system by creating a living, experimental model of the tropical zones of Earth (Fig. 11.6). Excluded were not only temperate and arctic regions, but the upper atmosphere, deep geologic strata, and deep ocean biosphere minus the freezing zones (27). Therefore, its goals included studying the technologies required which included a complete global communications center and their ecological impacts on the complex agricultural system, the five wilderness biomes, and on human and animal health.

3.1.2.3. Design Requirements for a Biosphere

An artificial biosphere, because of its size, cannot depend on nature to supply certain needs, for example, tides, winds, and rains. A "technosphere" must be designed that can supply, measure, and control these functions performed by the moon, sun, and vast weather systems in Earth's biosphere. For example, in Biosphere 2, wind generation by air handlers helped

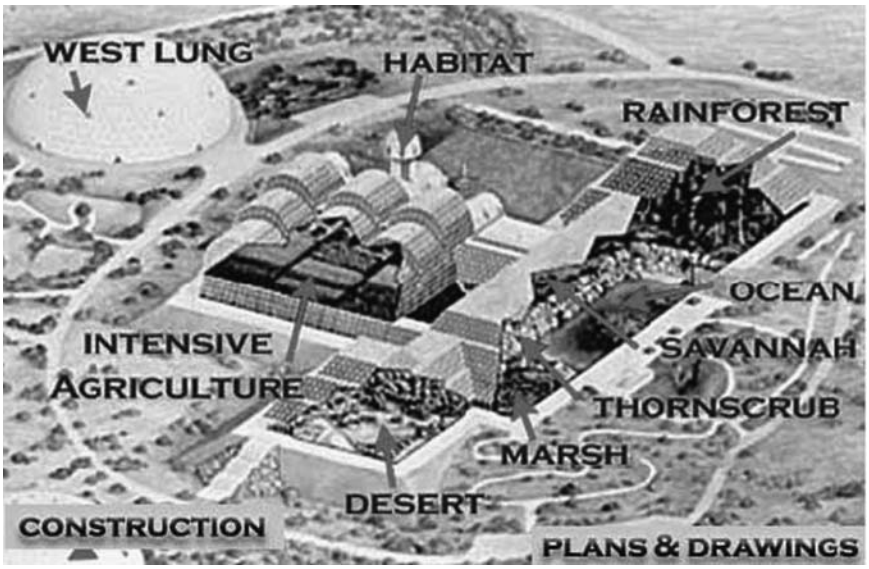

Fig. 11.6. Plan of Biosphere 2, showing the human habitat and intensive agriculture; the five wilderness biomes: rainforest, desert, ocean, and marsh; and the variable volume "lungs."

regulate temperatures in the biomes and its airflow was also necessary for wind-pollinated plants. Above all, in order for an artificial biosphere to exist, a container must be designed that will allow recycling to occur with minimal loss or input of outside material. In Biosphere 2 that meant stringent engineering which resulted in a leak rate of air of less than 10% a year. Leak rates were monitored through depletion determinations of several trace gases (SF6, He and Kr), which were spiked into the atmosphere of Biosphere 2. The degree of air-tightness was much greater than those in the previous closed systems allowing exact tracking of essential life element cycles (28, 29). Water, food, and waste had to and did recycle during the 3 years and Biosphere 2 operated as a research facility for closed ecological systems. Buildup of trace elements from technogenic, biogenic and anthropogenic sources must be monitored and not allowed to exceed the minimum exposure limits (although there is still little information about the long-term tolerable limits for many trace gases) (30, 31).

On the other hand, desired throughputs of energy and information must be engineered into the container. The energy source(s) must be distributed to the different regions of the biosphere so that the proper temperature differentials are maintained, the proper light is received, and the tides and winds perform their ecological functions. The energy sink(s) must reliably get rid of heat excesses. If the system is healthy, some of the energy input will be stored by the biosphere as increased free energy in its biomass, complex molecules, and information. In the case of Biosphere 2, which started out at its initial closure in September 1991 with about 15 tons of biomass, by the end of its first 2 year mission, plant growth had been so robust that biomass had doubled.

Biospheric design must take a two-pronged approach to its life systems: One, the selection and layout of its biomes and ecosystems; two, the selection of its species and individuals

in those species. All five kingdoms must be represented with humans representing a sixth kingdom in design load if not taxonomically (32). The most important of these kingdoms in terms of the work performed in a biosphere are the bacteria, prokaryotes, and eukaryotes, which are found in great number in both aerobic and anaerobic levels of the soils and water bodies. All the functional suites of bacteria must be represented, since they are essential for the successful completion (cycling) of biogeochemical elements essential for life (6). Fortunately, once sufficient diversity of microbes is present, natural processes operate to increase microbes that use particular elements as food stuffs. For example, the methanogens increase in response to an abundance of methane and help ensure completion of cycling feedback loops (33–35).

The initial set chosen of each species from the prokaryotes, eukaryotes, plants, animals, and fungi must contain enough members to ensure reproduction under the given conditions of the new biosphere. The determination of this number needed to ensure viable survival is a major part of biospheric research and design. Biosphere 2 was designed with nearly 4,000 species (not including microbial diversity) allowing for a loss of 20–30% of original biodiversity as ecological communities adapted (21). This species surplus was built into the initial state conditions in order to allow real competition to occur, and thus allow the system to self-organize (4).

Biomes are the key design levels in making biospheres sustainable and thriving. In our planetary biosphere, they constitute the most sustainable large functional units. The Russian biologist Kamshilov noted, "The stability of the biosphere as a whole, and its ability to evolve, depend. . . on the fact that it is a system of relatively independent biogeocoenoses (biomes) . . . which compete for habitat, substance, and energy and so provides for the evolution of the biosphere as a whole (36)." Biomes provide integrative matrices for maximizing numbers of econiches, stable and complex food chains, and varied biochemical cycling routes. Continuous monitoring assessed the health of humans, the other species, the different biomes' integrity, and changes in the cycles and compositions of air and water and how that affected humans or biomes. To assist the health of biomes, the crew ("biospherians") must function as an analogue to "keystone predators" responsible for maintaining balances in each biome by assisting threatened important species and controlling invasive species (37, 38).

Once the number of humans, biomes and their species, and the support technospheric systems are determined, then the needed volume of the container can be calculated in order to provide carbon dioxide and oxygen ratios desired during the different seasonal cycles. Biosphere 2 was modeled on subtropical seasonal cycles. Carbon dioxide minimums and maximums cycled from 300 to 4,200 ppm annually, and CO_2 flux between day and night could reach over 500 ppm (Figs. 11.7 and 11.8) (20, 39). Except for the coral reef, which is affected by the pH, which rises with elevated atmospheric carbon dioxide levels, the carbon dioxide could have been designed to reach levels as high as 10,000 ppm without human health problems.

The cycle of overriding interest and concern in biospheric operation is that of carbon dioxide. The ratio of living biomass (to reach 60–70 tons in Biosphere 2 after 10 years) and soil material (some 30,000 tons, from 1 m deep in the agricultural biome to up to 5 m in the rainforest) to atmospheric mass was far greater than those in the planetary biosphere. This

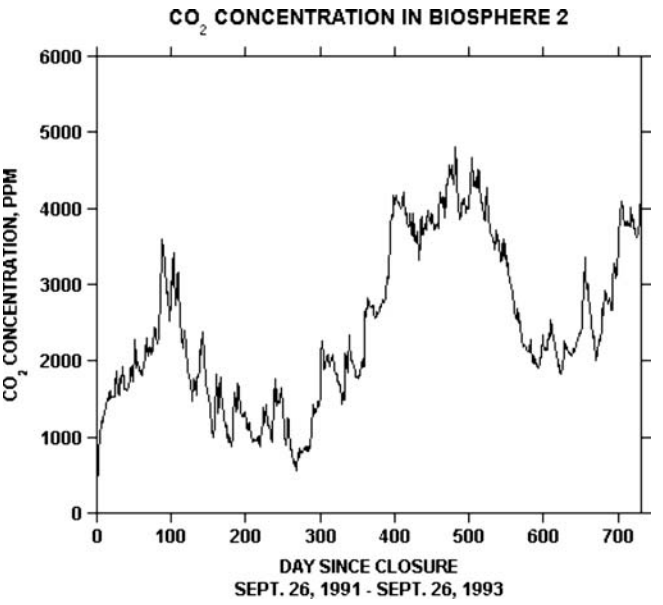

Fig. 11.7. Carbon dioxide average daily concentration in the Biosphere 2 atmosphere during the Mission 1 two-year closure experiment, 1991–1993.

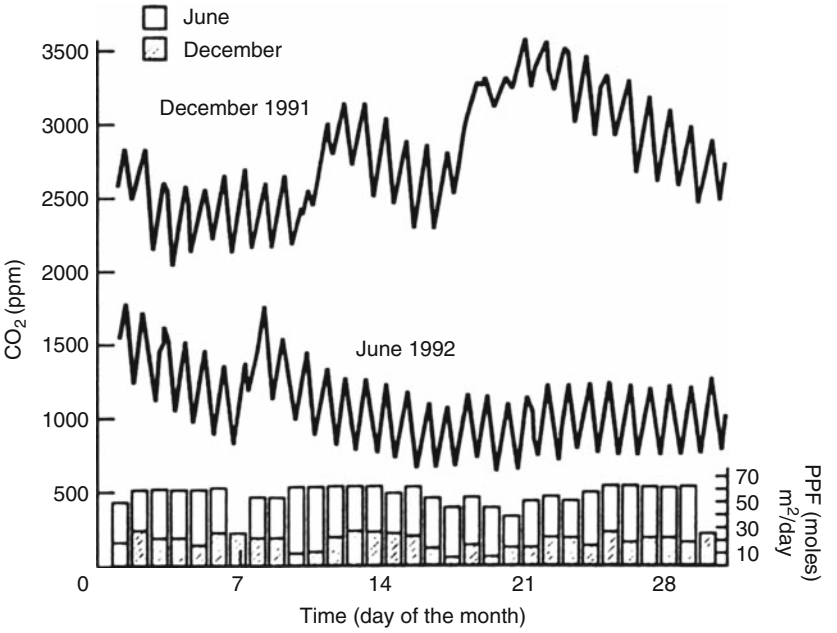

Fig. 11.8. Day-night oscillation of CO_2, about 500 ppm, is shown in this graph of a winter and a summer month. The histogram at the *bottom* also shows total daily light for the winter month (*shaded bar*) and for the summer month (*clear bar*).

higher ratio, even at the beginning 15 tons of biomass, results in a far shorter residence time for CO_2 in artificial biospheres than in Earth's biosphere, an estimated 4 days as contrasted to 3–10 years (21). Therefore, to operate small artificial systems the CO_2 sources (people, animals, soil microbes, compost, liquid waste recycle wetlands) must match uptake (plants and algae) rather closely (1, 4, 40).

After making the first approximations to human number, biomass, biodiversity, biomes, atmospheric volume, and technosphere (including sealing), the kinds and volumes of dryland and wetland soils and rock substrate (e.g., limestone for coral reef, "parent rock" underlying topsoil), design must provide and recycle the nutrients for the life forms and to balance the atmospheric cycle. For example, deserts require a somewhat basic soil, rainforests, a somewhat acidic soil. Wetland (paddy) rice varieties need anaerobic soils, while root crops prefer light, sandy soils. The challenge of creating biospheric systems on the Moon and Mars will be to learn to amend local regolith and soil to make them suitable for agriculture and support of a range of organisms and ecosystems (19, 23, 25, 41). Worms as well as bacteria and fungi play a key role in making productive soils. In addition, fungi imperfecta, mycorrhizae, play an enormous role in soil health and nutrient recycling through composting of inedible crop residues and litter decomposition by natural detritivores is a crucial requirement for a sustainable system (23, 42, 43). Biosphere 2 was designed as a soil-based system because soils play a major role in purifying and recycling the atmosphere as well as in controlling the rate of water use and providing support and nutrients to the plants. They enable the use of time-tested and low-energy methods of recycling of inedible crop matter; wastewater treatment through wetland plants; and increase the diversity of microbes able to be supported (44). Soils also give a rich and satisfying aroma to humans and provide essential aesthetic and even spiritual experiences.

Creating a sustainable high yield agricultural system with low time requirement (less than 1/3 of biospherian time) to plant, harvest, measure, process, and cook is one of the most difficult design tasks in making a habitable biosphere. The chief technical requirement is that the agriculture must supply a complete nutritional diet. This agriculture must be sustainable without gradual loss of vital nutrients if the human health is to be maintained properly. The design of the Biosphere 2 agricultural system, the Polynesian sweet potato and pig, the East Asian rice and fish, the Indian wheat and chicken, and tree fruits, such as banana and papaya, seen in Mayan and Ugandan farms were integrated in a 0.2 hectare system that used over 80 crop varieties. Biosphere 2 was the first instance of a closed ecological life support system in which a complete nutritional diet was the goal and in which domestic animals were successfully included. The diet for the eight crew members of Biosphere 2 included milk (from African pygmy goats), eggs (from the system's domestic chickens), meat (from the goats, chickens, and Ossabaw feral pygmy pigs), and fish (from Tilapia grown as in the rice/azolla water fern paddies). In addition, a wide range of vegetables, grains, starches, and fruit are grown. This agriculture kept eight biospherians leading a strenuous life in top health and work morale for 2 years (40, 45, 46). The reliance on ambient sunlight reduced by 50–55% in passing through the glazed envelope also limits area productivity and might differ in space applications where advantage may be taken of enhanced artificial light techniques to boost yields (19, 21, 47).

In small closed systems like man-made biospheres, the agricultural system cannot use toxic chemicals such as biocides or pesticides, but must develop non-polluting methods of controlling crop diseases and harmful insects, and nutrients from the food produced must be returned to keep the soil fertile (23, 48). Processing, storage, and culinary systems that do not waste vital nutrients and that present the dishes at table in an appetizing form must be developed. No artificial biosphere could survive paying the energetic and pollution costs of modern agriculture and, indeed, one of the main applications of biospheres can be in the development of a comprehensive sustainable agriculture.

After the first approximations of atmosphere, soils, life forms, human needs, agricultural system, and technospheric scale and content, biospheric design work must begin to calculate water needs and how to control its flows and arrange the size and location of its reservoirs. The four subsystems are potable water, wastewater recycling from the human habitat and farm animals, irrigation water for the crops, and rain and stream/pond/marine water for the wilderness biomes. In Biosphere 2, sunlight drove evapotranspiration and technical systems were installed to condense atmospheric humidity to produce high-quality, low-salt water for drinking and for agriculture and wilderness biomes. The technosphere must take the place of many ecological functions in man-made biospheric systems through storage and pumping of water to where it is needed.

Finally, to complete the basic design program, a hierarchy of computerbased monitoring and control must be developed to track the performance of the entire system and its key components. This system must operate by "mission rules," which specify the tolerable range of environmental parameters. The five minimum functional levels are: (a) Point sensing and activation, (b) local data acquisition and control, (c) system supervisory monitoring and controls (though much of a biosphere's operations are self-organizing), (d) global monitoring and historical archives, (e) telecommunications between crew and monitor stations inside and those on the outside. An alarm system which will activate either manual or AI intervention as necessary must be included. Final biospheric design results from a number of iterations of integrations of the above vectors until it satisfies the critical economic, ecological, and human criteria.

3.1.2.4. The Use of Test Modules in Design

A note of caution: In engineering a biospheric scale apparatus with its attendant cost requirements, it is advisable to make a test module to develop skills in making and living in closed ecological life systems and to test probable sub-contractors. The Biosphere 2 Test Module (Fig. 11.9) was the first closed life system that had complete wastewater and water recycle with very low leak rate, but its size prevented total supply of food support for longer than a month's occupation by a single experimenter (33, 34). There was a scale jump of 500:1 to go from the Biosphere 2 Test Module to Biosphere 2.

Important findings were made during experimentation from 1987 to 1989 with the Biosphere 2 Test Module. The total system remained quite productive and the many microbial and fungal vectors were held in balance by competition despite concerns that a soil-based system might fuel fungal growth or microbial pathogens and was able to recycle its carbon dioxide at atmospheric levels far below hazardous levels. Experimenters in the closure experiments

Fig. 11.9. Biosphere 2 Test Module.

reported that living inside a closed ecological system was more than survivable: It was very enjoyable, Engineering challenges included achieving the desired leak rate and use of the variable volume chamber (lung), and working out the practicality of demanding specifications far beyond normal engineering requirements with newly invented technologies (19, 33, 34).

3.1.2.5. BIOSPHERIC LABORATORIES AND EXPERIMENTAL ECOLOGY

Biomass in the analogue wilderness biomes continued to increase during the period when Biosphere 2 was operated as a closed ecological system (1991–1994), with woodland canopies rapidly developing in rainforest, savannah, and marsh. The Biosphere 2 desert biome self-organized in an unexpected way and resulted in a community dominance shift from cacti/succulents to shrubs/annuals. Ocean water clarity was greatly improved with the installation of protein skimmers constructed from materials available inside Biosphere 2 and post-closure studies showed the maintenance of most coral species and the start of many new coral colonies. Overall, fewer species appear to have been lost than anticipated, though what level of biodiversity will be maintained in the various biomes was a question designed to be studied over the long-term operation of Biosphere 2 (4, 20).

Biospheric laboratories can provide data and model experiments for the field of restoration ecology, since the issues of small numbers of organisms and small areas and the sustainability of critical biodiversity are shared with many natural areas (49). In Biosphere 2, this was especially illustrated by the creation of a very flourishing mangrove marsh system, a system that had been thought quite difficult to restore. More fundamentally, biospheric laboratories can contribute to the transformation of ecology from a descriptive to an experimental science because all the variables can be measured starting from known initial state conditions and

integrating ongoing energy and information inputs. Experimental biospheres can be to the life sciences what cyclotrons are to physics.

3.1.2.6. Variety of Purposes for Biospheric Experiments

Biospheric experiments can yield valuable insights on the interactions between natural ecosystems and global technical systems (27). They can also operate as unique test facilities for long-term space stations, travel, and space settlements where inhabitants must operate bioregenerative and technical systems as a synergy. Learning to integrate advanced technical systems with complex life systems can be of immense educational value, both in hands-on training of a managerial corps for complex projects, a corps able to handle the difficulties of contemporary life and in providing general principles for the general public by outreach education. Another use is to take advantage of the isolation of biospheric systems for conducting potentially dangerous experiments on new chemicals, pollutants or genetically modified life forms to see their impact on complex ecosystems. Smaller scale closed life systems cannot provide this scale and quality of results. Deciding which of the possible results are aimed for is a fundamental design control (Allen et al, 2003). Spin-off benefits from biospheric experiments include valuable insights as to human behavior, cultural formation, and ways for humans to integrate their sciences, arts, and enterprises into a harmonious dynamic. This potentially integrated world has been called by Vernadsky and others as a noosphere, or a world of intelligence (25, 40).

3.1.3. Mars on Earth® Closed Ecological System Project

The Mars on Earth® (MOE) Project is a simulation of a four-person life support system for a Mars Base that is located on Earth. Phase 1 of the project includes the design, construction, and operation of a prototype life support base – the Mars Base Modular Biosphere – that will support a crew of four people. This closed life support system will provide a test bed for developing space-based life support systems, such as water and wastewater recycling, food production, air purification, etc. that will be needed to undertake a manned mission to Mars (50). Project location is still to be determined.

The Mars on Earth research and development agriculture system consists of six modular units each with a footprint of 110 sq m (total 660 m^2). Approximately 478 m^2 will be used for growing crops leaving approximately 182 m^2 for access, equipment, and processing. The units will be soil-based using soils produced by amending simulants of Mars surface soil, since the goal is to use in situ planetary resources. The goal of the Mars on Earth facility is to produce a complete diet and to recycle all waste products, including human waste from the crew while maintaining an atmospheric balance suitable for plant growth and human habitation. Six modular units provide both variety in temperature regime and safety in redundancy. The units will share air and water circulation system, but be linked in such a way that they could operate independently or in case of damage be isolated from the rest of the system.

Crop composition is outlined below (Table 11.3). Ten basic crops can provide 3,000 Kcal, 79 g of protein, and 35 g of fat per person per day. These crops would be supplemented with vegetables, herbs, and spices, from a 32.5 m^2 area. Table 11.4 shows some estimated yield

Table 11.3
Projected diet based on ten major crops, Mars on Earth® Prototype Space Life Support System (43)

Crop	% of diet	Kcal/ person/ day	Crop wt/calorie content (Kcal/g)	Gams of crop per day	Protein content grams	Protein from crop/day	Fat con- tent/ gram	Fat from crop/day
Rice	15%	450	3.5	128.57	0.13	16.71	0.01	1.29
Wheat	10%	300	3.3	90.91	0.13	11.82	0.02	1.82
Sweet potato	25%	750	1.06	707.55	0.01	7.08	0.0028	1.98
Peanut	5%	150	5.84	25.68	0.26	6.68	0.48	12.33
Soybean	5%	150	4.02	37.31	0.08	2.99	0.18	6.72
Pinto bean	10%	300	3.42	87.72	0.24	21.05	0.0086	0.75
Winter squash	7.50%	225	0.634	354.89	0.01	3.55	0.001	0.35
Beet root	7.50%	225	0.445	505.62	0.01	5.06	0.0002	0.1
Banana	10%	300	0.6	500.00	0.006	3.00	0.02	10
Papaya	5%	150	0.26	576.92	0.003	1.73	0.0007	0.4
Total		3,000		3,015.18		79.66		35.74

Table 11.4
Projected light levels, yield and estimated cropping area for Mars on Earth® Biological Life Support System (43)

Crop	Kcal required for 4 crew daily	Best yield Bio ($kg\,m^{-2}\,d^{-1}$)	Light level ($mol^{-1}\,m^{-2}\,d^{-1}$)	Correction factor for 50 $mol^{-1}\,m^{-2}\,d^{-1}$	Extrapolated Yield in 50 $mol^{-1}\,m^{-2}\,d^{-1}$ $kg\,m^{-2}\,d^{-1}$	Extrapolated yield in $Kcal/m^{-2}\,d^{-1}$	Area 4 required for feeding crew
Wheat	1,200	0.0024	16	3	0.0073	24.38	49.22
Rice	1,800	0.0057	25	2	0.0114	40.55	44.39
Sweet potato	3,000	0.0160	25	2	0.0320	33.89	88.51
Peanut	600	0.0014	25	2	0.0028	16.32	36.77
Soybean	600	0.0013	25	2	0.0026	10.64	56.41
Pinto Bean	1,200	0.0037	25	2	0.0074	25.36	47.32
Beet (root)	900	0.0232	25	2	0.0464	20.45	44.01
Winter squash	900	0.0425	25	2	0.0850	54.32	16.57
Banana	1,200	0.0498	25	1	0.0498	29.64	40.48
Papaya	600	0.1084	25	1	0.1084	28.68	20.92
	12,000						445

Add 33 sq m of salad greens and other leafy vegetables, $= 478\,m^2$.

Fig. 11.10. The Laboratory Biosphere facility, Santa, Fe, New Mexico (52).

figures and necessary crop growing area with a light input of 50 mol m^{-2} d^{-1}. After further research, design scenarios may call for less growing area to supply a Mars exploration crew of 4–5 (43). The diet utilizes the ten crops chosen; wheat, rice, sweet potato, peanut, soybean, pinto, beetroot, winter squash, banana, and papaya because of their success and suitability in the Biosphere 2 experiments. They are hardy, dependable, and relatively easy to harvest and process with a minimum of equipment. Supplemented with fruits and vegetables from the vegetable area and horticulture understory, they can form the basis of a healthy vegetarian diet (45, 51).

3.1.3.1. Experimentation in the "Laboratory Biosphere," Santa Fe, New Mexico

In preparation for the Mars on Earth Project, the "Laboratory Biosphere" (Fig. 11.10), a new closed ecological system test bed was designed, constructed, and put into operation by the initial consortium of Global Ecotechnics Corporation, the Institute of Ecotechnics and the Biosphere Foundation. Currently the facility is owned and operated by the Global Ecotechnics Corporation in Santa Fe, New Mexico (52). The facility was initially sealed in April 2002; and three experiments using soybeans, wheat and sweet potato as the chamber's crops were conducted between May 2002 and January 2004 (53). The Laboratory Biosphere was created as a test bed to continue experiments with a sustainable soilbased agriculture system unlike most bioregenerative systems, which use hydroponic systems dependent on the supply of nutrient solution. Because of the small volume of the system (34–43 m^3), (Table 11.5), developing mechanisms to keep parameters like carbon dioxide within acceptable limits is critical. Recycling of nutrients within the system to maintain soil fertility and the ability of

Table 11.5
Component volume and mass of Laboratory Biosphere Closed Ecological Facility, Santa Fe, New Mexico (52)

Component	Volume, m^3	Mass, kg
Fixed air	33.6	32
Variable air (lung)	0–9	0–8
Soil (dry)	1.46	1,650
Water	0.3–0.5	300–500
Plants (variable)	0–0.02	0–20 (estimate)

the inherent complex ecology of soils to handle trace gas buildups are primary research goals. Other objectives include studies of short and long-term exchanges of carbon dioxide, oxygen, nitrogen, NO_x, and methane between soil, plants and atmosphere, the impact of cultivation (tillage) on soil/atmospheric exchanges, investigation and development of strategies to return nutrients to the soil to maintain fertility, (e.g., shredding biomass vs. composting) and the impact on soil chemistry of returning leachate water to the soil as irrigation water. Integration of automated sensors and controls in the system with real-time modeling has importance for operation, research, and educational outreach programs. The Laboratory Biosphere is also intended to test and develop a "cybersphere" (network of shared intelligence) that may be scaled up for the Mars on Earth project as well as having potential applications for natural ecosystems and global biosphere research.

3.2. Russian Research in Closed Ecosystems

The history and methodology of experimental work on creating closed ecological systems in Russia owes its inspiration to the concepts of "Russian Cosmism," an important philosophical system in Russia during the nineteenth century. In the beginning of the twentieth century, K.E. Tsiolkovsky, a forerunner of Russian cosmonautics, wrote that planet Earth was like a "cradle" for human civilization and commented that it was impossible for humanity, to, stay in the cradle forever. Tsiolkovsky's concept of a "greenhouse" for space flights is amazingly close to modern experimental findings on creating closed ecological systems (54). V.I. Vernadsky, elaborating the concept of the biosphere as a planetary-scaled essentially closed material cycle, was convinced that it would be possible to sustain life indefinitely in a system with a closed material cycle.

Experimental work on creating biological life support systems on the basis of closed loop, internal material cycling, started in the Soviet Union in the 1960s.

3.2.1. Experimental Facilities of IBMP (Moscow)

The Institute of Biomedical Problems (IBMP), founded in Moscow, organized a specialized department to work out the biotechnological foundations for biological life support systems. General biological concepts of closed ecological ecosystems and their space applications were developed (55, 56).

Microalgae were chosen for the first experiments as the main metabolic balancing link (to coordinate with the human metabolism). It was assumed that the CO_2 produced by humans would be removed by the algae, which would incorporate its carbon into their structure. The O_2 produced by algae would be released for human use. Also, it was assumed that humans could directly utilize algae as a food.

The first experimental installation was constructed in the IBMP. It allowed direct gas exchange, and later water exchange, between a human and microalgal culture. Five experiments were conducted, in which three cultivators (15 l in volume each), containing a culture of 10–12 g/l density of dry matter, continuously provided requirements of the human, placed in the sealed cabin 5 m^3 in volume, for atmosphere and water regeneration during 29–32 days. During the experiments, a CO_2 imbalance, amounting to 5–17% per day, was recorded, as a result of the difference between the human respiratory quotient and the assimilatory quotient of microalgae. The gas closure of the "human–microalgae" system reached 90%. Carbon monoxide and methane concentrations, and quantity of microorganisms in the atmosphere also were stabilized (56).

Next steps to close the system further were to be connected by closing the trophic cycle. Unfortunately, microalgae could not be extensively used as food (The biochemical composition of algal biomass, containing over 60% of proteins and nucleic acids, does not conform to human nutritional requirements, not less than 60% of which must be supplied by carbohydrates).

This was the main reason, which motivated early Russian researchers to include higher plants as a traditional source of food for humans. A greenhouse was part of the Ground Experimental Complex (GEC). Environment in the GEC was regenerated principally by physicochemical processes; the greenhouse was a source of fresh food (The greenhouse, 15 m^2 in area, daily produced 482 g of dry biomass, including 86 g of edible biomass: 54 g of wheat and 32 g of vegetables). Only 18% of the produced biomass was used for food. It was obvious that transformation of unused plant biomass to food was necessary in order to increase material closure of the system (57).

The principles of intensive cultivation of autotrophic and heterotrophic organisms as BLSS (biological life support systems) components were established and laboratory prototypes of BLSS incorporating humans were designed and tested. Six ground-based experiments were performed in which atmosphere and water were regenerated in closed ecosystems, including unicellular algae, higher plants, and one to two human subjects. These systems were designed to fully regenerate atmosphere, water and grow some of the crews' food (58). Creation of closed ecosystems for a space crew requires test verification of various components of these systems during orbital flights. To this end, an effort was made to devise associated experimental equipment and start investigations on growth, development, and cultivation of biological species (animals, lower and higher plants) as BLSS components in microgravity.

Some animals were choen to be included in the human life support as producers of animal protein: Possible candidates were quail and fish (59). For the first time, animals were tested in real space flights, using short bio-satellite flights and onboard Mir Space Station (60).

Since 1990, the orbital complex MIR has witnessed several incubator experiments for the determination of spaceflight effects on embryogenesis of Japanese quail. First, viable chicks

that had completed the whole embryological cycle in MIR microgravity hatched out in 1990; it became clear that newborns would not be able to adapt to microgravity unaided. There were eight successful incubations of chicks in the period from 1990 to 1999.

The experiments were marked by high mortality rate at various phases of embryonic development and a number of developmental abnormalities. The number of embryos with these abnormalities varied from 12 to 26% in different experiments and did not correlate with the total number of dead embryos; however, the types of abnormalities were the same as may happen during incubation of eggs on Earth (microphthalmia, ectopia, abnormal development of limbs). At present, there is no clarity about causes of the developmental abnormalities in space embryos. The experiments on bird embryogenesis in microgravity showed that embryonic development of birds does not depend on the gravity factor. It is necessary to study the whole cycle of development of embryo from the moment of egg insemination and early stages of zygote division. This is the only way to fully evaluate the significance of the gravity factor for avian embryogenesis, and the role of shifts in the egg macrostructure occurring in microgravity as one of possible causes of developmental abnormalities (61).

The initial attempt to grow leaf cabbage and radishes in a mini "greenhouse Svet" (a small plant growth unit developed for spaceflight experimentation) onboard space station Mir was made in 1990. The experiment showed normal morphogenesis of plants in microgravity. However, space plants were conspicuously behind ground controls in their growth and development rate, and so they were significantly smaller. This was attributed, among other reasons, to peculiarities of moisture transfer in capillary/porous media (artificial soils) in the zero-g environment. In preparation for the fundamental biological research on the MIR/NASA program, Russian and the U.S. investigators joined their efforts to develop the Greenhouse Environmental Monitoring System (GEMS) enabling monitoring and control of environmental parameters of plants. Outfitting of greenhouse Svet with GEMS allowed investigators to keep track of all plant growth parameters and to adjust water supply to the crop in Mir experiments (61).

The main purposes of the experiments with Greenhouse-4 and -5 were to investigate the reproductive function of wheat in microgravity, and to grow crops of several generations of wheat. The goal was achieved with the use of the well-designed cultivation technology and ethylene-resistant wheat species USU-Apogee. The experiments were further proof that in microgravity wheat growth and development proceeds on the same pattern as on Earth. The period of crop development to harvest as a whole was not extended. Neither were the individual phases of wheat development. Experiment Greenhouse-4 yielded almost 500 seeds from 12 plants.

A second generation of space seeds was obtained in experiment Greenhouse-5. The series of Greenhouse experiments on space station Mir showed the importance of compensating for the altered physical conditions in microgravity and providing the plants needs. Plant organisms can make up for the lack of the gravity vector by other trophic reactions and ensure productivity comparable with ground controls.

Space investigations of organisms – candidate components of closed ecosystems of life support – give grounds to assert the feasibility of development of LSS based on the biological turnover of substances for manned spacecraft. Autotrophs are able to sustain normal

functioning within LSS in the zero-gravity environment, given appropriate equipment is designed to offset the microgravity-induced changes in ambient conditions and provide for plant organisms. Introduction of heterotrophs in space LSS is a more formidable problem since gravity is a very unusual condition for congenital reflexes and instincts of newborns, the absence of which creates grave difficulties for living in microgravity.

Missions to Mars will be the longest duration human space flight that we can anticipate in the next decades. The Martian LSS may include a greater scope of biological processes to regenerate environment than merely one greenhouse. Ground-based BLSS tests gave proof of the high efficiency of unicellular algae in oxygen and water regeneration (58). Five ground-based experiments were conducted at IBMP to study closely the human–algae-mineralization life support model. The system for one person was 15 m^3 in volume and contained 45 l of algal suspension (dry algae density = 10–12 g/l of water), water volume, including the algal suspension was equal to 59 l. More sophisticated models in which unicellular algae were replaced partially by higher plants (crop area = 15 m^2) were tested in three experiments with a duration of 1.5 to 2 months. Algae, as a primary BLSS component in the, ground-based experiments, were able to fully regenerate atmosphere and water, and to provide the following: Partial closure of the nitrogen cycle due to complete consumption of the human urinal nitrogen by unicellular algae; purification of the pressurized chamber atmosphere from various water-soluble gaseous admixtures through adsorption and utilization in a photoreactor filled with algae and associated microorganisms (a photoreactor is a hydrobiological filter); optimization of the airborne ions and aerosol balance in the atmosphere (negatively charged ions normally dominate in pressurized chamber atmosphere); stabilization of water-insoluble gaseous admixtures in atmosphere (methane, carbon monoxide, and others) as a result of their adsorption on the surface of algal and microbial cells, and subsequent withdrawal together with the biomass yield, ousting from the microbial populations, by competition with foreign microflora to the algobacterial ecosystem in the photoreactor microflora, including human pathogens. In other words, though simple by structure, BLSS based on unicellular algae carries out a multitude of functions, including regeneration of atmosphere and water, as well as some other specific functions through which the human environment becomes healthier (58). Polyfunctionality of the biological regeneration of the human environment is another argument for its integration into space life support systems.

Life support systems with the use of a biological material cycle for humans outside the realm of Earth's biosphere can be divided into two classes: (a) For interplanetary vehicles (IPV) and (b) for planetary outposts (PO). IPV LSS may represent one or another embodiment of the system in which unicellular algae will constitute the autotrophic element and participate in regeneration of the environment alone with a cluster of biological and physical–chemical systems. Microgravity is the factor that will, in many respects, define the look of future IPV LSS as it will require the development of innovative technologies and techniques to ensure normal system operation, particularly of the biological component. The reason why unicellular algae are given preference as the basic IPV LSS autotrophic element is that their population, in contrast to higher plants, possess a number of very important features, which are the presence of much greater numbers of organisms, short life cycle (hours), tolerance to stress factors, autoselection, a huge diversity of metabolic functions and, as mentioned above, polyfunctionality as a environment-forming element (62).

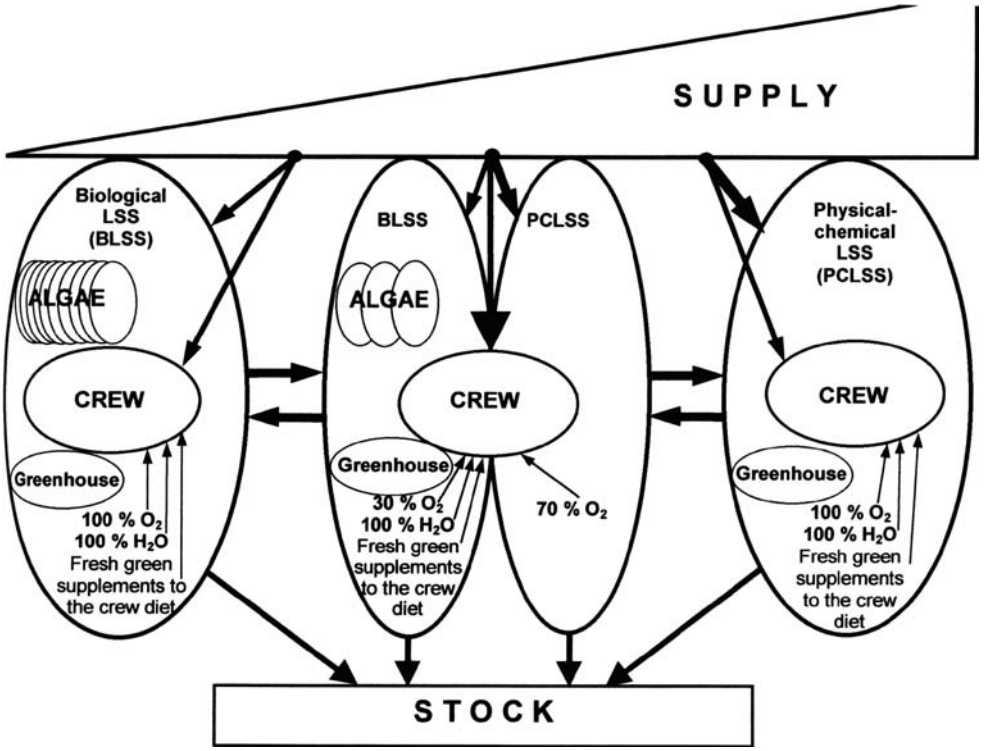

Fig. 11.11. Functional diagram of LSS of a space vehicle outbound to Mars (IBMP) (61).

For a long period of time, any future Martian crew will be separated from the biosphere of Earth, and therefore, LSS should be highly robust and reliable, and fully redundant. Redundancy can be achieved by installation on the Martian vehicle of two systems with different regeneration machinery, i.e., based on both physical–chemical and biological processes, each one will have the property of serving all the physical needs of the Martian crew. The best plan is to make the two systems function concurrently, each one fulfilling a specified portion of the regenerative functions.

The functional diagram of LSS is shown in Fig. 11.11. It is based on the assumption that urine processing by unicellular algae will result in a 30% supply of the autotrophic element from the total crew demand in oxygen. The urine will be fully consumed by growing and developing algae. That is why it is believed that nominally the LSS biological component will regenerate 100% of water, 30% of oxygen and consume 30% of CO_2. In case of physical–chemical underproduction of oxygen, within 24 h oxygen production by unicellular algae can be doubled. The size of stored reserves will be largely determined by the requirements of physical–chemical LSS, for in view of the technologies used on the present-day orbital stations, water electrolysis will remain the main source of oxygen for the crew. The diagram (Fig. 11.11) is considered to be very preliminary and will require more careful development work and more integrated studies, particularly at transition periods when one of the LSS

components starts increasing its rate of production. However, this composition of LSS for an expedition to Mars will enable the resolution of many issues, including robustness and reliability of the LSS functioning during long periods of absence of any connection with Earth's biosphere, and adequate sustenance of the human environment (62).

The construction of the real full BLSS for space crews cannot be done without international cooperation in the theoretical and experimental groundwork for using these systems in space.

3.2.2. Experiments with Bios-3 (Institute of Biophysics, Krasnoyarsk)

In all experimental ecosystems implemented before Bios-3, the human test subject participated in the system only as a metabolic link. Transferring control to within the system required the development of new technological variants and bioregenerative techniques: It was necessary to reduce the hours spent on technological operations, to minimize the types of analyses essential to system governance, and to prepare the system inhabitants to work independently in maintaining the life support system. Bios-3 was constructed to implement and to research experimental ecosystems with internal control.

The experimental complex Bios-3 is illustrated in Figs. 11.12 and 11.13. Bios-3 is enclosed in a stainless steel, hermetically-sealed, rectangular, welded housing of dimensions $14.9 \times 9 \times 2.5$ m. It is partitioned into four equal and airtight compartments. The higher plants (grown in phytotrons) are accommodated in two of these compartments, one is occupied by the unicellular algae growing in cultivators, and the last is inhabited by a crew of three people. In the crew's compartment, there are three individual cabins, a kitchen–dining room, and a room equipped with a shower and a toilet. One of the cabins can be materially sealed to study the metabolism of the person located within it. The toilet serves as the entrance to the whole complex through an entryway compression airlock. The crew's compartment also includes a common area that functions as a laboratory, studio, and recreational room (16).

The overall dimensions of the complex are 315 m^3. The volume of each compartment is about 79 m^3. All the compartments of the complex are joined by airtight doors, and there are airtight doors that open to the outside of the complex from each compartment. Each door is designed to be opened by one person from the inside and the outside, when necessary, in no more than 20 s. This was extremely important for maintaining the safety of the test subjects: They were able to leave Bios-3 without delay and without external help in cases of danger from fire, etc. (Figs. 11.12–11.14).

Experiments testing prolonged human life in the Bios-3 Life Support System were conducted from 1970 to 1980. What follows are some results of the Bios-3 experiment that began on December 24, 1972 and which ended according to schedule on the 180th day on June 22, 1973. It consisted of three phases, each 2 months long, which differed in their mass transfer characteristics.

During the first phase of the experiment, the system consisted of two phytotrons containing both a wheat culture and a selection of vegetables and a living compartment for the crew. All crew requirements for gas and water were satisfied by the higher plants. Graywater (sinks, showers, laundry) from the living compartment was pumped into the wheat's nutrient medium. Solid and liquid wastes from the crew were removed from the Bios-3 complex. Crew food

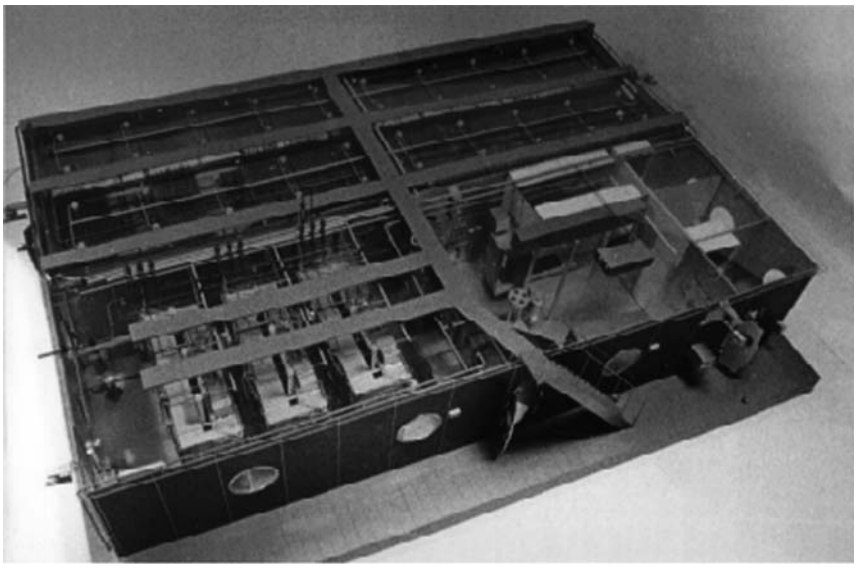

Fig. 11.12. General view of the Bios-3 (model with transparent roof). Front left – algal compartment; right – crew compartment; back – two higher-plant compartments. Light sources are on the roof of installation. Ladders and gangways on the roof are for servicing light sources. On the front wall, to the right – entrance of one of crew's cabins. To the right and left of it – airlocks for passing tools, chemical reagents, and other things in and out (16).

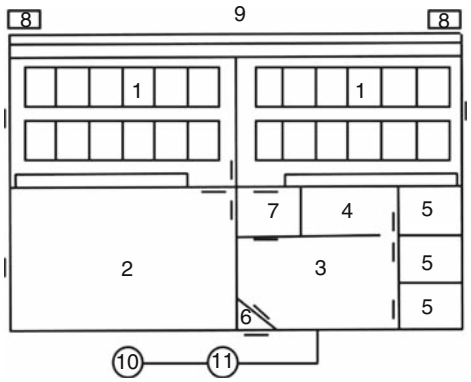

Fig. 11.13. BIOS – 3. Design of the Bios-3 experimental system. 1 – phytotrons; 2 – algal cultivator compartment; 3 – living quarters; 4 – kitchen–dining-room; 5 – cabins; 6 – toilet lock; 7 – vestibule; 8 – pumps of the cooling system for light sources; 9 – watering collector of the heat exchange wall of phytotrons; 10 – pressurization compressor; 11 – bacterial filter (16).

requirements were satisfied in two ways. The grain (bread) and vegetables produced by the higher plants provided fresh food, and the freeze-dried (lyophilized) food stores stocked in the Bios-3 complex at the beginning of the experiment were sources of animal protein and other processed food (16).

Fig. 11.14. Harvesting in the Bios-3 phytotron crop section (16).

During Phase II of the experiment, one phytotron was removed from the system, and a compartment containing *Chlorella* cultivators was introduced in its place. The cultivator photoreactor enabled the system to attain a higher degree of closure with respect to gas and water exchange. Human gas and water exchange requirements were satisfied by the combined photosynthetic activity of the photoreactors and the phytotron. Crew liquid wastes were consumed by the photoreactors; the solid wastes were dried so that water could be returned to the system. The nutrition system supplying the plants remained as it was in Phase I.

In Phase III, the phytotron containing wheat and vegetables was replaced with a phytotron containing only a selection of vegetable crops.

System mass transfer with the surrounding environment and between links was studied and described as the daily movement of mass throughout the system. The human role in the mass transfer of an autonomous system did not differ from the same human role in a nonautonomous ecological system. The crew fit into the closed ecosystem like a single ecosystem link with characteristic rates of consumption of resources (air, food, water) and the production of metabolic byproducts, gaseous, liquid, and solid. A link composed of three people rather than one person is advantageous since temporary fluctuations in an individual's metabolism are smoothed over by the total metabolism of the three people taken together.

Trace element dynamics in the system was a special research topic (The trace elements were: iron, lead, nickel, chromium, aluminum, titanium, molybdenum, boron, copper, zinc, and manganese). It was found that macroelement dynamics – for instance, the dynamics of nitrogen and phosphorous – are controllable and balanced, as opposed to trace element dynamics that cannot be easily governed. Trace elements were emitted from certain

components in the system, mainly from building materials, after which they accumulate elsewhere in the system. For instance, trace elements were accumulated in the edible parts of plants. During the length of the experiment, no toxic effects were discovered due to uncontrolled trace element dynamics, but the concentration of some of them in the solutions circulated within the system rose or fell two- to tenfold. Any imbalance in the movement of these trace elements poses a potential threat to the continued health and survival of the system. For future longterm missions, it will be necessary to pay special attention to the trace element dynamics of a closed system and to the ways to control them.

The closure of the material exchange cycle in the system is evaluated by the cumulative index $R = (1 - \mathrm{m/M}) \times 100\%$ (Where M = daily crew requirements, m = daily ecosystem requirements). System closure during Phase I for the "humans-higher plants" experiment was evaluated at 82% ($m = 6128.2\,\mathrm{g/day}$, $M = 34395.8\,\mathrm{g/day}$). When a third link, *Chlorella*, that consumes human liquid excretions, was introduced into the system, then closure grew to 91% (16).

The composition of the atmosphere regenerated by the system was characterized by the presence of a series of organic volatiles and heightened carbon dioxide content – from 0.4 to 1% for short-lived spikes to 2.3% at various phases of the experiment. Volatile concentrations quickly reached equilibrium values and fluctuated around these values during the course of the remainder of the experiment.

An exception to this were the short-lived spikes in carbon monoxide concentrations or other carbon compounds, which occur during emergency situations and which are subsequently corrected by the crew. After the system returned to its normal state, CO concentration was maintained at a low equilibrium level by internal system procedures. These observations demonstrated that there were not only producers, but also consumers of CO in the system. Levels of CO could be maintained at steady-state by processes internal to the system only if this were the case. This conclusion is applicable to other toxic gases measured in Bios-3's atmosphere (16).

At various stages of the experiment, the condensate water collected from the green plants in the phytotrons and algal cultivators served as a source of drinking and sanitary/cleaning water. Water was procured from these sources and then purified over ion-exchanging resins and charcoal. After this final purification, this water satisfied health standards for drinking water.

The food consumed (rations and vitamins included) in a system containing two phytotrons provided three people with 26% of their carbohydrates, 14% of their proteins, and 2.3% of their fats. The foods yielded from plants grown in the phytotrons – the bread baked by the test subjects and the vegetables – did not differ in their biochemical composition and taste characteristics from high quality food produced by ordinary agricultural methods. Inclusion of these products in the diet boosts crew morale. During all phases of experiments, it was found that it was possible to maintain a balanced atmospheric composition by manipulating the biochemical composition of the crew diet within physiologically acceptable norms. Some results on the microflora dynamics, including microflora exchange are presented in the chapter in this book devoted to Microbial Ecology of CES (Somova et al. this volume).

Table 11.6
Calculation of daily gas exchange of the crew in one of the Bios-3 experiments (63)

	I stage			II–III Stages		
Indicator	Proteins	Fats	Carbohydrates	Proteins	Fats	Carbohydrates
Composition of ration (g)	230	230	1150	165	165	825
Assimilability of foodstuffs (%)	86.9	96.9	99.4	86.0	86.9	99.4
Assimilated quantity of foodstuffs (g)	200	223	1143	143	160	820
Quantity of O_2 necessary to oxidize 1 g of substance (l)	0.966	2.019	0.829	0.966	2.019	0.966
Quantity of CO_2 formed during oxidation of 1 g of substance (l)	0.774	1.427	0.829	0.774	1.427	0.829
Quantity of O_2 necessary to oxidize assimilated substances (l)	193	450	948	138	323	680
Quantity of CO_2 formed during oxidation of assimilated substances (l)	155	318	948	111	228	680
Total quantity of consumed O_2 (l)		1,591			1,141	
Total quantity of released CO_2 (l)		1,421			1,019	
Respiratory coefficient		0.893			0.893	

Comprehensive medical examinations of test subjects during the half-year experiment and for an extended period after the closure experiment revealed neither any worsening of their health nor any deviations of their physiological parameters from the original state. So, it was concluded "that the habitat generated in Bios-3 is adequate for human physiological and ecological requirements, and a healthy human can stay in this biological life support system for quite a long time" (16).

Table 11.6 gives the results of the gas exchange during the 1977 four-month closure in Bios-3 (63).

3.3. European Research on Closed Ecological Systems

European efforts have included much work on microgravity issues of biological development, essential to the successful translation of ground-based CELSS to space, and work on basic physiological responses of plants to environmental factors (such as that of André and

associates at CNRS, Cadarache, France (64). Binot and colleagues at the European Space Technology Center at Noordwijk, the Netherlands are studying various microbial systems as elements in spacecraft life support systems. Closed ecological system research by the European Space Agency under the "MELiSSA" program is being conducted at the University of Barcelona, Spain and is summarized in the chapter on the microbial aspects of CES by Somova et al (this volume) and below.

3.3.1. The Closed Equilibrated Biological Aquatic System

The Closed Equilibrated Biological Aquatic System (C.E.B.A.S.) was developed by V. Blüm and co-workers at the Ruhr-University of Bochum (RUB). It was originally designed as an aquarium for long-term zoological space research. Research on the idea of a balance, sustainable system utilizing aquaculture of plants and fish began in 1985 in ground-based, open experimental format. As it eventually developed, C.E.B.A.S. can be described as a closed ecological system based on an engineered aquatic ecosystem, which contains fish and/or water snails, bacteria which are capable of oxidizing ammonia and water plants, which add oxygen and are a food source for the other tropic levels. It serves as a model for aquatic food production modules, which are not seriously affected by microgravity and other space conditions. Its space flight version, the so-called C.E.B.A.S. MINI-MODULE was successfully tested in spaceflight on the STS-89 and STS-90 (NEUROLAB) Space Shuttle missions.

The balance of the ecosystem relies on the management of the green plants, which as the autotrophic producers through photosynthesis produce organic compounds by the conversion of light energy into chemical energy. This process also produces oxygen, which is essential for the metabolic respiration of the animals (the fish and snails) and the bacterial microbes, all of whom function as the consumers in the closed ecosystem. The consumers in turn produce carbon dioxide, which is required by the plants for their photosynthesis. The nitrogen cycle is kept cycling since the consumers excrete ammonia, which is then converted by bacterial action into nitrate, which can be used by the plants (Fig. 11.15).

The live-bearing fish *Xiphophorus helleri*, the swordtail used in the vertebrate models of the experimental apparatus and the water snail *Biomphalaria glabrata*, used in the invertebrate version, were chosen after evaluation of their adaptive and physiological features, which make them extremely suitable to address the scientific goals. The plant bioreactor is designed for rootless, buoyant higher water plants with a high capacity for nitrate and phosphate ion uptake. *Ceratophyllum demersum* (horn-weed) was chosen as the most suitable species.

After initial development of C.E.B.A.S., it has been further developed as a test bed for animal/plant biomass production systems for Earth application (Fig. 11.16), as well as a very promising approach for bioregenerative life support systems for human food production and water and oxygen regeneration in space. The scientific program was then extended and currently four German and three U.S. American universities are involved in the research project. The scientific areas cover biomineralization (snails: W. Becker, U. Marxen) University of Hamburg; fishes: S. Doty, Hospital of Special Surgery, New York); CES-analysis (V. Blüm, M. Andriske, RUB); embryology (snails: W. Becker, fishes: V. Blüm, F. Paris, RUB); immunology (fishes: R. Goerlich, University of Düsseldorf); microbiology (W. Rueger, RUB);

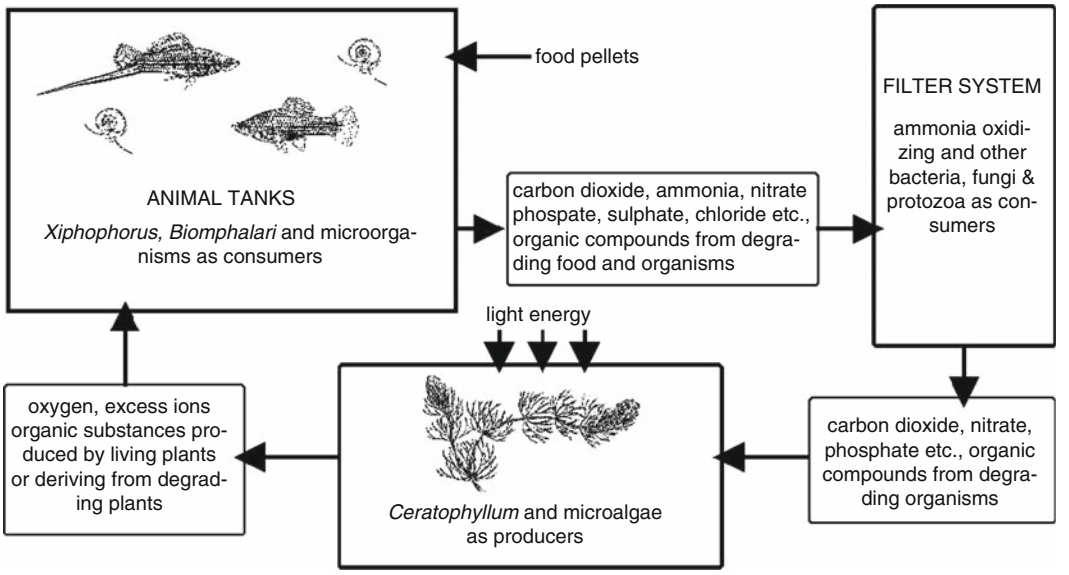

Fig. 11.15. Functional relationships of the basic components of the C.E.B.A.S. The animal tank is combined with an aquatic plant bioreactor and with a bacteria filter, which contains ammonia oxidizing bacteria (65).

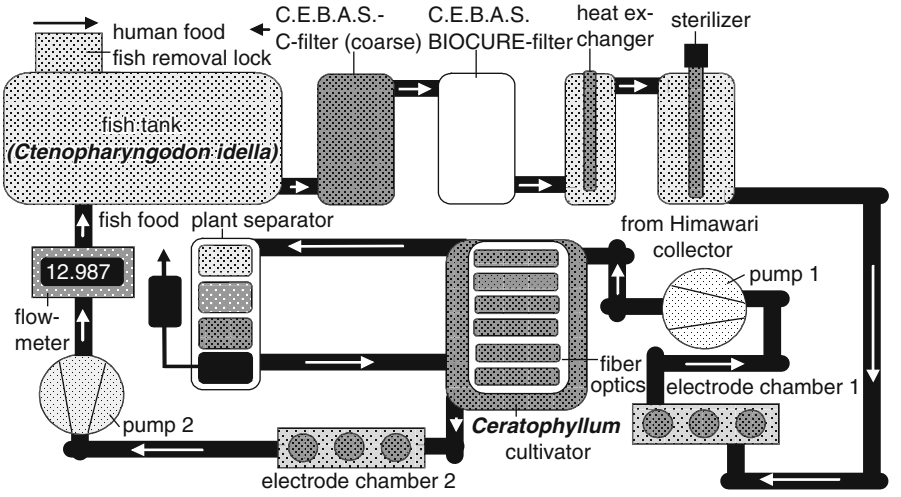

Fig. 11.16. Schematic of a variant of C.E.B.A.S. for high intensity production of fish and plants in terrestrial applications (65).

neurobiology (fishes: H. Rahmann, R. Anken, University Stuttgart-Hohenheim); plant morphology/physiology (H. Hollaender-Czytko, D. Voeste, RUB); statocyst/vestibular research (snails and fishes: M. Wiederhold, University of Texas, San Antonio); and reproductive endocrinology/physiology (fishes: V. Blüm, F. Paris, RUB, M.P. Schreibman, Brooklyn College, New York). They are closely linked to applied projects in the field of innovative combined animal–plant aquaculture (V. Blüm and co-workers) (65).

The C.E.B.A.S. exists in two different versions: The original C.E.B.A.S. with a total volume of about 150 l and the C.E.B.A.S. MINI-MODULE, which is operative in several types with volumes between 7.7 and 10 l. The first completed mid-term tests were over 9 and 12 months. The latter passed numerous tests up to 3 months duration. The C.E.B.A.S. MINI-MODULE was developed to fit into a space shuttle middeck locker tray as a spaceflight version. After numerous successful tests of the laboratory models, the spaceflight hardware was constructed by OHB Systems in Bremen.

The results of the space experiments with the C.E.B.A.S. MINIMODULE confirmed the initial hypothesis that aquatic organisms will not be seriously impacted by microgravity and other space environmental factors. A brief summary of major findings included (66–70):

- The fish behaved normally, orienting themselves with respect to the light source and tank walls, and exhibited normal patterns of feeding and domination/hierarchical fighting. Their overall bodily coordination was unimpaired. Loop swimming was only observed for a brief period during a docking maneuver. Reproductive system was functional as evidenced by birth of new fish in space and embryonic and early development of juvenile fish was normal. There were no peritoneal fat deposits which indicates insufficient nutrition.
- The snails adhered to the substrate, and when they floated free in the water stream, they stretched their bodies out of their shells, made contact with other floating snails and adhered to each other. Embryonic development and biomineralization of the shell were also unimpaired.
- Ground control plants and the spaceflight water plants behaved similarly. Photosynthesis was excellent and there was high biomass production. In the STS-90 mission, reductions in productivity were attributed to self-shading. Both spaceflight and ground-control plants flowered. "The only morphological change observed was a irregular arrangement of starch grains in the cells of the xylem–phloem sheath of spaceflown plants, which is radial in ground plants" (65).
- The bacteria in the microbial filter also performed well. In the STS-89 mission, bacterial density was comparable to those in natural ponds. In the STS-90 missions, a much higher bacterial density was attributed to higher mortality of juvenile fishes, thus reducing the major predator.

3.3.2. The MELiSSA (Micro-Ecological Life Support System Alternative) Project

A major effort in bioregenerative closed ecological systems for life support is the Micro-Ecological Life Support System Alternative (MELiSSA) of the European Space Agency with numerous European, Canadian, and other international cooperation. The basic concept behind MELiSSA is to conjoin the metabolic abilities of both microorganisms and higher plants in a series of steps to create a closed loop, producing air, water, and food for astronauts in space. It should, when complete, be another powerful tool for research in the behavior of artificial ecosystems and for developing the capacity for life support on long-duration space missions. Key processes being developed are the recycling of waste (inedible biomass, feces, and urine), carbon dioxide and minerals and the production of food, fresh water, and air revitalization.

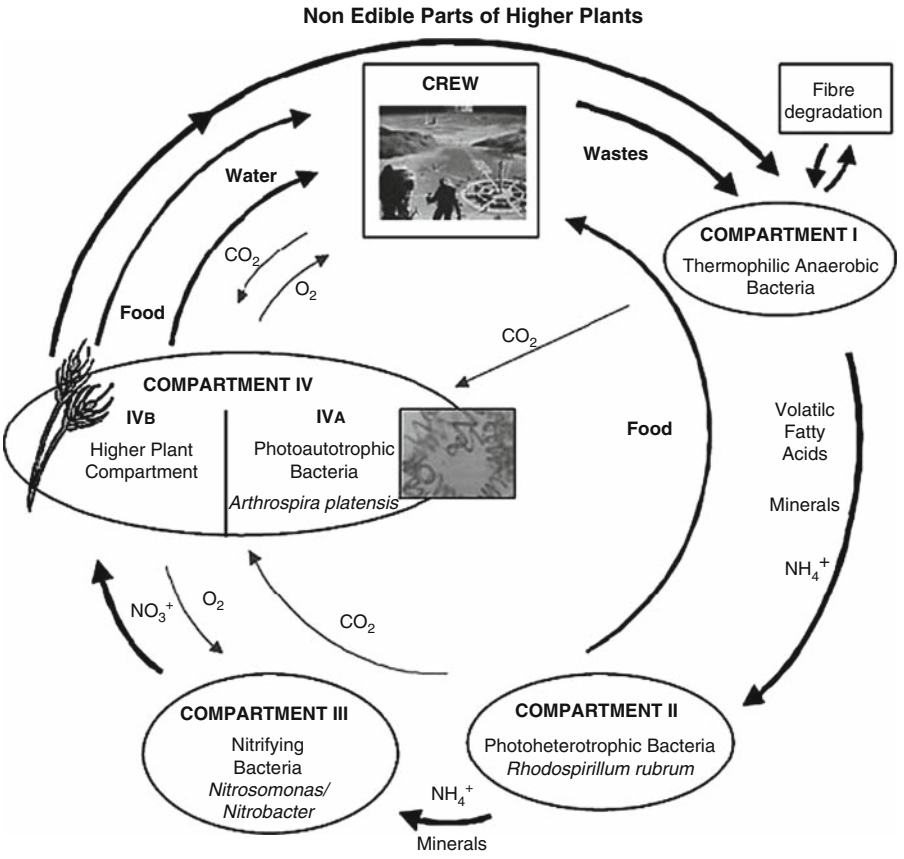

Fig. 11.17. Diagram layout of MELiSSA Loop (Microecological Life Support System Alternative) European Space Agency (http://www.estec.esa.nl/ecls/melissa/newmelissaloop.html).

Project design calls for the integration of various sub compartments of MELiSSA at a facility at the university of Barcelona, Spain. MELiSSA is being researched and built in various research and industrial labs in Europe and Canada. Construction of the project began in 1995, and now early versions of two of the three fermentation chambers are operational at the integrated facility in Barcelona. The complete pilot plant is expected to be fully operational by 2005 (http://extids.estec.esa.nl/melissa) (Fig. 11.17).

The closing of the loop in MELiSSA is conceived of as being effected through the interconnections of five internal compartments. Compartment 1 is the Liquefying Compartment where the wastes produced by the consumers (the crew) such as feces, urea, non-edible crop residues and non-edible microbial byproducts are collected and then anaerobically transformed into more usable forms, such as ammonia, hydrogen gas, CO_2, and minerals. The likelihood is that Compartment 1 will operate at high temperatures (55° C), using thermophilic fermentative bacteria to perform the microbial degradation of the waste materials. Research on the optimal mix of thermophilic bacteria has increased the rates of proteolysis from 15 to 70% using a

consortium of bacteria. To improve this degradation level, several technologies are currently studied (subcritical oxidation, fungi, rumen bacteria, hyperthermophilic bacteria). Compartment 2 is the Photoheterotrophic compartment. Here, 2 subcomponents, photoautotrophs and photoheterotrophs, will eliminate the liquid waste products of Compartment 1. Recent research has led to the expectation that the very successful substrate degradation obtained with *R. rubrum* may lead to the necessity of a single second compartment, the photoheterotrophic one. Next step is the nitrifying step, accomplished in Compartment 3. Here, ammonia from wastes is converted to nitrates, which are more easily utilized by higher plants and autotrophic algae/bacteria. It is expected that a mix of *Nitrosomas* and *Nitrobacter* will be used in Compartment 3. Since this is essentially a fixed bed reactor, the importance of the hydrodynamic factors, loading/rate reactions and overall stochiometry of the process must be well understood and managed. The fourth compartment has two sections, an algae compartment with the cyanobacteria: *Arthrospira platensis* and the Higher Plant (HP) compartment. These compartments are essential for the regeneration of oxygen and the production of food. Eight candidate food crops are being studied: Wheat, tomato, potato, soybean, rice, spinach, onion, and lettuce. Research at the University of Guelph in Canada is examining biomass production rates, as well as mineral and nutritive content. Research into optimizing environmental considerations (light, nutrients, humidity), development of supportive technology for higher plant production and sensor development are also being undertaken (71) http://extids.estec.esa.nl/melissa.

3.4. Japanese Research in Closed Ecological Systems

Japanese research started under the leadership of Nitta and Oguchi of the National Aerospace Laboratory in Tokyo, initially concentrated on gas recycling systems, involving oxygen and carbon dioxide separation and concentration, water recycling systems, plant and algae physiology and cultivation techniques, as well as animal and fish physiology and breeding (72).

In the 1990s, a large and ambitious project "Closed Ecological Experimental Facility" (CEEF) was supported by the Japanese state, and the Institute of Environmental Sciences facility was founded on the Island of Honshu in Rokkasho, a small township in the prefecture Aomori.

Since, the, 1990s, great strides toward creating artificial closed ecological systems have been made by Japanese researchers, supported by generous financial aid from industry and government. It is evident that Japanese society, with a very high population density and insular psychology, is able to comprehend the crucial importance of biospheric problems for civilization. In a period of a few years, the project CEEF – Closed Ecology Experiment Facilities – has been designed and basically implemented (Fig. 11.18).

CEEF consists of a connected series of different subsystems: (a) For the cultivation of plants, Closed Plantation Experiment Facility, (b) for domestic animals, the Closed Animal Breeding (c) for the crew of two, the Habitat Experiment Facility, and (d) a Closed Geo-Hydrosphere Experiment Facility, which are schematically shown in Figs. 11.18 and 11.19. The material circulated in CEEF is controlled strictly in the materially-sealed closed system by air-conditioners and material processing subsystems. Only energy and information are exchanged with the outside. Each facility can be operated independently or linked with

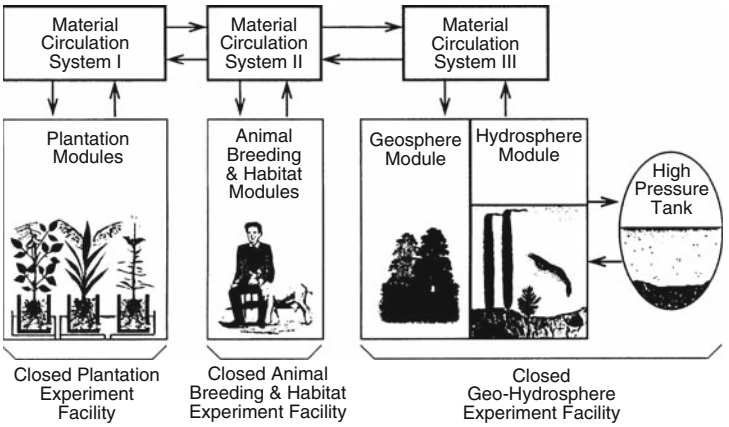

Fig. 11.18. Closed ecology experimental facility (CEEF) (From Closed Ecology Experiment Facilities (CEEF) (73).

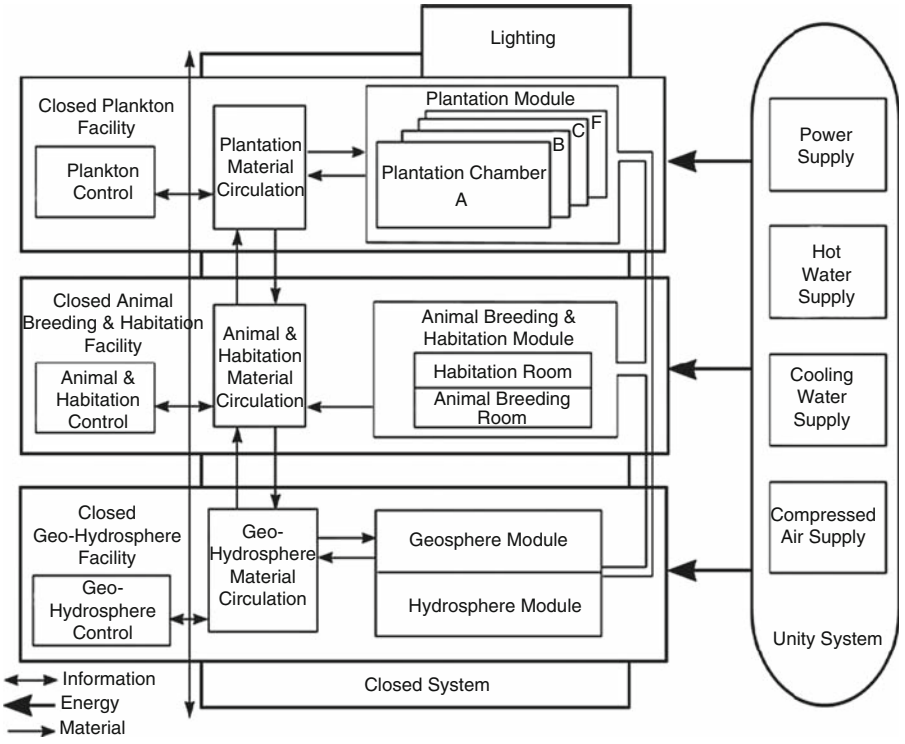

Fig. 11.19. System Configuration of Closed Ecology Experiment Facilities (CEEF) (73).

another facility. The subsystems of CEEF are a unique tool for the environmental sciences and other fields of research such as test beds for life support systems for human and Mars base application, the global change problem and furthering the solutions for a pollution-free or "zero-emission society" (73).

There are two basic important objectives for the CEEF facility. One is the topical problem of thorough investigation of the migration of radioactive elements by the metabolic pathways in ecosystems. Another objective is to model global change, specifically the ecological consequences of global warming. Thus, the closed ecological system is increasingly perceived not only as a means to support human life in a hostile environment – in space – but primarily as a tool for the experimental investigation of the problems of the Earth's biosphere.

Special emphasis was placed on designing a physicochemical subsystem to form a closed loop of the material circulation of biological processes via the mineralization of wastes and end products to return the elements to the biological cycling. These technologies are called the Artificial Material Processing Equipment.

The largest industrial companies of Japan took part in manufacturing equipment and elaborating technology for the facility. CEEF and its subsystems are described in detail in publications of the Institute for Environmental Sciences (74).

Comparison of the complex Bios-3, operating in Siberia, and the system CEEF allows the conclusion that they complement each other. CEEF can separate the closed material cycle to quantify the flow rates and dynamics, of, numerous individual components, which can then be analyzed individually. Bios-3 (as well as successor biospheric systems like Biosphere 2) has made it possible to investigate the properties of ecosystems that emerge when they get closed, and that are not peculiar to any of its elements taken separately (16).

An integrated system is far simpler to operate owing to the multitude of natural, ecological cyclic processes occurring in it and auto regulatory feedbacks. This process is often called the "self-organizing principle" of natural ecosystems (e.g., (75)). As an example of a complication caused by the separation of functions in CEEF, we can mention that to avoid disruption of metabolic closure, the personnel servicing the system inside is provided with specially designed suits, similar to spacesuits, so that their respiration does not mix with the system gas exchange. In Bios-3, however, the crew services and controls the system entirely, their respiration being part of the system metabolism. However, to study particular processes, it is inconvenient, and sometimes absolutely impossible, to use the integral system. For this purpose, it would be expedient to turn to the Japanese "analytical" system separated into subsystems. Thus, the results, which will be obtained by the experiments on the closure in CEEF will add to the knowledge gained in other closed ecological systems and biospheric systems as well as the ongoing studies of natural ecosystems and the Earth's biosphere.

The CEEF facility is still under development, although plant cultivation experiments with a 120 m^2 planting bed have been conducted. In addition to specific research, the facility is designed as a "mini-Earth" to allow investigation of processes analogous to those in the Earth's biosphere, such as the dynamics of radioactive isotopes in agricultural, terrestrial, and marine environments (74).

In the past few years, the team working on CEEF also presented their first experimental data which includes understanding how higher crop plants and domestic animals might respond to

environmental changes. The research leaders at CEEF think the facility could help in predicting the effect of small temperature rises, perhaps due to global warming, on rice growth. Nitta has also investigated the potential impact on the carbon cycle of small amounts of radioactive carbon dioxide that will likely be released into the air by the Rokkasho-Mura nuclear fuel reprocessing plant, which is currently under construction and could still be modified. This use of closed ecological systems for conducting experiments in controlled conditions shows the potential of the new discipline of biospherics as an appropriate predictive and experimental tool before such experiments are released unwittingly and tried on the global biosphere.

All of these unique facilities have a common fundamental goal – to model the biosphere – and a common practical objective – to create closed human life support systems. It is very complicated and costly both to construct these facilities and to use them for experiments. Since this work is important for humanity as a whole, it is necessary to coordinate the experiments at the preparatory stages and while analyzing the results, following the example of atomic physicists cooperating in using the few nuclear-particle accelerators existing in the world. The importance of the work on creation of artificial closed ecosystems for humanity as a whole and the complexity and high cost of experiments – all make further international cooperation in this field of knowledge imperative (4, 16, 19, 25, 37).

4. CONCLUSION

Tsiolkovsky, the Russian space visionary who laid the basis for modern rocketry and astronautics, also foresaw the need for regenerative life support systems in the spacecraft: "The supply of oxygen for breathing and food would soon run out, the byproducts of breathing and cooking contaminate the air. The specifics of living are necessary – safety, light, the desired temperature, renewable oxygen, a constant flow of food" (54).

The expansion of human presence into space, both in the microgravity conditions of orbital space and on lunar or planetary surfaces, will in one sense be but the latest in the series of expansionary advances of life. With man's growing technical ability to create living spaces out of contact with the Earth's biotic regeneration (as in submarines, high altitude aircraft, and spacecraft), and to voyage to extraplanetary ones, a new chapter in this biospheric expansion stands ready to be opened. The creation, initially ground-based and later off the Earth, of simple closed ecological life support systems, and eventually of stable and evolving biospheric systems, will mark the transition of life from one-planet phenomena to the one capable of permanent expansion into the Solar System and beyond.

Permanent human presence in space and a number of ambitious long-duration missions are beginning to emerge as significant goals in the evolving international space agenda. This changing framework of space development has had a number of important inputs, including the U.S. National Commission on Space, (41) which looked at the next 50 years in space and outlined a coherent set of objectives, which could build an effective space infrastructure. These studies emphasized the importance of bioregenerative life support as a key enabling technology. In 1988, the U.S. Congress amended NASA's charter to include permanent human presence as a legitimate part of its activity. This emerging American space agenda is similar to what many saw as the central focus of the Russian (then Soviet) space program whose

accomplishments include the series of Salyut then Mir space stations, which operated for decades. Soviet plans, now derailed by lack of financial resources, included plans for lunar bases early in the twenty-first century, to be followed by manned exploration, and finally, Mars bases. For many years, the motto of the Soviet space life scientists had been: "On Mars we must grow our own apples!" Economic pressures make cooperative international ventures increasingly attractive. International cooperation in space is also valuable as a way of aligning all people of the Earth and inspiring them with our shared, grander historic and evolutionary challenges. The strategy of "evolutionary expansion" into space as opposed to space spectaculars with no infrastructural increase (known as "footprints and flags") is beginning to dominate space exploration planning.

This far-reaching space agenda requires, and is producing, a shift in life support away from the type of technologies that were developed for the sprint missions to the Moon or for short duration spaceflights. It is now becoming clear that bioregenerative life support is one of the chief technologies that can make possible our long-term future in space. It will be a huge undertaking to translate ground-based test bed work into plausible space-based systems. Living in space will also require better understanding of radiation hazards and defenses, measures to deal with microgravity and reduced gravitational effects on living systems, and the ability to utilize extraterrestrial materials. But, what is becoming clear to space planners and the public alike is that bioregenerative life support systems are the key to be able to *live* in space.

The Russian pioneer of the science of the biosphere, V.I. Vernadsky, saw that the challenge of our time was the harmonization of what he termed the "technosphere" with the biosphere. He foresaw the emergence of the "noosphere," a sphere of intelligence, which humanity must develop since our impacts on the global biosphere are so powerful.

H.T. Odum is considered the father of "Ecological Engineering," a new discipline, which seeks a symbiotic mix of man-made and ecological self-design that maximizes productive work of the entire system (including the human economy and the large-scale environmental system) (75). By minimizing human manipulation and the use of machinery, ecological engineering solutions aim to increase material recycling, enhance efficiency, reduce costs, and maximize the contributions of ecological processes in the total system. An important application of ecological engineering is the design of interface ecosystems, such as constructed wetland sewage treatment systems (as were developed by Wolverton (76) for NASA's life support systems, and further developed for Biosphere 2 (48)), to handle byproducts of the human economy and to maximize the performance of both the human economy and natural ecosystems (77).

H.T. Odum and E.P. Odum, the founders of Systems Ecology, tried in vain during the early days of space life support development in the 1960s to get a model of complex, high diversity species systems able to self-organize, accepted as a viable path of development. Instead the prevailing mode that has been extremely highly engineered systems minimizes such ecological diversity and robustness (78). It is perhaps time to heal this historical division within the field of space life support systems, recognizing the strengths that both approaches represent.

The opportunity and challenge for those working on bioregenerative technologies, CELSS and closed ecological systems for space life support is starkly underscored by their necessity

to achieve successful recycling and stability of their systems in volumes far smaller than those of Earth's natural ecosystems, and with vastly accelerated cycling times. This means that there is enormous necessity for intelligent design to make small closed ecological systems function properly. In the coming decades, the opportunity exists for this work to become ever more relevant to the parallel efforts to understand the Earth's biosphere and to transform the human endeavor to a sustainable basis. We live in a virtually materially closed ecological system on Earth – and to live long-term in space, we will need to create new closed ecological systems. Learning to sustain, recycle and harmoniously live within our world(s) is the overriding challenge we face both on Earth, and if we are to live in space, whether in space stations or on lunar and planetary surfaces. The stakes are huge: We must learn from both efforts to prosper and evolve (79–82).

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12
Natural Environmental Biotechnology

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CONTENTS

Abstract Aquaculture or the production of aquatic organisms (both flora and fauna) under controlled conditions has been practiced for centuries, primarily for the generation of food, fiber, and fertilizer. The water hyacinth and a host of other organisms like duckweed, seaweed, midge larvae, and alligator weeds are used for wastewater treatment. Water hyacinth system, wetland system, evapotranspiration system, rapid rate filtration, slow rate system, overland flow system, and subsurface infiltration have also been applied. This chapter describes the above applications and explains their practice, limitations, design criteria, performance, and costs.

Key Words Natural waste treatment • aquatic organisms • water hyacinth • weeds • wetland • infiltration • design and performance.

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1. AQUACULTURE TREATMENT: WATER HYACINTH SYSTEM

1.1. Description

Aquaculture or the production of aquatic organisms (both flora and fauna) under controlled conditions has been practiced for centuries, primarily for the generation of food, fiber, and fertilizer. The water hyacinth (*Eichhornia crassipes*) appears to be the most promising organism for wastewater treatment and has received the most attention (1). However, other organisms are being studied. Among them are duckweed, seaweed, midge larvae, alligator weeds, and a host of other organisms. Water hyacinths are large fast-growing floating aquatic plants with broad, glossy green leaves and light lavender flowers. A native of South America, water hyacinths are found naturally in waterways, bayous, and other backwaters throughout the South. Insects and disease have little effect on the hyacinth and they thrive in raw, as well as partially treated, wastewater. Wastewater treatment by water hyacinths is accomplished by passing the wastewater through a hyacinth-covered basin (Fig. 12.1), where the plants remove nutrients, BOD_5, suspended solids, metals, etc. Batch treatment and flow-through systems, using single and multiple cell units, are possible. Hyacinths harvested from these systems have been investigated as a fertilizer/soil conditioner after composting, animal feed, and a source of methane when anaerobically digested (2).

1.2. Applications

Water hyacinths are generally used in combination with (following) lagoons, with or without chemical phosphorus removal. A number of full-scale systems are in operation. Most often considered for nutrient removal and additional, treatment of secondary effluent (1–3). Also, research is being conducted on the use of water hyacinths for raw and primary treated wastewater or industrial wastes, but present data favor combination systems. Very good heavy metal uptake by the hyacinth has been reported. Hyacinth treatment may be suitable for seasonal use in treating wastewaters from recreational facilities and those generated from processing of agricultural products. Other organisms and methods with wider climatological applicability are being studied. The ability of hyacinths to remove nitrogen duringactive

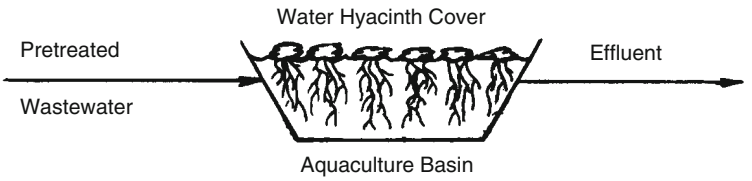

Fig. 12.1. Aquaculture treatment: water hyacinth system. (*source:* US EPA).

growth periods and some phosphorus and retard algae growth provides potential applications in (2, 3):

(a) The upgrading of lagoons
(b) Renovation of small lakes and reservoirs
(c) Pretreatment of surface waters used for domestic supply
(d) Storm water treatment
(e) Demineralization of water
(f) Recycling fish culture water, and
(g) For biomonitoring purposes.

1.3. Limitations

Climate or climate control is the major limitation. Active growth begins when the water temperature rises above 10°C. and flourishes when the water temperature is approximately 21°C. Plants die rapidly when the water temperature approaches the freezing point; therefore, greenhouse structures are necessary in northern locations. Water hyacinths are sensitive to high salinity. Removal of phosphorus and potassium is restricted to the active growth period of the plants.

Metals such as arsenic, chromium, copper, mercury, lead, nickel, and zinc can accumulate in hyacinths and limit their suitability as a fertilizer or feed material. The hyacinths may also create small pools of stagnant surface water which can breed mosquitoes. Mosquito problems can generally be avoided by maintaining mosquito fish in the system. The spread of the hyacinth plant itself must be controlled by barriers since the plant can spread and grow rapidly and clog affected waterways. Hyacinth treatment may prove impractical for large treatment plants due to land requirements. Removal must be at regular intervals to avoid heavy intertwined growth conditions. Evapotranspiration can be increased by two to three times greater than evaporation alone.

1.4. Design Criteria

Ponds, channels, or basins are in use. In northern climates covers and heat would be required. Harvesting and processing equipment are needed. Operation is by gravity flow and requires no energy. Hyacinth growth energy is supplied by sunlight. All experimental data is from southern climates where no auxiliary heat was needed. Data is not available on heating requirements for northern climates, but it can be assumed proportional to northern latitude of location and to the desired growth rate of hyacinths.

Design data vary widely. Table 12.1 shows the design criteria for water hyacinth systems (4). The following ranges refer to hyacinth treatment as a tertiary process on secondary effluent (2):

(a) Depth should be sufficient to maximize plant rooting and plant absorption
(b) Detention time depends on effluent requirements and flow, range 4–15 days
(c) Phosphorus reduction, 10–75%
(d) Nitrogen reduction, 40–75%
(e) Land requirement is usually high, i.e., 2–15 acres/MG/d (2.14–16.04 $m^2/m^3/d$)

Table 12.1
Design criteria for water hyacinth systems

Factor	Aerobic nonaerated	Aerobic nonaerated	Aerobic aerated
Influent wastewater	Screened or settled	Secondary	Screened or settled
Influent BOD_5 (mg/L)	130–180	30	130–180
BOD_5 loading (kg/ha-d)	40–80	10–40	150–300
Expected effluent (mg/L)			
BOD_5	<30	<10	<15
SS	<30	<10	<15
TN	<15	<5	<15
Water depth (m)	0.5–0.8	0.6–0.9	0.9–1.4
Detention time (days)	10–36	6–18	4–8
Hydraulic loading (m^3/ha-d)	> 200	<800	550–1,000
Harvest schedule	Annually	Twice per month	Monthly

Source: US EPA (4).

1.5. Performance

Process appears to be reliable from mechanical and process standpoints, subject to temperature constraints. In tests on five different wastewater streams including raw wastewater and secondary effluents, the following removals were reported (2):

(a) BOD_5: 35–97%
(b) TSS: 71–83%
(c) Nitrogen: 44–92%
(d) Total *P*: 11–74%.

Takeda and Co-workers (3) reported using aquaculture wastewater effluent for strawberry production in a hydroponic system which reduced the final effluent phosphorus concentration to as low as 0.1 mg/L which meets the stringent phosphorus discharge regulations. There is also evidence that in aquaculture system coliform, heavy metals, and organics are also reduced, as well as pH neutralization.

Hyacinth harvesting may be continuous or intermittent. Studies indicate that average hyacinth production (including 95% water) is on the order of 1,000–10,000 lb/d/acre (1,121–11,210 kg/d/ha). Basin cleaning at least once per year results in harvested hyacinths. For further detailed information on water Hyacinth systems the reader is referred to references (5–13).

2. AQUACULTURE TREATMENT: WETLAND SYSTEM

2.1. Description

Aquaculture-wetland systems for wastewater treatment include natural and artificial wetlands as well as other aquatic systems involving the production of algae and higher plants

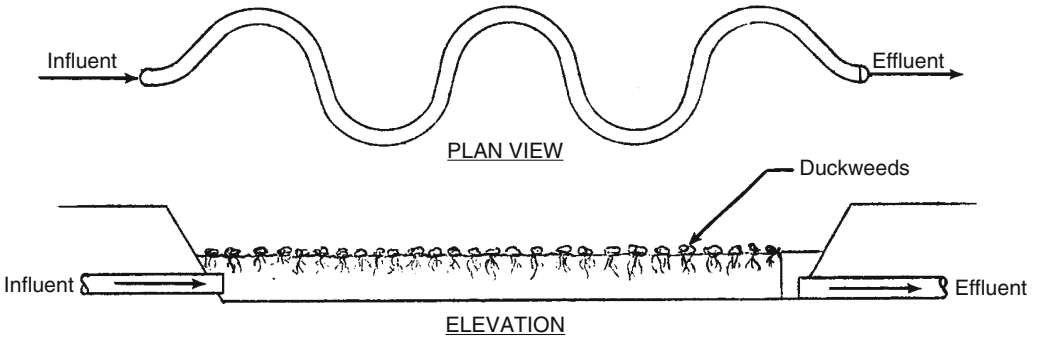

Fig. 12.2. Aquaculture treatment: wetland system. (*source:* US EPA).

(both submerged and emergent), invertebrates and fish. Natural wetlands, both marine and freshwater, have inadvertently served as natural waste treatment systems for centuries; however, in recent years marshes, swamps, bogs, and other wetland areas have been successfully utilized as managed natural "nutrient sinks" for polishing partially treated effluents under relatively controlled conditions. Constructed wetlands can be designed to meet specific project conditions while providing new wetland areas that also improve available wildlife wetland habitats and the other numerous benefits of wetland areas. Managed plantings of reeds (e.g., *Phragmites* spp.) and rushes (e.g., *Scirpus* spp. and *Schoenoplectus* spp.) as well as managed natural and constructed marshes, swamps, and bogs have been demonstrated to reliably provide pH neutralization and reduction of nutrients, heavy metals, organics, BOD_5, COD, SS, fecal coliforms, and pathogenic bacteria (2, 4).

Wastewater treatment by natural and constructed wetland systems is generally accomplished by sprinkling or flood irrigating the wastewater into the wetland area or by passing the wastewater through a system of shallow ponds, channels, basins, or other constructed areas where the emergent aquatic vegetation has been planted or naturally occurs and is actively growing (see Fig. 12.2). The vegetation produced as a result of the system's operation may or may not be removed and can be utilized for various purposes (2):

(a) Composted for use as a source of fertilizer/soil conditioner
(b) Dried or otherwise processed for use as animal feed supplements, or
(c) Digested to produce methane.

2.2. Constructed Wetlands

Constructed wetlands are classified as a function of water flow (2, 4): surface and subsurface which are known as free water surface (FWS) and subsurface flow system (SFS) (also termed vegetated submerged bed, VSB). When simply expressed, constructed wetland treatment technology makes artificial receiving water and its vegetation part of the treatment process. In comparison to algae, the higher forms of plants – life-floating (duckweed, water

hyacinths), submerged, and emergent (cattails, rushes, and reeds) – perform less efficiently per unit weight of biomass.

FWS constructed wetland treatment conceptually relies on attached growth bacterial performance, receiving oxygen from the evapotranspiration response of the aquatic vegetation. Practically, the dominant bacterial action is anaerobic. The ammonium and nitrogen removal mechanisms (14–17) are a combination of aerobic oxidation, particulate removal, and synthesis of new plant protoplasm.

An FWS wetland is nothing more than a lagoon, except that a far greater expanse is needed to maximize the productivity per unit area. In practice, very large systems may achieve significant, if not complete, nitrogen oxidation, with surface reaeration contributing to the oxygen supply. Some nitrification and denitrification undoubtedly occurs in all systems.

If it is assumed that the wetland vegetation will not be harvested, as is the case with natural wetland systems, its capacity for nitrogen control is finite, reflecting the site-specific vegetation and the ability to expand in the available space. Thus, the bigger the natural wetland that is called part of the process, the better, since there is dilution of the wastewater to the point that it is no longer significant in comparison to the naturally occurring background flow and water quality.

Constructed FWS wetlands yield a managed vegetative habitat that becomes an aquaculture system. Examination of the evolution of this technology shows the emergence of concepts that include organic load distribution or artificial aeration to avoid aesthetic nuisances, and emphasis on plants that grow the fastest. Duckweed and water hyacinth systems (classified as aquaculture) have been reported to achieve long-term total nitrogen residuals of less than 10 mg/L and may be manageable, with harvesting and sensitive operation, to values of less than 3 mg/L on a seasonal, if not sustained, basis.

Submerged-flow constructed wetlands are simply horizontal-flow gravel filters with the added component of emergent plants within the media. They have been classically used for BOD removal following sedimentation and/or additional BOD and SS removal from lagoon effluents as with FWS approaches. This technology has the potential for high-level denitrification when a nitrified wastewater is applied; the naturally occurring environment promotes anoxic (denitrification) pathways for oxidized nitrogen elimination.

Ultimately, the success or failure of the wetland approach for nitrogen control may rest with the harvest of the vegetation, the need for backup (so that areas under harvest have the backup of areas in active growth), and often natural seasonal growth and decay cycles. If biomass production is an unacceptable goal, the designer should think of a more tolerant mixed vegetation system that minimizes the need to harvest the accumulated vegetation and maximizes the promotion of concurrent or staged nitrification and denitrification in some fashion. Conceptually, the optimization has to begin with promotion of nitrogen oxidation systems that may be shallow (better aeration for attached and suspended bacterial growth) with vegetation that minimizes light penetration and avoids as much algal growth as possible. Cyclic staging, recycle, forced aeration, and/or mixing represent some of the enhancements that naturally follow (17).

2.3. Applications

Several full-scale systems are in operation or under construction (18). Wetlands are useful for polishing treated effluents. They have potential as a low cost, low-energy-consuming alternative or addition to conventional treatment systems, especially for smaller flows. Wetlands have been successfully used in combination with chemical addition and overland flow land treatment systems. Wetland systems may also be suitable for seasonal use in treating wastewaters from recreational facilities, some agricultural operations, or other waste-producing units where the necessary land area is available (18). Potential application as an alternative to lengthy outfalls extended into rivers, etc. and as a method of pretreatment of surface waters for domestic supply, storm water treatment, recycling fish culture water and biomonitoring purposes.

2.4. Limitations

Temperature (climate) is a major limitation since effective treatment is linked to the active growth phase of the emergent vegetation. Tie-ins with cooling water from power plants to recover waste heat have potential for extending growing seasons in colder climates. Enclosed and covered systems are possible for very small flows.

Herbicides and other materials toxic to the plants can affect their health and lead to poor treatment. Duckweeds are prized as food for waterfowl and fish and can be seriously depleted by these species. Winds may blow duckweeds to the shore if wind screens or deep trenches are not employed. Small pools of stagnant surface water which can allow mosquitoes to breed can develop, but problems can generally be avoided by maintaining mosquito fish or a healthy mix of aquatic flora and fauna in the system. Wetland systems may prove impractical for large treatment plants due to the large land requirements. They also may cause loss of water due to increases in evapotranspiration.

2.5. Design Criteria

Natural or artificial marshes, swamps, bogs, shallow ponds, channels, or basins could be used. Irrigation, harvesting and processing equipment are optional. Aquatic vegetation is usually locally acquired.

Design criteria are very site and project specific. Available data vary widely. Values below refer to one type of constructed wetland system used as a tertiary process on secondary effluent (2):

(a) Detention time = 13 days
(b) Land requirement = 8 acres/MG/d = 8.55 $m^2/m^3/d$
(c) Depth may vary with type of system, generally 1–5 ft. = 0.30–1.52 m

2.6. Performance

Process appears reliable from mechanical and performance standpoints, subject to seasonality of vegetation growth. Low operator attention is required if properly designed.

Table 12.2
Nutrient removal from natural wetlands

Project	Flow (m^3/d)	Wetland type	Percent reducion TDP[a]	NH_3-N	NO_3-N	TN[b]
Brillion Marsh, WI	757	Marsh	13	–	51	–
Houghton Lake, MI	379	Peatland	95	71	99[c]	–
Wildwood, FL	946	Swamp/Marsh	98	–	–	90
Concord, MA	2,309	Marsh	47	58	20	–
Bellaire, MI	1, 136[d]	Peatland	88	–	–	84
Coots Paradise, Town of Dundas, Ontario, Canada	–	Marsh	80	–	–	60–70
Whitney Mobile Park, Home Park, FL	≈ 227	Cypress Dome	91	–	–	89

Source: US EPA (4).
[a] Total dissolved phosphorus.
[b] Total nitrogen.
[c] Nitrate and nitrite.
[d] May–November only.

Tables 12.2 and 12.3 illustrate the capacities of both natural and constructed wetlands for nutrient removal (4). In test units and operating artificial marsh facilities using various wastewater streams, the following removals have been reported for secondary effluent treatment (10-day detention) (2):

(e) BOD_5, 80–95%
(f) TSS, 29–87%
(g) COD, 43–87%
(h) Nitrogen, 42–94% depending upon vegetative uptake and frequency of harvesting
(i) Total P, 0–94% (high levels possible with warm climates and harvesting)
(j) Coliforms, 86–99%
(k) Heavy metals, highly variable depending on species.

There is also evidence of reductions in wastewater concentrations of chlorinated organics and pathogens, as well as pH neutralization without causing detectable harm to the wetland ecosystem.

Residuals are dependent on the type of system and whether or not harvesting is employed. Duckweed, for example, yields 50–60 lb/acre/d (dry weight) (53.46–64.15 m^2/m^3d) during peak growing period to about half of this figure during colder months. For further detailed information on wetland systems the reader is referred to references (19–23).

Table 12.3
Nutrient removal from constructed wetlands (4)

Project	Flow (m^3/d)	Wetland type	BOD_5 (mg/L)		SS (mg/L)		Percent reduction		Hydraulic surface loading rate (m^3/ha-d)
			Influent	Effluent	Influent	Effluent	BOD_5	SS	
Listowel, Ontario (12)	17	FWS[a]	56	10	111	8	82	93	–
Santee, CA (10)	–	SFSI[b]	118	30	57	5.5	75	90	–
Sidney, Australia (13)	240	SFS	33	4.6	57	4.5	86	92	–
Arcata, CA	11,350	FWS	36	13	43	31	64	28	907
Emmitsburg, MD	132	SFS	62	18	30	8.3	71	73	1,543
Gustine, CA	3,785	FWS	150	24	140	19	84	86	412

Source: US EPA.
[a] Free water surface system.
[b] Subsurface flow system.

3. EVAPOTRANSPIRATION SYSTEM

3.1. Description

Evapotranspiration (ET) system is a means of on-site wastewater disposal that may be utilized in some localities where site conditions preclude soil absorption. Evaporation of moisture from the soil surface and/or transpiration by plants is the mechanism of ultimate disposal. Thus, in areas where the annual evaporation rate equals or exceeds the rate of annual added moisture from rainfall and wastewater application, ET systems can provide a means of liquid disposal without danger of surface or groundwater contamination.

If evaporation is to be continuous, at least three conditions must be met (2):

(a) There must be a continuous supply of heat to meet the latent heat requirement, approximately 590 cal/g of water evaporated at 15°C
(b) A vapor pressure gradient must exist between the evaporative surface and the atmosphere to remove vapor by diffusion, convection, or both. Meteorological factors, such as air temperature, humidity, wind velocity, and radiation influence both energy supply and vapor removal
(c) There must be a continuous supply of water to the evaporative surface. The soil material must be fine-textured enough to draw up the water from the saturated zone to the surface by capillary action but not so fine as to restrict the rate of flow to the surface.

Evapotranspiration is also influenced by vegetation on the disposal field and can theoretically remove significant volumes of effluent in late spring, summer, and early fall, particularly if large silhouette, good transpiring bushes and trees are present.

A typical ET bed system (Fig. 12.3) consists of a $1\frac{1}{2}$ to 3 ft (0.45 to 0.91 m) depth of selected sand over an impermeable plastic liner. A perforated plastic piping system with rock cover is often used to distribute pretreated effluent in the bed. The bed may be square-shaped on relatively flat land, or a series of trenches on slopes. The surface area of the bed must be

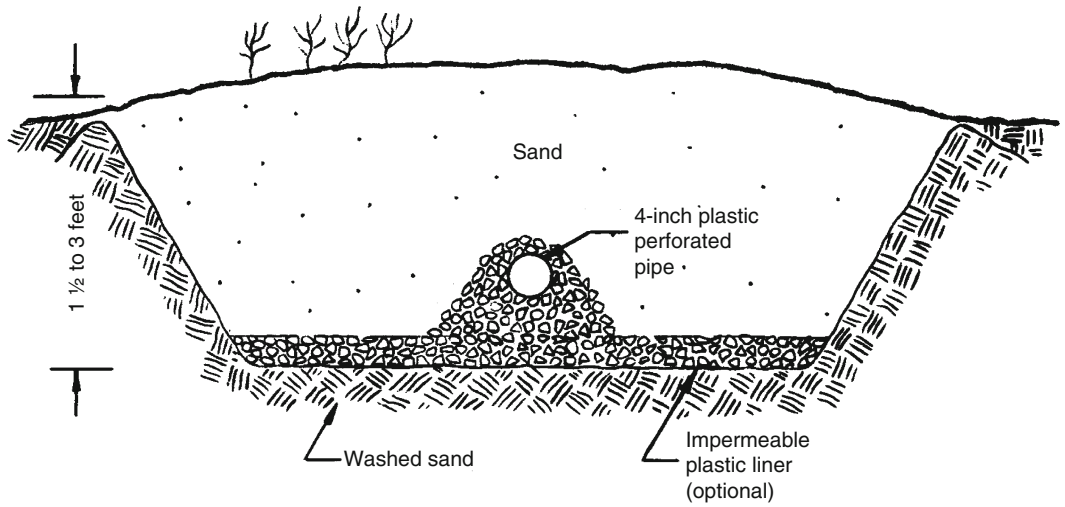

Fig. 12.3. Section through an evapotranspiration bed. (*source:* US EPA). (Conversion factor: 1 inch = 2.54 cm)

large enough for sufficient ET to occur to prevent the water level in the bed from rising to the surface.

Beds are usually preceded by septic tanks or aerobic units to provide the necessary pretreatment. Given the proper subsurface conditions, systems can be constructed to perform as both evapotranspiration and absorption beds. Nearly three-fourth of all the ET beds in operation was designed to use both disposal methods. Mechanical evaporators have been developed, but are not used at full scale.

3.2. Applications

There are approximately 4,000–5,000-year-round evapotranspiration beds estimated to be in operation in the United States, particularly in the semiarid regions of the Southwest.

ET beds are used as an alternative to subsurface disposal in areas where these methods are either undesirable due to groundwater pollution potential or not feasible due to certain geological or physical constraints of land. The ET system can also be designed to supplement soil absorption for sites with slowly permeable soils. The use of ET systems for summer homes extends the range of application, which is otherwise limited by annual ET rates. Since summer evaporation rates are generally higher and plants with high transpiration rates are in an active growing state, many areas of the country can utilize ET beds for this seasonal application.

3.3. Limitations

The use of an evapotranspiration system is limited by climate and its effect on the local ET rate. In practice, lined ET bed systems are generally limited to areas of the country where pan evaporation exceeds annual rainfall by at least 24 in. The decrease of ET in winter at middle end high latitudes greatly limits its use. Snow cover reflects solar radiation, which reduces ET. In addition, when temperatures are below freezing more heat is required to change frozen water to vapor. When vegetation is dormant, both transpiration and evaporation are reduced. An ET system requires a large amount of land in most regions. Salt accumulation may eventually eliminate vegetation and thus, transpiration. Bed liner (where needed) must be kept water-tight to prevent the possibility of groundwater contamination. Therefore, proper construction methods should be employed to keep the liner from being punctured during installation.

3.4. Design Criteria

Design of an evapotranspiration bed is based on the local annual weather cycle. The total expected inflow based on household wastewater generation and rainfall rates is compared with an average design evaporation value established from the annual pattern. It is recommended to use a 10-year-frequency rainfall rate to provide sufficient bed surface area (2). A mass balance is used to establish the storage requirements of the bed. Vegetative cover can substantially increase the ET rate during the summer growing season; but may reduce evaporation during the nongrowing season. Uniform sand in the size range of D_{50} of approximately 0.10 mm is capable of raising water approximately 3 ft to the top of the bed. The polyethylene liner thickness is typically greater than or equal to 10 milllion. Special attention should be paid to

storm water drainage to make sure that surface runoff is drained away from the bed proximity by proper lot grading.

3.5. Performance

Performance is a function of climate conditions, volume of wastewater, and physical design of the system. Evapotranspiration is an effective and reliable means of domestic wastewater disposal. An ET system that has been properly designed and constructed is an efficient method for the disposal of pretreated wastewater and requires a minimum of maintenance. Healthy vegetative covers are aesthetically pleasing and the large land requirement, although it limits the land use, it does conserves the open space. Neither energy is required, nor is head loss of any value incurred.

3.6. Costs

The following site-specific costs serve to illustrate the major components of an evapotranspiration bed in Boulder, Colorado with an annual net ET rate in the range of $0.04\,\text{gpd/ft}^2$ (0.0032 m^3/min/ha) (2). A 200-gpd (757 Lpd) household discharge would require a 2-ft (0.6 m) deep bed with an area of approximately $5,000\,\text{ft}^2$ (464.5 m^2). Costs have been adjusted to current value (2009) of US Dollars using the Cost Index for Utilities shown in Appendix A (24).

Construction cost	
Building sewer with 1,000-gal (3,785-L) septic tank, design and permit	$1,700
Excavation and hauling ($375\,\text{yd}^3$) (286.71 m^3)	$2,500
Liner ($5,200\,\text{ft}^2$) (483.08 m^2)	$1,600
Distribution piping (625 ft) (190.5 m)	$700
Sand ($340\,\text{yd}^3$) (260 m^3) and gravel ($38\,\text{yd}^3$) (29.05 m^3)	$4,300
Supervision and labor	$1,200
Total	$12,000
Annual operation and maintenance cost:	
Pumping septage from septic tank (every 3–5 years)	$12–48
Total	$12–48

The construction cost for this particular system would be approximately $\$2.40/\text{ft}^2$, ($\$25.83/\text{m}^2$) which is consistent with a reported national range of $\$1.80–3.86/\text{ft}^2$ ($\$19.37–41.55/\text{m}^2$). The cost of an evapotranspiration bed is highly dependent on local material and labor costs. As shown, the cost of sand is a significant portion of the cost of the bed. The restrictive sand size requirement makes availability and cost sensitive to location.

If an aerobic pretreatment unit is used instead of the septic tank, add $700–7,000 to the construction cost and an amount of $144–495/year to the annual operation and maintenance cost.

4. LAND TREATMENT: RAPID RATE SYSTEM

The land-based technologies have been in use since the beginning of civilization. Their greater value may be the use of the wastewater for beneficial return (agricultural and recharge)

in water-poor areas, as well as nitrogen control benefits. If nitrogen control benefits are desired, some key issues arise concerning the type of plant crop with its growing and harvesting needs and/or the cycling of the water application and restorative oxygenation resting periods. Native soils and climate add the remaining variables.

Generally, the wastewater applications are cyclic in land-based technologies, making some form of storage or land rotation mandatory to ensure the restorative oxygenation derived from the resting period. Surface wastewater applications allow additional beneficial soil aeration (plowing, tilling, and raking), which can become mandatory for the heavily loaded systems after an elapsed season, or number of loading cycles. Actual surface cleaning programs, to remove the plastic, rubber, and other debris found in pretreated municipal wastewaters, also may be necessary, although not at the frequency used for beneficial soil aeration.

In this and the following sections detailed information on the four most common land-based technologies will be provided. Subsurface, slow, and rapid infiltration systems do not discharge to surface waters and conceptually may allow a more relaxed nitrogen control standard in comparison to the overland flow system, depending on local ground-water regulations.

4.1. Description

Rapid rate infiltration was developed approximately 100-years ago and has remained unaltered since then. It has been widely used for municipal and certain industrial wastewaters throughout the world. Wastewater is applied (see Fig. 12.4) to deep and permeable deposits such as sand or sandy loam usually by distributing in basins or infrequently by sprinkling, and is treated as it travels through the soil matrix by filtration, adsorption, ion exchange precipitation, and microbial action (25). Most metals are retained on the soil; many toxic organics are degraded or adsorbed. An underdrainage system consisting of a network of drainage pipe buried below the surface serves to recover the effluent, to control groundwater mounding, or to minimize trespass of wastewater onto adjoining property by horizontal subsurface flow. To recover renovated water for reuse or discharge underdrains are usually intercepted at one end of the field by a ditch. If groundwater is shallow, underdrains are placed at or in the groundwater to remove the appropriate volume of water (2). Thus, the designed soil depth, soil detention time and underground travel distance to achieve the desired water quality can be controlled. Effluent can also be recovered by pumped wells.

Basins or beds are constructed by removing the fine-textured top soil from which shallow banks are constructed. The underlying sandy soil serves as the filtration media. Underdrainage is provided by using plastic, concrete (sulfate resistant if necessary), or clay tile lines. The distribution system applies wastewater at a rate which constantly floods the basin throughout the application period of several hours to a couple of weeks. The waste floods the bed and then drains uniformly away, driving air downward through the soil and drawing fresh air from above. A cycle of flooding and drying maintains the infiltration capacity of the soil material. Infiltration diminishes slowly with time due to clogging. Full infiltration is readily restored by occasional tillage of the surface layer and, when appropriate, removal of several inches from the surface of the basin. Preapplication treatment to remove solids improves distribution system reliability, reduces nuisance conditions, and may reduce clogging rates. Common preapplication treatment practices include the following:

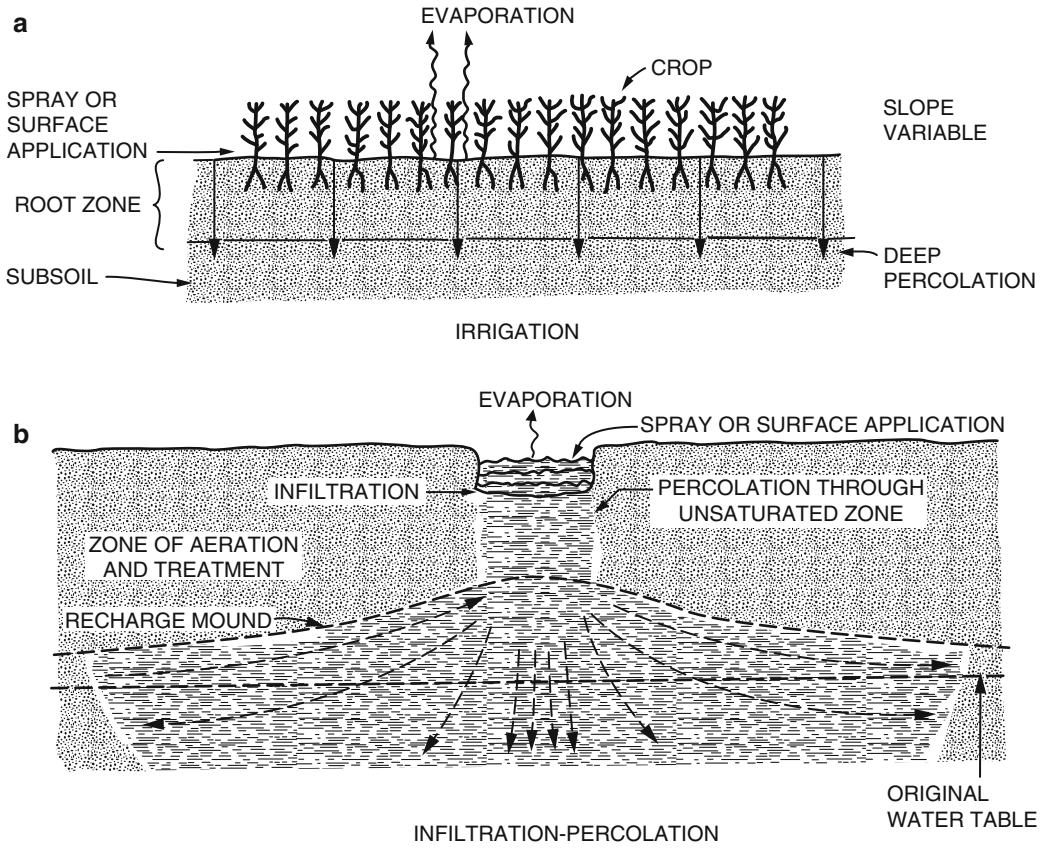

Fig. 12.4. Flow diagram of land treatment using rapid rate system. (a) Irrigation, (b) infilteration–percolation. (*source:* US EPA).

(a) Primary treatment for isolated locations with restricted public access (26)
(b) Biological treatment for urban locations with controlled public access
(c) Storage is sometimes provided for flow equalization and for nonoperating periods.

Nitrogen removals are improved by (17, 27):

(a) Establishing specific operating procedures to maximize denitrification
(b) Adjusting application cycles
(c) Supplying an additional carbon source
(d) Using vegetated basins (at low rates)
(e) Recycling portions of wastewater containing high nitrate concentrations, and
(f) Reducing application rates.

Rapid rate infiltration systems require relatively permeable, sandy-to-loamy soils. Vegetation is typically not used for nitrogen control purposes but may have value for stabilization and maintenance of percolation rates. The application of algae-laden wastewater to rapid

infiltration systems is not recommended because of clogging considerations but could be considered with attendant additional tolerance for surface maintenance, drying, and soil aeration needs.

4.2. Applications

Rapid infiltration is a simple wastewater treatment system, that is (2):

(a) Less land intensive than other land application systems and provides a means of controlling groundwater levels and lateral subsurface flow
(b) It provides a means of recovering renovated water for reuse or for discharge to a particular surface water body
(c) It is suitable for small plants where operator expertise is limited
(d) It is applicable for primary and secondary effluent and for many types of industrial wastes, including those from breweries, distilleries, paper mills, and wool scouring plants (26, 28, 29).

In very cold weather the ice layer floats atop the effluent and also protects the soil surface from freezing. Generated residuals may require occasional removals of top layer of soil. The collected material is disposed of onsite.

4.3. Limitations

The rapid infiltration process is limited by (2):

(a) Soil type
(b) Soil depth
(c) The hydraulic capacity of the soil
(d) The underlying geology, and
(e) The slope of the land.

Nitrate and nitrite removals are low unless special management practices are used.

4.4. Design Criteria

The design criteria for rapid rate system can be summarized as follows (2):

(a) Field area: 3–56 acres/MG/d (3.2–59.9 $m^2/m^3/d$)
(b) Application rate: 20–400 ft/year, 4–92 in./wk (6.1–121.9 m/year; 10.2–233.7 cm/wk)
(c) BOD_5 loading rate: 20–100 lb/acre/d (22.4–112.1 kg/ha/d)
(d) Soil depth: 10–15 ft (3–4.6 m) or more
(e) Soil permeability: 0.6 in/h (1.5 cm/h) or more
(f) Hydraulic loading cycle: 9 h to 2 weeks application period, 15 h to 2 weeks resting period
(g) Soil texture sands, sandy barns
(h) Basin size: 1–10 acres (0.4046–4.046 ha); at least 2 basins/site
(i) Height of dikes: 4 ft (1.22 m); underdrains 6 ft (1.83 m) or more deep
(j) Application techniques: flooding or sprinkling
(k) Preapplication treatment: primary or secondary.

Designs can be developed that foster only nitrification or nitrification and denitrification (17, 27). Nitrification is promoted by low hydraulic loadings and short application periods (1–2 days) followed by long drying periods (10–16 days). Denitrification can vary from 0 to

Table 12.4
Loading cycles for high rate infiltration systems

Loading cycle objective	Applied wastewater	Season	Application period (d[a])	Drying period (d)
Maximize infiltration rates	Primary	Summer	1–2	5–7
		Winter	1–2	7–12
	Secondary	Summer	1–3	4–5
		Winter	1–3	5–10
Maximize nitrogen removal	Primary	Summer	1–2	10–14
		Winter	1–2	12–16
	Secondary	Summer	7–9	10–15
		Winter	9–12	12–16
Maximize nitrification	Primary	Summer	1–2	5–7
		Winter	1–2	7–12
	Secondary	Summer	1–3	4–5
		Winter	1–3	5–10

Source: US EPA (25).
[a]Regardless of season or cycle objective, application periods for primary effluent should be limited to 1–2 days to prevent excessive soil clogging.

80%. For significant denitrification, the application period must be long enough to ensure depletion of the soil (and nitrate nitrogen) oxygen. Higher denitrification values predictably track higher BOD: nitrogen ratios. Enhancement may be promoted by recycling or by adding an external driving substrate (methanol). Nitrogen elimination strategies also may reduce the drying period by about half to yield lower overall nitrogen residuals with higher ammonium-nitrogen concentrations. Suggested loading cycles (25) to maximize infiltration rates, nitrogen removal, and nitrification rates are given in Table 12.4.

4.5. Performance

The effluent quality is generally excellent where sufficient soil depth exists and is not normally dependent on the quality of wastewater applied within limits. Well designed systems provide for high quality effluent that may meet or exceed primary drinking water standards. Percent removals for typical pollution parameters are (2):

(a) BOD_5, 95–99%
(b) TSS, 95–99%
(c) Total N, 25–90%
(d) Total P, 0–90% until flooding exceeds adsorptive capacity (30)
(e) Fecal Coliform, 99.9–99.99 + % (31).

The process is extremely reliable, as long as sufficient resting periods are provided. However, it has a potential for contamination of groundwater by nitrates. Heavy metals could be eliminated by pretreatment techniques as necessary. Monitoring for metals and toxic organics is needed where they are not removed by pretreatment. The process requires long-term

commitment of relatively large land areas, although small by comparison to other land treatment systems (32, 33).

4.6. Costs

The construction and operation and maintenance (O & M) costs are shown in Figs. 12.5 and 12.6, respectively (2). The costs are based on 1973 (Utilities Index = 149.36, EPA Index 194.2, ENR Index = 1, 850) figures. To obtain the values in terms of the present (2009) value of the US Dollars, using the Cost Index for Utilities (Appendix A), multiply the costs by a factor of 3.82 (24).

Assumptions applied in preparing the costs given in Figs. 12.5 and 12.6:

(a) Application rate, 182 ft/year. (55.5 m/year)
(b) Construction costs include field preparations (removal of brush and trees) for multiple unit infiltration basins with 4 ft (1.2 m) dike formed from native excavated material, and storage is not assumed necessary.
(c) Drain pipes buried 6–8 ft (1.8–2.4 m) with 400 ft (121.9 m) spacing, interception ditch along length of field, and weir for control of discharge; gravel service roads and 4-ft (1.2 m) stock fence around perimeter.
(d) O & M cost includes inspection and unclogging of drain pipes at outlets; annual tilling of infiltration surface and major repair of dikes after 10 years; high pressure jet cleaning of drain

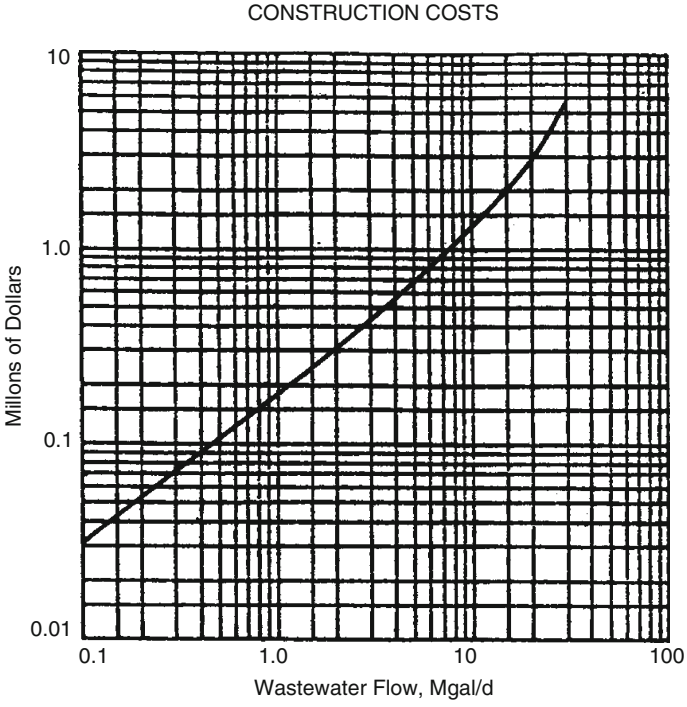

Fig. 12.5. Construction costs for rapid rate system. (*source:* US EPA). 1 Mgal/d = 1 MGD = 3.785 MLD = 43.8 L/s

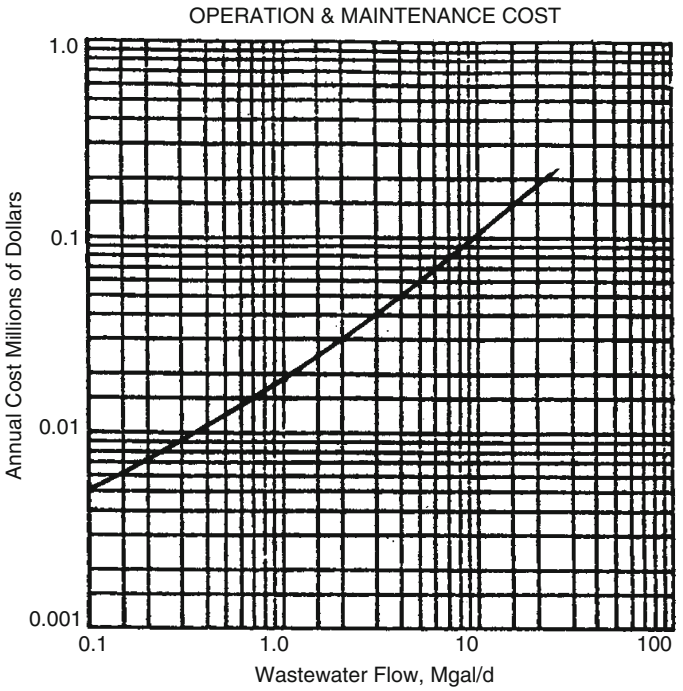

Fig. 12.6. Operation and maintenance costs of rapid rate system. (*source:* US EPA). 1 Mgal/d = 1 MGD = 3.785 MLD = 43.8 L/s

pipes every 5 years, annual cleaning of interceptor ditch, and major repair of ditches, fences and roads after 10 years.

(e) Costs of pretreatment-monitoring wells, land, and transmission to and from pretreatment facility not included.

5. LAND TREATMENT: SLOW RATE SYSTEM

5.1. Description

Slow rate land treatment represents the predominant municipal land treatment practice in the United States. In this process, wastewater is applied to vegetated soils that are slow to moderate in permeability (clay barns to sandy barns) and is treated as it travels through the soil matrix by filtration, adsorption, ion exchange, precipitation, microbial action, and by plant uptake. Wastewater can be applied in various ways including (a) sprinklers, (b) flooding, and (c) ridge and furrow methods as illustrated in Fig. 12.7. An underdrainage system consisting of a network of drainage pipes buried below the surface may be used to recover the effluent, to control groundwater, or to minimize trespass of leachate onto adjoining property by horizontal subsurface flow. To recover renovated water for reuse or discharge, underdrains are usually intercepted at one end of the field by a ditch. Underdrainage for groundwater control is installed as needed to prevent waterlogging of the application site or to recover the renovated water for reuse. Proper crop management also depends on the drainage conditions. Sprinklers

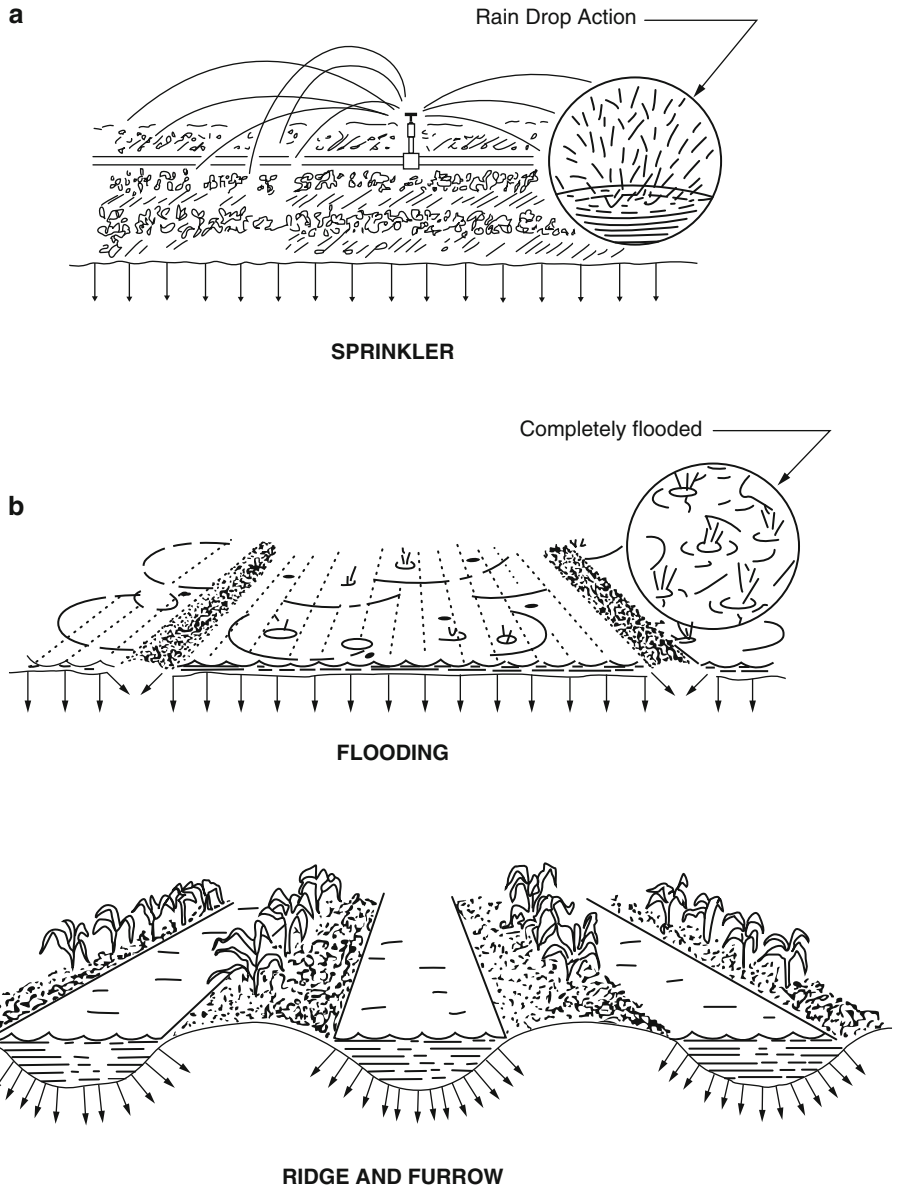

Fig. 12.7. Flow diagram of land treatment using slow rate system. (**a**) Sprinkler distribution, (**b**) flooding, and (**c**) ridge and furrow. (*source:* US EPA).

can be categorized as hand moved, mechanically moved, and permanent set, the selection of which includes the following considerations (2):

(a) Field conditions (shape, slope, vegetation, and soil type)
(b) Climate

(c) Operating conditions, and
(d) Economics.

Vegetation is a vital part of the process and serves to extract nutrients, reduce erosion, and maintain soil permeability. Considerations for crop selection include:

(a) Suitability to local climate and soil conditions
(b) Consumptive water use and water tolerance
(c) Nutrient uptake and sensitivity to wastewater constituents
(d) Economic value and marketability
(e) Length of growing season
(f) Ease of management, and
(g) Public health regulations.

Common preapplication treatment practices include the following:

(a) Primary treatment for isolated locations with restricted public access and when limited to crops not for direct human consumption
(b) Biological treatment plus control of coliform to 1,000 MPN/100 mL for agricultural irrigation, except for human food crops to be eaten raw
(c) Secondary treatment plus disinfection to 200 MPN/100 mL fecal coliform for public access areas (parks).

Wastewaters high in metal content should be pretreated to avoid plant and soil contamination. Table 12.5 shows the wastewater constituents that have potential adverse effects on crops (25). Forestland irrigation is more suited to cold weather operation, since soil temperatures are generally higher, but nutrient removal capabilities are less than for most field crops.

5.2. Applications

Slow rate systems produce the best results of all the land treatment systems. Advantages of sprinkler application over gravity methods include (34):

(a) More uniform distribution of water and greater flexibility in range of application rates
(b) Applicability to most crops
(c) Less susceptibility to topographic constraints, and
(d) Reduced operator skill and experience requirements.

Underdrainage provides a means of recovering renovated water for reuse or for discharge to a particular surface water body when dictated by senior water rights and a means of controlling groundwater. The system also provides the following benefits:

(a) An economic return from the use of water and nutrients to produce marketable crops for forage, and
(b) Water and nutrient conservation when utilized for irrigating landscaped areas.

5.3. Limitations

The slow rate process is limited by (2):

(a) Soil type and depth
(b) Topography

Table 12.5
Potential adverse effects of wastewater constituents on crops

Problem and related constituent	Constituent level			Crops affected
	No problem	Increasing problems	Severe problems	
Salinity (EC_w) (mmho/cm)	<0.75	0.75–3.0	>3.0	Crops in arid climates only
Specific ion toxicity from root absorption				
Boron (mg/L)	<0.5	0.5–2	2.0–10.0	Fruit and citrus trees – 0.5–1.0 mg/L; field crops – 1.0–2.0 mg/L; grasses – 2.0–10.0 mg/L
Sodium (adj–SAR[a])	<3	3.0–9.0	>9.0	Tree crops
Chloride (mg/L)	<142	142–355	>355	Tree crops
Specific ion toxicity from foliar absorption				
Sodium (mg/L)	<69	>69	–	Field and vegetable crops under sprinkler
Chloride (mg/L)	<106	>106	–	application
Miscellaneous				
NH_4-N + NO_3-N (mg/L)	<5	5–30	30	Sugarbeets, potatoes, cotton, grains
HCO_3 (mg/L)	<90	90–520	>520	Fruit
pH (units)	6.5–8.4	4.2–5.5	<4.2 and >8.5	Most crops

Source: US EPA (25).
[a] Adjusted sodium adsorption ratio.

(c) Underlying geology
(d) Climate
(e) Surface and groundwater hydrology and quality
(f) Crop selection, and
(g) Land availability.

Crop water tolerances, nutrient requirements, and the nitrogen removal capacity of the soil–vegetation complex limit hydraulic loading rate (35). Climate affects growing season and will dictate the period of application and the storage requirements. Application ceases during period of frozen soil conditions. Once in operation, infiltration rates can be reduced by sealing of the soil. Limitations to sprinkling include adverse wind conditions and clogging of nozzles.

Slopes should be less than 15% to minimize runoff and erosion. Pretreatment for removal of solids and oil and grease serves to maintain reliability of sprinklers and to reduce clogging. Many states have regulations regarding preapplication disinfection, minimum buffer areas, and control of public access for sprinkler systems.

The process requires long-term commitment of large land area; i.e., largest land requirement of all land treatment processes (36). Concerns with aerosol carriage of pathogens, potential vector problems, and crop contamination have been identified, but are generally controllable by proper design and management.

5.4. Design Criteria

The design criteria for slow rate system can be summarized as follows (2):

(a) Field area: 56–560 acres/MG/d (59.9–598.8 $m^2/m^3/d$)
(b) Application rate: 2–20 ft/year, 0.5–4 in./wk (0.61–6.1 m/year, 1.27–10.16 cm/wk)
(c) BOD_5 loading rate: 0.2–5 lb/acre/d (0.2–5.6 kg/ha/d)
(d) Soil depth: 2–5 ft (0.6–1.5 m) or more
(e) Soil permeability: 0.06–2.0 in./h (0.15–5.08 cm/h)
(f) Minimum preapplication treatment: primary
(g) Lower temperature limit: 25°F (−3.9 °C)
(h) Particle size of solids: less than one-third of the sprinkler nozzle diameter
(i) Underdrains: 4–8-inch (10.1–20.3 cm) diameter, 4–10-ft (1.2–3.0 m) deep, 50–500-ft (15.2–152.4 m) apart; pipe material: plastic, concrete (sulfate-resistant, if necessary), or clay.

5.5. Performance

Effluent quality is generally excellent and consistent regardless of the quality of wastewater applied (37). Percent removals for typical pollution parameters when wastewater is applied through more than 5 ft (1.5 m) of unsaturated soil are:

(a) BOD_5: 90–99 + %
(b) TSS: 90–99 + %
(c) Total N: 50–95% depending on N uptake of vegetation
(d) Total P: 80–99%, until adsorptive capacity is exceeded (38)
(e) Fecal Coliform: 99.99 + % when applied levels are more than 10 MPN/100 mL.

This treatment is capable of achieving the highest degree of nitrogen removal. Typically, nitrogen losses due to denitrification (15–25%), ammonia volatilization (0–10%) and soil immobilization (0–25%) supplement the primary nitrogen removal mechanism by the crop (17). The balance of the nitrogen passes to the percolate. Typical design standards require preservation of controlling depths to ground water and establishing nitrogen limits in either the percolate or ground water as it leaves the property site. Nitrogen loading to the ground water is often the controlling consideration in the design. For further detailed information on slow rate infiltration systems the reader is referred to refs. (39–44).

5.6. Costs

The construction and O & M costs are shown in Figs. 12.8 and 12.9, respectively (2). The costs are based on 1973 (Utilities Index = 149.36, EPA Index 194.2, ENR Index = 1, 850)

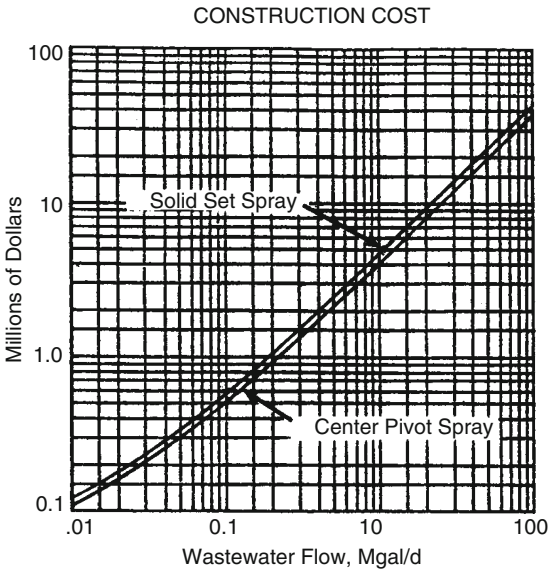

Fig. 12.8. Construction cost of slow rate system. (*source:* US EPA). 1 Mgal/d = 1 MGD = 3.785 MLD = 43.8 L/s

figures. To obtain the values in terms of the present value (2009) of the US Dollars, using the Cost Index for Utilities (Appendix A), multiply the costs by a factor of 3.82 (24).

Assumptions applied in preparing the costs given in Figs. 12.8 and 12.9: (Here 1 in = 2.54 cm; 1 ft = 0.3048 m; 1 acre = 0.4046 ha; 1 MG = 3.785 ML; 1 psi = 6.8948 kPa; 1 gpm = 3.785 Lpm)

(a) Yearly average application rate: 0.33 in./d
(b) Energy requirements: Solid set spray distribution requires 2,100 kwh/year/ft of TDH/MG/d capacity. Center pivot spraying requires an additional 0.84×10^6 kwh/year/acre (based on 3.5 d/wk operation) for 1 MG/d or larger facilities (below 1 MG/d, additional power = 0.84–1.35×10^6 kwh/year/acre)
(c) Clearing costs are for brush with few trees using bulldozer-type equipment
(d) Solid set spraying construction costs include: lateral spacing, 100 ft; sprinkler spacing, 80 ft along laterals; 5.4 sprinklers/acre; application rate, 0.20 in./h; 16.5 gpm flow to sprinklers at 70 psi; flow to laterals controlled by hydraulically operated automatic valves; laterals buried 18 in.; mainlines buried 36 in.; all pipe 4-in diameter and smaller is PVC; all larger pipe is asbestos cement (Total dynamic head = 150 ft).
(e) Center pivot spraying construction costs include: heavy-duty center pivot rig with electric drive; multiple units for field areas over 40 acres; maximum area per unit, 132 acres; distribution pipe is buried 3 ft deep
(f) Underdrains are spaced 250 ft (76.2 m) between drain pipes. Drain pipes are buried 6–8-ft (1.8–2.4 m) deep with interception ditch along length of field and weir for control of discharge.
(g) Distribution pumping construction costs include: structure built into dike of storage reservoir; continuously cleaned water screens; pumping equipment with normal standby facilities; piping and valves within structure; controls and electrical work

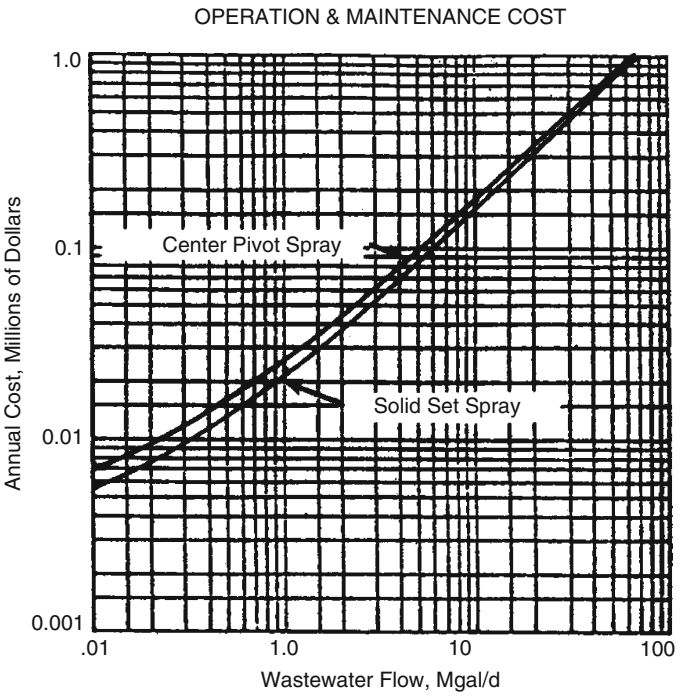

Fig. 12.9. Operation and maintenance cost of slow rate system. (*source:* US EPA). 1 Mgal/d = 1 MGD = 3.785 MLD = 43.8 L/s

(h) Labor costs include inspection and unclogging of drain pipes at outlets and dike maintenance
(i) Materials costs include for solid set spraying: replacement of sprinklers and air compressors for valve controls after 10 years; for center pivot spraying, minor repair parts and major overhaul of center pivot rigs after 10 years; high pressure jet cleaning of drain pipes every 5 years, annual cleaning of interceptor ditch, and major repair of ditches after 10 years; distribution pumping repair work performed by outside contractor and replacement parts; scraping and patching of storage receiver liner every 10 years
(j) Storage for 75 days is included; 15-ft or 4.5 m dikes (12-ft or 3.66 m wide at crest) are formed from native materials (inside slope 3:1, outside 2:1); rectangular shape on level ground; 12-ft or 3.66 m water depth; multiple cells for more than 50 acre or 20.2 ha size; asphaltic lining; 9-in. or 22.9 cm riprap on inside slope of dikes
(k) Cost of pretreatment, monitoring wells, land, and transmission to and from land treatment facility not included.

6. LAND TREATMENT: OVERLAND FLOW SYSTEM

6.1. Description

Wastewater treatment using the overland flow system is relatively new. It is now extensively used in the food-processing industry. Very few municipal plants are in operation and most are in warm, dry areas. Wastewater is applied over the upper reaches of sloped terraces and is

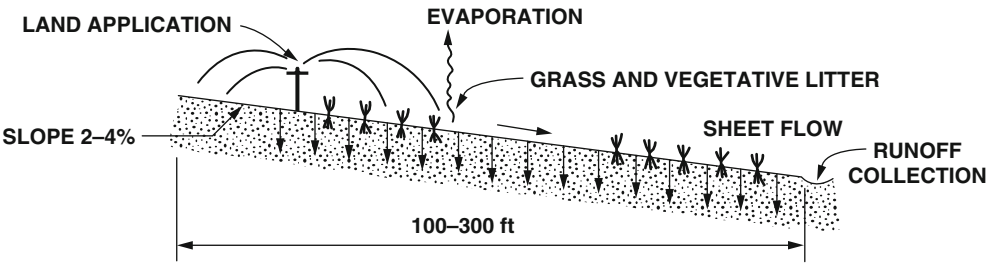

Fig. 12.10. Land treatment using overland flow system. (*source:* US EPA). 1 ft = 0.3048 m

treated as it flows across the vegetated surface to runoff collection ditches (see Fig. 12.10). The wastewater is renovated by physical, chemical, and biological means as it flows in a thin film down the relatively impermeable slope.

A secondary objective of the system is for crop production. Perennial grasses (Reed Canary, Bermuda, Red Top, tall fescue, and Italian Rye) with long growing seasons, high moisture tolerance, and extensive root formation are best suited to overland flow. Harvested grass is suitable for cattle feed. Biological oxidation, sedimentation and grass filtration are the primary removal mechanisms for organics and suspended solids. Nitrogen removal is attributed primarily to nitrification/denitrification and plant uptake. Loading rates and cycles are designed to maintain active microorganism growth on the soil surface. The operating principles are similar to a conventional trickling filter with intermittent dosing. The rate and length of application is controlled to minimize severe anaerobic conditions that result from overstressing the system. The resting period should be long enough to prevent surface ponding, yet short enough to keep the microorganisms in an active state. Surface methods of distribution include the use of gated pipe or bubbling orifice. Gated surface pipe, which is attached to aluminum hydrants, is aluminum pipe with multiple outlets. Control of flow is accomplished with slide gates or screw adjustable orifices at each outlet. Bubbling orifices are small diameter outlets from laterals used to introduce flow. Gravel may be necessary to dissipate energy and ensure uniform distribution of water from these surface methods. Slopes must be steep enough to prevent ponding of the runoff, yet mild enough to prevent erosion and provide sufficient detention time for the wastewater on the slopes. Slopes must have a uniform cross slope and be free from gullies to prevent channeling and allow uniform distribution over the surface. The network of slopes and terraces that make up an overland system may be adapted to natural rolling terrain. The use of this type of terrain will minimize land preparation costs. Storage must be provided for nonoperating periods. Runoff is collected in open ditches. When unstable soil conditions are encountered or flow velocities are erosive, gravity pipe collection systems may be required. Common preapplication practices include the following: screening or comminution for isolated sites with no public access; screening or comminution plus aeration to control odors during storage or application for urban locations with no public access (45, 46). Wastewaters high in metal content should be pretreated to avoid soil and plant contamination.

A common method of distribution is with sprinklers. Recirculation of collected effluent is sometimes provided and/or required. Secondary treatment before overland flow permits reduced (as much as 2/3 reduction) land requirements. Effluent disinfection is required where stringent fecal coliform criteria exist.

6.2. Application

Because overland flow is basically a surface phenomenon, soil clogging is not a problem. High BOD_5 and suspended solids removals have been achieved with the application of raw comminuted municipal wastewater. Thus, preapplication treatment is not a prerequisite where other limitations are not operative. Depth to groundwater is less critical than with other land systems. It also provides the following benefits: an economic return from the reuse of water and nutrients to produce marketable crops or forage; and a means of recovering renovated water for reuse or discharge. This type of applications is preferred for gently sloping terrain with impermeable soils.

6.3. Limitations

The process is limited by soil type, crop water tolerances, climate, and slope of the land. Steep slopes reduce travel time over the treatment area and thus, treatment efficiency. Flat land may require extensive earthwork to create slopes. Ideally, slope should be 2–8%. High flotation tires are required for equipment. Cost and impact of the earthwork required to obtain terraced slopes can be major constraints. Application is restricted during rainy periods and stopped during very cold weather (47). Many states have regulations regarding preapplication disinfection, minimum buffer zones and control of public access.

6.4. Design Criteria

The design criteria for overland Flow system can be summarized as follows (2):

(a) Field area required: 35–100 acres/MG/d (37.4–106.9 m^2/m^3/d)
(b) Terraced slopes: 2–8%
(c) Application rate; 11–32 ft/year, 2.5–16 in./wk (3.3–9.8 m/year, 6.4–40.6 cm/wk)
(d) BOD_5 loading rate: 5–50 lb/acre/d (5.6–56 kg/ha/d)
(e) Soil depth, sufficient to form slopes that are uniform and to maintain a vegetahve cover
(f) Soil permeability: 0.2 in/h (0.5 cm/h) or less
(g) Hydraulic loading cycle: 6–8-h application period, 16–181-week resting period
(h) Operating period: 5–6 d/wk
(i) Soil texture: clay and clay loams.

Below are representative application rates for 2–8% sloped terraces:

in./wk	Pretreatment	Terrace length (ft)
2.5–8	Untreated or primary	150
6–16	Lagoon or secondary	120

Here: 1 ft = 0.3048 m; 1 in/wk = 2.54 cm/wk

Table 12.6
Design loadings for overland flow systems

Preapplication treatment	Application rate ($m^3/h \cdot m$)	Hydraulic loading rate (cm/d)
Screening/primary	0.07–0.12[a]	2.0–7.0[b]
Aerated cell (1-day detention)	0.08–0.14	2.0–8.5
Wastewater treatment pond[c]	0.09–0.15	2.5–9.0
Secondary[d]	0.11–0.17	3.0–10.0

Source: US EPA (48).
[a] $m^3/h \cdot m \times 80.5 = gal/h \cdot ft.$
[b] $cm/d \times 0.394 = in./d.$
[c] Does not include removal of algae.
[d] Recommended only for upgrading existing secondary treatment.

Generally, 40–80% of applied wastewater reaches collection structures, lower percent in summer and higher in winter (southwest data). Table 12.6 shows the required pretreatment and allowed application and hydraulic rates (48)

6.5. Performance

Percent removals for comminuted or screened municipal wastewater over approximately 150 ft of 2–6% slope:

(a) BOD_5: 80–95%
(b) Suspended solids: 80–95%
(c) Total N: 75–90%
(d) Total P: 30–60%,
(e) Fecal coliform:90–99.9%.

The addition of alum $Al_2(SO_4)_3$, ferric chloride $FeC1_3$, or calcium carbonate $CaCO_3$ before application will increase phosphorus removals.

Little attempt has been made to design optimized overland flow systems with a specific objective of nitrogen control. Their performance depends on the same fundamental issues: nitrification–denitrification, ammonia volatilization, and harvesting of crops. When measured, overland flow systems designed for secondary treatment often reveal less than 10 mg/L total nitrogen (49). For further detailed information on overland flow systems the reader is referred to references (50–53).

6.6. Costs

The construction and O & M costs are shown in Figs. 12.11 and 12.12, respectively (2). The costs are based on 1973 (Utilities Index = 149.36, EPA Index 194.2, ENR Index = 1, 850) figures. To obtain the values in terms of the present value (2009) of the US Dollars, using the Cost Index for Utilities (Appendix A), multiply the costs by a factor of 3.82 (24).

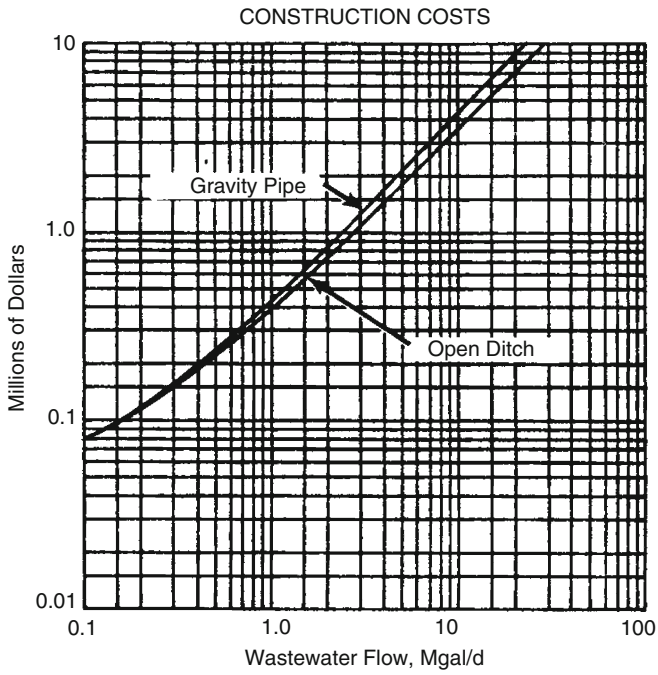

Fig. 12.11. Construction cost of overland flow treatment system. (*source:* US EPA). 1 Mgal/d = 1 MGD = 3.785 MLD = 43.8 L/s

Assumptions applied in preparing the costs given in Figs. 12.11 and 12.12: (Here 1 in = 2.54 cm; 1 ft = 0.3048 m; 1 acre = 0.4046 ha; 1 yd = 0.9144 m; 1 psi = 6.8948 kPa; 1 gpm = 3.785 Lpm)

(a) Storage for 75 days included.
(b) Site cleared of brush and trees using bulldozer-type equipment; terrace construction: 175–250-ft wide with 2.5% slope (1,400 yd/acre of cut). Costs include surveying, earthmoving, finish grading, ripping two ways, disking, land-planning, and equipment mobilization.
(c) Distribution system: application rate, 0.064 in./h; yearly average rate of 3 in./wk (8 h/d; 6 d/wk); flow to sprinklers, 13 gpm at 50 psi; laterals 70 ft from top of terrace, buried 18 in.; flow to laterals controlled by hydraulically operated automatic valves; mainlines buried 36 in.; all pipes, 4 in. diameter and smaller are made of PVC: all larger pipes are made of asbestos cement.
(d) Open Ditch Collection: network of unlined interception ditches sized for a 2 in/h storm; culverts under service roads; concrete drop structures at 1,000 ft intervals.
(e) Gravity Pipe Collection: network of gravity pipe interceptors with inlet/manholes every 250 ft along sub-mains; storm runoff is allowed to pond at inlets; each inlet/manhole serves 1,000 ft of collection ditch; manholes every 500 ft or 152.4 m along interceptor mains.
(f) O & M cost includes replacement of sprinklers and air compressors for valve controls after 10 years and either biannual cleaning of open ditches with major repair after 10 years or the periodic cleaning of inlets and normal maintenance of gravity pipe. Also includes dike maintenance and scraping and patching of storage basin liner every 10 years.

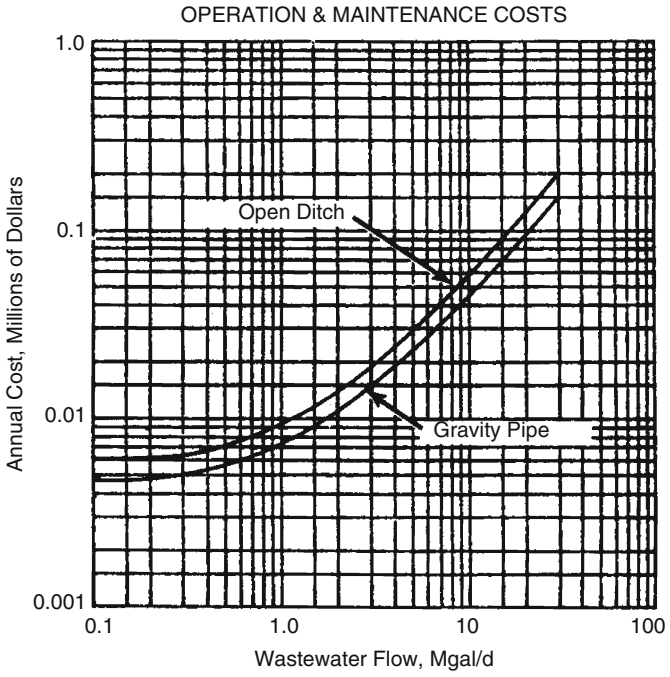

Fig. 12.12. Operation and maintenance cost of overland flow treatment system. (*source:* US EPA). 1 Mgal/d = 1 MGD = 3.785 MLD = 43.8 L/s

(g) Costs for pretreatment, land, transmission to site, disinfection, and service roads and fencing not included.

7. SUBSURFACE INFILTRATION

Subsurface infiltration systems are capable of producing a high degree of treatment; with proper design, they can provide a nitrified effluent, and denitrification can be achieved under certain circumstances. Keys to their success are the adequacy of the initial gravel infiltration zone for solids capture and the following unsaturated zone of native or foreign soils. Failure to provide an oxygenated environment by either resting or conservative loadings can lead to failure. Denitrification under gravity loading is likely to be small, but may be improved through pressure/gravity dosing concepts of liquid application to the trenches (54).

Subsurface infiltration wastewater management practices are embodied in the horizontal leach fields that routinely serve almost one-third of the United States population that use more than 20 million septic tanks in their individual nonsewered establishments and homes (2). In recent years, they have also been advanced for collective service in small isolated communities.

7.1. Description

A septic tank followed by a soil absorption field is the traditional on-site system for the treatment and disposal of domestic wastewater from individual households or establishments. The system consists of a buried tank where wastewater is collected and scum, grease, and settleable solids are removed by gravity separation, and a sub-surface drainage system where clarified effluent percolates into the soil. Precast concrete tanks with a capacity of 1,000 gallons (3785 L) are commonly used for house systems. Solids are collected and stored in the tank, forming sludge and scum 1ayers. Anaerobic digestion occurs in these layers, reducing the overall volume. Effluent is discharged from the tank to one of three basic types of subsurface systems, absorption field (54), seepage bed (54, 55), or seepage pits (56). Sizes are usually determined by percolation rates, soil characteristics, and site size and location. Distribution pipes are laid in a field of absorption trenches to leach tank effluent over a large area (Fig. 12.13). Required absorption areas are dictated by state and local codes. Trench depth is commonly about 24 in. or 60.96 cm to provide minimum gravel depth and earth cover. Clean, graded gravel or similar aggregate, varying in size from $\frac{1}{2}$ to $2\frac{1}{2}$ in. (1.27–6.35 cm), should surround the distribution pipe and extend at least 2 in. or 5.08 cm above and 6 in. or 15.24 cm below the pipe. The maintenance of at least a 2 ft (0.61 m) separation between the bottom of the trench and the high water table is required to minimize groundwater contamination. Piping typically consists of agricultural drain tile, vitrified clay sewer pipe, or perforated, nonmetallic pipe. Absorption systems having trenches wider than 3 ft are referred to as seepage beds. Given the appropriate soil conditions (sandy soils), a wide bed makes more efficient use of available land than a series of long, narrow trenches.

Many different designs may be used in laying out a subsurface disposal field. In sloping areas, serial distribution can be employed with absorption trenches by arranging the system so that each trench is utilized to its capacity before liquid flows into the succeeding trench. A dosing tank can be used to obtain proper wastewater distribution throughout the disposal area and give the absorption field a chance to rest or dry out between dosings. Providing two separate alternating beds is another method used to restore the infiltrative capacity of a system. Aerobic units may be substituted for septic tanks with no changes in soil absorption system requirements.

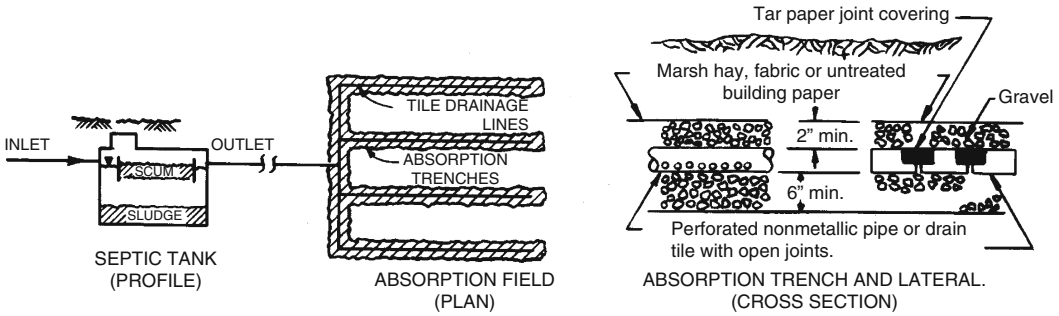

Fig. 12.13. Septic tank absorption field. (*source:* US EPA). $1'' = 1$ inch $= 2.54$ cm

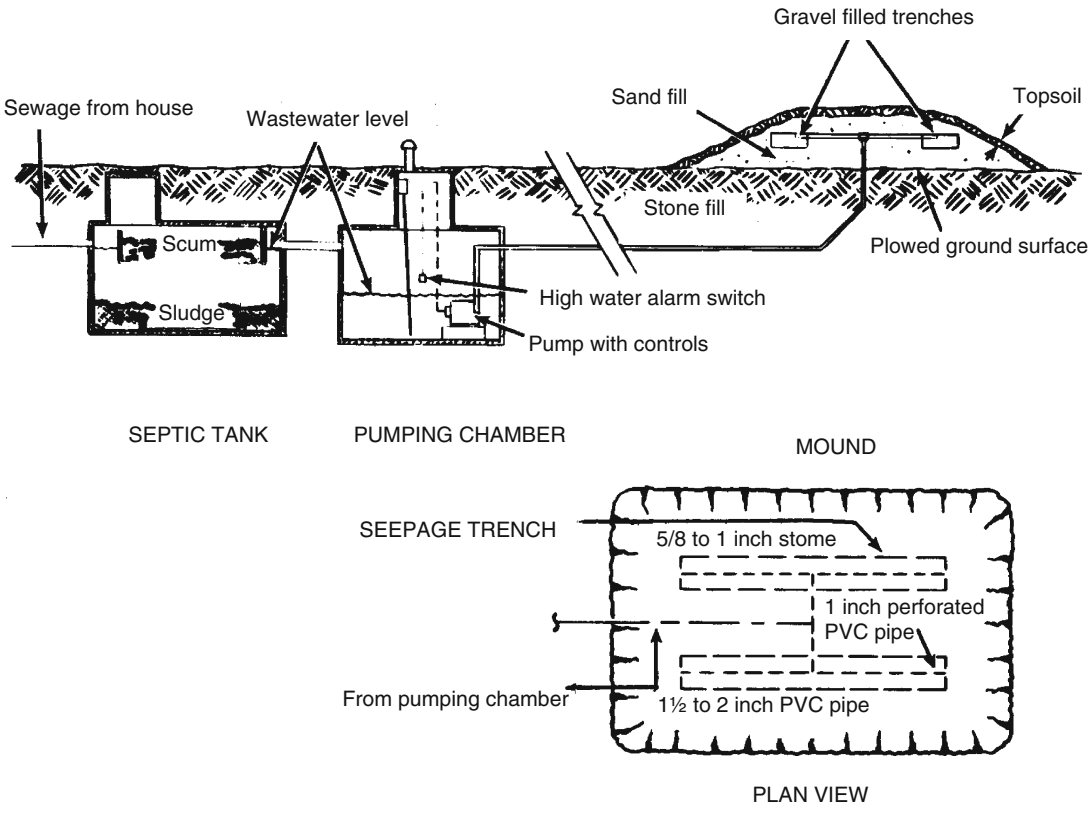

Fig. 12.14. Septic tank mound absorption field. (*source:* US EPA). 1 inch = 2.54 cm

In areas where problem soil conditions preclude the use of subsurface trenches or seepage beds, mounds can be installed (Fig. 12.14) to raise the absorption field above ground, provide treatment, and distribute the wastewater to the underlying soil over a wide area in a uniform manner (2, 57, 58). A pressure distribution network should be used for uniform application of clarified tank effluent to the mound. A subsurface chamber can be installed with a pump and high water alarm to dose the mound through a series of perforated pipes. Where sufficient head is available, a dosing siphon may be used. The mound must provide an adequate amount of unsaturated soil and spread septic tank effluent over a wide enough area so that distribution and purification can be effected before the water table is reached.

The mound system requires more space and periodic maintenance than conventional subsurface disposal system, along with higher construction costs. System cannot be installed on steep slopes, nor over highly (120 mm/in.) impermeable subsurface. Seasonal high groundwater must be deeper than 2 ft (0.61 m) to prevent surfacing at the edge of the mound (2). An alternative to the mound system is a new combined distribution and pretreatment unit to precede the wastewater application to the subsurface infiltration systems (59). The new system

is based on pumping of septic tank effluent to one or more units filled with lightweight clay aggregates. The wastewater is distributed evenly over the 2.3 m^2 surface of the pretreatment filter. The filter(s) effluent is then applied to the subsurface infiltration system.

7.2. Applications

Subsurface infiltration systems for the disposal of septic tanks effluents are used primarily in rural and suburban areas where economics are favorable. Properly designed and installed systems require a minimum of maintenance and can operate in all climates.

7.3. Limitations

The use of subsurface effluent disposal fields is dependent on the following factors and conditions (2):

(a) Soil and site conditions
(b) The ability of the soil to absorb liquid
(c) Depth to groundwater
(d) Nature of and depth to bedrock
(e) Seasonal flooding, and
(f) Distance to well or surface water.

A percolation rate of 60 mm/in. is often used as the lower limit of permeability. The limiting value for seasonal high groundwater should be 2 ft below the bottom of the absorption field. When a soil system loses its capacity to absorb septic tank effluent, there is a potential for effluent surfacing, which often results in odors and, possibly, health hazards.

7.4. Design Criteria

Absorption area requirements for individual residences are given in Table 12.7. The area required per bedroom is a function of the percolation rate, the higher the rate the smaller is the required area (2).

Design criteria for the mound system is as follows (2, 57, 58): Design flow 75 gal/person/d; 150 gal/bedroom/d. Basal area based on percolation rates up to 120 mm/in. Mound height at center is approximately 3.5–5 ft. Pump (centrifugal) must accommodate approximately 30 gpm at required TDH.

Properly designed, constructed, and operated septic tank systems have demonstrated an efficient and economical alternative to public sewer systems, particularly in rural and sparsely developed areas. System life for properly sited, designed, installed and maintained systems may equal or exceed 20 years.

7.5. Performance

Performance is a function of the following factors (2):

(a) Design of the system components
(b) Construction techniques employed
(c) Rate of hydraulic loading

Table 12.7
Required areas of subsurface infiltration absorption fields

Percolation rate (mm/in.)	Required area per bedroom (ft^2)
1 or less	70
3	100
5	125
10	165
15	190
30	250
45	300
60	330

Source: US EPA (2). 1 in. = 2.54 cm; 1 $ft^2 = 0.0929\ m^2$

(d) Geology and topography of the area
(e) Physical and chemical composition of the soil mantle, and
(f) Care given to periodic maintenance.

Pollutants are removed from the effluent by natural adsorption and biological processes in the soil zone adjacent to the field. BOD, SS, bacteria, and viruses, along with heavy metals and complex organic compounds, are adsorbed by soil under proper conditions. However, chlorides and nitrates may readily penetrate coarser, aerated soils to groundwater.

Leachate can contaminate groundwater when pollutants are not effectively removed by the soil system. In many well-aerated soils, significant densities of homes with septic tank-soil absorption systems have resulted in increasing nitrate content of the ground water. Soil clogging may result in surface ponding with potential aesthetic and public health problems. The sludge and scum layers accumulated in a septic tank must be removed every 3–5 years. For further detailed information on subsurface infiltration systems the reader is referred to references (60–65).

8. FACULTATIVE LAGOONS AND ALGAL HARVESTING

Simple regression-type ammonium and nitrogen removal models of facultative lagoons have been developed and reported with some suggestion of validation (66, 67). These identify pH to be of primary importance, based on an ammonia-stripping assumption. A pH rise occurs in the pond because carbon dioxide (CO_2) is the carbon source for the algae, which photosynthetically produce biomass and oxygen. The CO_2 source is largely from the aerobic (surface layers) and anaerobic stabilization (bottom layers and deposits) in the lagoon. With insufficient CO_2, the bicarbonate alkalinity will serve as the CO_2 source, and a significant pH rise can be experienced. Significant ammonia stripping does occur at a pH of greater than 8.5 (17).

The reported dependency of ammonia removal on pH could also be a surrogate parameter for an active algal biomass, and the actual ammonium and total nitrogen removals could reflect natural nitrification (using the photosynthetically produced oxygen), denitrification (bacterial use of the dormant algal biomass as the driving substrate during the nighttime hours), and algal synthesis during the daylight hours.

Facultative ponds should be designed to embrace and enhance the anaerobic reactions that produce CO_2 and, most important, methane (CH4), occurring in the bottom of the pond. Failure to do so will likely result in problems and, inevitably, the progressive buildup of solids and pass-through to the plant effluent. Many past problems with this biotechnology were associated with this consideration. The designer would be well-served by consulting the more fundamental publications regarding this technology (68–71).

Facultative ponds have the potential to achieve nitrogen oxidation down to the most stringent levels; their natural daytime to nighttime cycling of photosynthetic activity and aerobic to anoxic bacterial response provides a possible mechanism of nitrogen removal (72). Their liability: what to do with the algal biomass once generated. Procedures start with submerged drawoff outlet designs and consideration of chemical coagulation and/or filtration for tertiary algae removal (73). Regulatory standards may allow for a higher effluent SS. Pumped or submerged outlet removal and the sloped sidewalls of the lagoon allow for considerable flow equalization.

Facilities with an algal harvest approach (maximizing nitrogen removal by synthesis) can be designed to incorporate a number of concepts. The large lagoons at Sunnyvale and Stockton, California, return the subsequently removed algae to lagoons with adequate depth to ensure anaerobic activity. The systems have operated since the late 1970s with no residual removal. Alternatively, the pond design could be as shallow as is reasonable and well mixed, with the objective of maximizing light penetration. Algae-removal concepts abound (73–75) but are often unused on a sustained basis because of the uncertainty (and now a liability) concerning use or disposal of the harvested algae.

9. VEGETATIVE FILTER SYSTEMS

The intent of this section is to present design and maintenance criteria for runoff field application systems (commonly called vegetative filter systems). These relatively inexpensive systems can be effectively utilized to prevent feedlot runoff generated by small livestock management facilities from polluting streams, rivers, and other waters. Small livestock management operations typically do not have the economic resource, necessary to control their feedlot runoff with expensive lagoon-type zero-discharge systems. The vast majority of livestock management operations are relatively small and therefore, this system helps in preventing water pollution from livestock management facilities.

Runoff field application systems need attentive maintenance to function properly. Consistent failure on the part of the operator to maintain a runoff field application system in good operational condition could result in violations of regulations under the National Pollutant Discharge Elimination System (NPDES).

Designing an acceptable runoff field application system involves the following:

1. Meeting the conditions for system utilization.
2. Evaluating the planning considerations.
3. Meeting the component design criteria.
4. Meeting the specifications for vegetation establishment.
5. Providing the operator with operation and maintenance criteria.

9.1. Conditions for System Utilization

Runoff field application systems that are to be constructed and operated at a livestock management facility need to satisfy the following conditions:

1. The livestock management facility confines a maximum of 300 animal units
2. No NPDES permit is required for the facility
3. Sufficient land area with characteristics capable of meeting the design and maintenance criteria for runoff field application systems
4. The runoff field application system is maintained in good operational condition.

9.2. Planning Considerations

The following characteristics need to be addressed in planning a runoff field application system:

1. Slopes and soil material, vegetative species, and time of year for proper establishment of vegetation. Irrigation of the field application area, visual aspects, and other special needs should also be considered.
2. Location of settling basin.
3. Adequate drainage to insure satisfactory performance.
4. Provisions for preventing or designing for continuous or daily discharge of liquid waste to the field application area (e.g., provide temporary storage tanks for milking parlor wastewaters or provide alternate field application areas).
5. Provisions to allow harvesting activities without causing vegetative damage.
6. Provisions for excluding roof water and unpolluted surface water from the settling basin.
7. The need to mechanically distribute the flow uniformly across the top of the field application area.
8. Runoff field application systems designed to be located on soils with infiltration rates outside the range of 1.0–6.0 in./h (2.54–15.24 cm/h), are considered innovative designs.

9.3. Component Design Criteria

9.3.1. Settling Basin

1. Basin volume is obtained based on 4.5 $ft^3/100\,ft^2$ (0.12735 $m^3/9.29\,m^2$) of runoff area plus an additional 10% volume safety factor.
2. Ramp slope should not be steeper than 12:1 (H:V), with 15:1 being preferred.
3. Basin depth ranges from 2 to 4 ft. (0.61–1.22 m)
4. Settling basins located where groundwater tables rise to within 2 ft (0.61 m) of the surface should be provided with foundation drainage.
5. The settling basin riser pipe should be 1.5–2 ft (0.46–0.61 m) in diameter with vertical slots 1 in by 4 in (2.54 cm by 10.16 cm) high spaced at 120° intervals around the pipe. There should be 6

slots/ft of height with the bottom row of slots even with the settling basin floor. To avoid excess clogging, offset or locate the riser pipe as far as practicable from the inlet of the settling basin and attach a 3/3-in. mesh expanded metal screen cover over the top of the riser pipe. Provide a 3/3-in. mesh expanded metal screen ahead of the riser pipe so that all runoff entering the riser pipe must first cross this screen. Refer to diagram in Appendix J.

6. The settling basin ramp, floor, end-wall, and side-walls should be designed, constructed, and maintained to withstand normal operation practices involving power machinery.

9.3.2. Effluent Transport System

1. Pressurized effluent transport systems are designed by normal engineering hydraulic considerations including but not limited to static head, friction losses, flow velocity, and pipe diameter.
2. Gravity flow effluent transport systems may be designed as pipes flowing full or as open channels. The design velocity is 2 ft/s (0.61 m/s) or greaser to prevent solids deposition. Minimum pipe capacity is based on the design flow rate (Q_f) over the field application area. The design feedlot runoff volume (VR) is calculated as shown in Appendix B. Design flow rate (Q_f) can be obtained from the graph in Appendix G.
3. Closed pipes used for effluent transport systems are to be provided with some means of cleaning by rodding or flushing.

9.3.3. Junction Box

1. A junction box needs to be provided at the intersection of the effluent transport system and distribution manifold to dissipate the energy of the anticipated hydraulic jump from the effluent transport system discharge and to proportionally split the flow to the distribution manifold(s).
2. The recommended junction box design specifications are provided in Appendix I.
3. The junction box should be provided with a removable cover to allow entry for maintenance and prevent entry of objects that would interfere with the operation of the runoff field application system.

9.3.4. Distribution Manifold

1. Pressurized distribution manifolds shall be designed by normal engineering considerations including but not limited to static head, friction losses, flow velocity and pipe diameter.
2. Gravity flow distribution manifolds should be less than 50-ft (15.24 m) long each and at least 2 ft (0.61 m) shorter than the width of the field application area.
3. The following must be considered in the distribution manifold design: construction material, length, capacity, Slope (level), solids removal and cleaning and location of junction box.
4. Recommended design of distribution manifolds is provided in Appendix H.
5. Distribution manifolds must be anchored securely while in operation.

9.3.5. Runoff Field Application Area

1. The runoff field application area is to be located on gently sloping soils of moderate permeability supporting a heavy stand of grass vegetation and designed to operate by overland flow.
2. Slopes are shaped to cause applied runoff to flow uniformly across the design width for the entire length of the field application area.
3. The uniform sheet flow should move downslope through the field application area flow length at a velocity that will provide a minimum contact time of 2 h. Appendix E, gives minimum flow lengths needed to provide a contact time of 2 h at various slopes.
4. Field application areas should have a minimum width of 20 ft (6.1 m) and a maximum width of 100 ft (30.48 m).

5. The range of soil infiltration rates specified in the planning considerations (1.0–6.0 in./h) (2.54–15.24 cm/h) insures that the infiltration capacity of the field application area will equal or exceed the volume of feedlot runoff to be infiltrated for the 1 year – 2-h design rainstorm event. The following equation is used for designing the field application area:

$$\text{FAA} = \frac{\text{VR}(12)}{(2\,\text{h})\text{SI} - 1.69} \quad \text{when: } l \leq \text{I} \leq 6.0\,\text{in/h} \leq 15.24\text{cm/h}$$

where:
FAA = field application area, ft^2
VR = volume of runoff, ft^3
SI = soil infiltration rate, in./h

6. The procedure for determining VR and test to determine SI are provided in Appendixes B and C, respectively. Here 1 $ft^2 = 0.0929\,m^2$; 1 $ft^3 = 0.0283\,m^3$; 1 in/h = 2.54 cm/h.

9.4. Specifications for Vegetation Establishment

The following specifications apply to all runoff field application systems:

1. All trees, stumps, brush, rocks, and similar materials that can interfere with installing the field application area should be removed. The materials are disposed of in a manner that is consistent with standards for maintaining and improving the quality of the environment and with proper functioning of the field application area.
2. All areas disturbed during construction have to be vegetated.
3. To aid in the establishment of vegetation, feedlot runoff should be prevented from entering the field application area through the use of temporary diversions until vegetation is established to a minimum height of 4 in. and 90% ground cover.
4. Immediately before seedbed preparation, the following minimum amounts of starter fertilizer should be applied:
   - Nitrogen (N) – 120 lb/acre (134.52 kg/ha) of actual nitrogen
   - Phosphorus (P) – 120 lb/acre (134.52 kg/ha) of P_2O_5
   - Potassium (K) – 120 lb/acre (134.52 kg/ha) of K_2O.
5. Apply limestone, if necessary, for the species to be grown.
6. Incorporate the required lime and fertilizer and prepare a firm seedbed to a depth of 3 in. The seedbed should be free from clods, stones, or other debris that might hamper proper seeding.
7. Select one of the following mixtures and seed according to the rate shown:
   - Reed canarygrass – 25 lb/acre. (28 kg/ha)
   - Mixture reed canarygrass and tall fescue – 15 lb/acre (16.8 kg/ha) of each species.
   - Use of species other than reed canarygrass or tall fescue is considered an innovative design.
8. Apply seed uniformly at a depth of 1/4 to 1/2 in. (0.64 to 1.27 cm) with a drill (band seed) or cultipacker type seeder or broadcast seed uniformly and cover to a depth of 1/4 to 1/2 in. (0.64 to 1.27 cm) with a cultipacker or harrow. If a drill or cultipacker seeder is used, seed across the slope or cut channel.
9. Seeding dates shall be either
   - Early spring to May 15.
   - May 15 to August 1, provided sufficient water is provided for germination and vigorous growth.
   - August 1 to September 10.

10. Mulch with clean straw using 2 tons of mulch per acre (4.48 metric tons of mulch per hectare). The mulch must be uniformly spread over the seeded area.
11. Anchor the mulch by one of the following methods:
    - Press it into the soil to a 2-in. (5.1 cm) depth by using a serrated straight disk or a dull farm disk set straight. Cross the slope perpendicular to the direction of the flow of water, or
    - Apply netting on top of the mulch and anchor it with staples.

9.5. Operation and Maintenance Criteria

The following operation and maintenance criteria apply as best management practices to all runoff field application systems:

1. Protect the field application area from damage by farm equipment, traffic, and livestock. Livestock must be fenced out of the runoff field application area.
2. Avoid damaging the field application area with herbicides.
3. Fertilize the field application area when necessary to establish growth.
4. Harvest when the forage is at the proper state of maturity for maximum quality feed. No harvesting should occur after September 15. Use the following guide for cutting stages and minimum cutting height for the species seeded:
    - Reed canarygrass – cut at early boot stage to heading – minimum cutting height 6 in (15.24 cm).
    - Reed canarygrass – tall fescue mixture – cut at early boot to heading – minimum cutting height 6 in.
5. Repair damage caused by erosion or equipment immediately so the runoff field application system will continue to perform properly. A shallow furrow on the contour across the field application area can be used to reestablish sheet flow.
6. To prevent excess organic solids from entering the field application area:
    - Scrape feedlot regularly: however, do not scrape waste into settling facilities, but place in separate manure stacking area away from settling basin.
    - Drainage from manure stacking facilities should be directed to settling basin or contained.
    - Remove solids from the settling basin when 2–4 in (5.1–10.2 cm) accumulate.
    - Scrape lot frequently during early spring. At least once each 7 days is recommended.
7. If organic wastes accumulate on the field application area and are damaging vegetation, redistribute wastes,
8. Remove solids that accumulate in the effluent transport system, junction box and distribution manifold regularly.
9. Solids removed from runoff field application system components shall be disposed of pursuant to local regulations
10. Periodic soil testing of the field application area is suggested to determine changes in phosphorus, potassium, and pH levels.
11. Each spring, relevel the distribution manifold and restore the design slope on other pipes.
12. When vegetation of a kind other than reed canarygrass or tall fescue infests 20% or more of the field application area, the infested area should be re vegetated.

9.6. Innovative Designs

It is strongly suggested that any operator contemplating use of runoff field application systems not designed, constructed, or maintained in accordance with the design criteria

contained in this section should receive prior approval from the Agency for such system. The Agency will approve innovative designs should the operator present clear, cogent, and convincing proof that the technique has a reasonable and substantial chance for meeting the requirements.

Examples of innovative designs are:

1. Settling basin designed at less than 4.5 $ft^3/100\,ft^2$ (0.12735 $m^3/9.29\,m^2$) of drainage area.
2. Settling channel used instead of settling basin.
3. Use of terraces for field application area.
4. Riser pipe designed differently.
5. Use of vegetation other than tall fescue or reed canarygrass.
6. Greater than 300 animal units on feedlot.
7. Distribution manifold designed for full pipe flow driven by gravity.
8. Not providing a junction box.
9. Application of materials other than feedlot runoff, rainfall, or milking parlor washwaters to the runoff field application system (for example, silage leachate, sewage, pesticides, oil, refuse).
10. Use of field application area smaller than provided in this design or with less than 2-h contact time.
11. Use of soils on runoff field application area with infiltration rates outside the range of 1.0–6.0 in/h (2.54–15.24 cm/h)
12. Use of field application area widths greater than 100 ft. (30.48 m)

9.7. Outline of Design Procedure

1. Collect site-specific data
   Types and areas (ft^2) contributing drainage
   Slope of field application area
   Soil infiltration rate (SI) of field application area
2. Calculate runoff volume and total drainage area from Appendix B
3. Settling basin design
   4.5 $ft^3/100\,ft^2$ (0.12735 $m^3/9.29\,m^2$) of drainage area +10% extra volume
   Dimensions from Appendix J
4. Field application area design

$$\mathrm{FAA} = \frac{\mathrm{VR}(12)}{(2\,\mathrm{h})\mathrm{SI} - 1.69}\ \mathrm{ft}^2.$$

   Dimensions from Appendix E
5. Calculate flow onto field application area
   Flows from Appendix G
   or
   $Q_f = 0.0026$ (FAA) (gpm)
6. Effluent transport system design from Appendix F
7. Junction box design from Appendix I
8. Distribution manifold design from Appendix H.

9.8. Procedure to Estimate Soil Infiltration Rate

Soil infiltration rate for a runoff field application area can be determined by the following modified cylinder infiltrometer method:

1. *Preparing the test site:* Drive a rigid, leak-proof container approximately 6 in (15.24 cm) into the ground taking care to avoid disturbing the soil as much as possible. This container should be approximately 2 ft (0.61 m) long by at least 10 in (25.4 cm) wide and may be of any suitable material. A metal pipe is recommended (see Appendix C).
2. *Saturation and swelling of the soil:* Before conducting the test, saturate the soil for at least 4 h, but preferably 8 h, by refilling the container with clean water as needed.
3. *Testing:* At the time of the test, adjust the water level to 12 in (30.48 cm) above the soil surface. Allow the water level to drop 6 in (15.24 cm) and then commence measuring the drop in water level at 15-min intervals until all the water has infiltrated. Repeat testing.
4. *Recording Results:* Record results of all tests as the total minutes required for the last 6 in (15.24 cm) of water to infiltrate (min/in). Average the two tests at each site.
5. *Soil infiltration rate:* The soil infiltration rate (SI) is calculated at each site:

$$\text{SI} = \frac{36}{\text{min/in.}} = \text{in./h.}$$

6. *Average soil infiltration rate:* Average the soil infiltration rates from each testing site to calculate the SI value for the runoff field application area.

These tests must not be made on frozen ground and include a safety factor in Part 5 to compensate for inherent inaccuracies in this procedure.

Obtain the Table of engineering properties – Physical and Chemical Properties for Permeability from a modern USDA–SCS soil survey for the county where the runoff field application system will be installed.

1. Locate the soil name and map symbol for the field application area on the map sheets.
2. On the Physical and Chemical Properties Table locate the surface layer permeability rate.
3. At the surface layer use the average value of the permeability range to obtain SI.

9.9. Procedure to Determine Slopes

The slope must be determined at the site of the runoff field application area to be able to use design Appendix E. Many methods are available to determine slope but all methods are based on the fact:

$$\text{Slope} = \frac{\text{rise}}{\text{run}} = \frac{\Delta y}{\Delta x}.$$

The following procedure can be used to determine slope.

1. Obtain a 40-ft (12.19 m) length of string or wire with a 25 ft (7.62 m) section marked off (if you use nylon, measure the 25 ft (7.62 m) with a steel tape because nylon stretches when pulled taut); carpenter's line level from a hardware store: a stake: a rod about 8 ft (2.43 m) long (an 8 ft. 2 in. × 4 in. or 2.44 m 50.8 mm × 101.6 mm works well); a tape measure; a notebook and an assistant.
2. Set up your notes and refer to Appendix D
3. Stake one end of the string at point 1 and attach the other end to the rod so that there is 25 ft (7.62 m) between the stake and rod, and the string can slide up and down the rod. With the string taut, level the string in the center using the line level and record the rise at point 2 in your notes by measuring the string height at the rod.

4. Repeat step 3 all the way down the field and calculate the slope by:

$$\text{Slope} = \frac{\text{A}(100)}{\text{B}} = \%.$$

5. Use the % slope for Appendix E.

10. DESIGN EXAMPLE

A livestock producer had 300 head of feeder cattle on a concrete feedlot (see Fig. 12.15) and wanted to install a runoff field application system to control feedlot runoff which entered a nearby stream.

Solution:

1. Site specific data
   (a) From Fig. 12.15 and procedure in Appendix B:
   Concrete Feedlot Area $= 20.038\,\text{ft}^2 = 0.46$ acres
   Roof Area $= 4,792\,\text{ft}^2 = 0.11$ acres
   All other drainage is to be diverted from the feedlot and field application area with gutters and curbs.

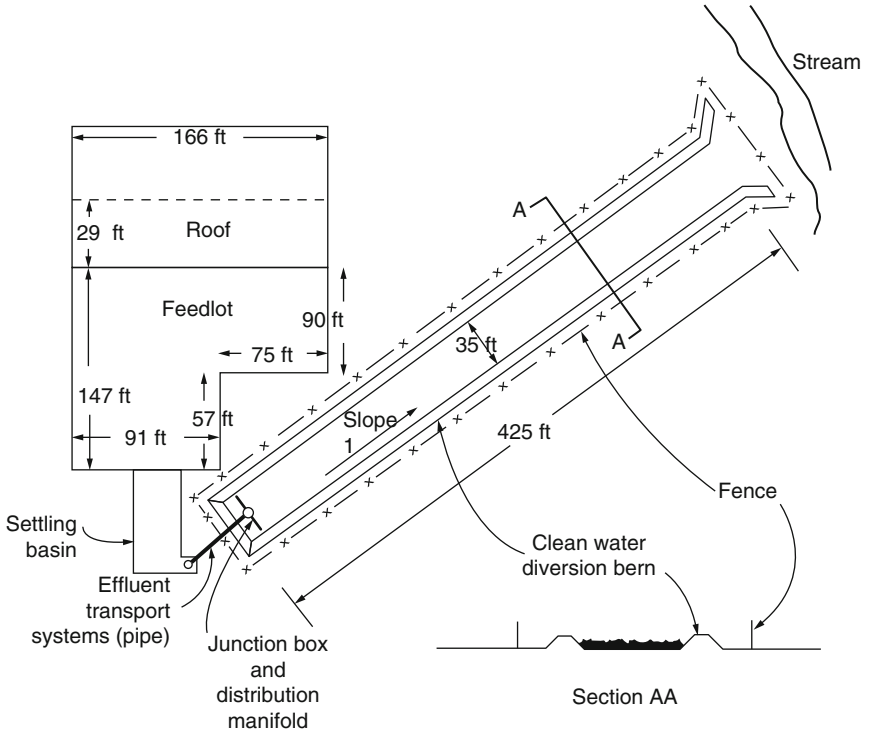

Fig. 12.15. Plan for sample design example (76). 1 ft = 0.3048 m

(b) From the collected slope data and figure in Appendix D, the s1ope of the field application area = 1.0%.
(c) From the soil survey for the county the infiltration rate (SI) of the field application area = 2.0 in./h

2. From Appendix B, Calculate runoff volume and total drainage area.
Roof 4, 785 $ft^2 \times 0.1408 = 674\ ft^3$
Feedlot $20.037\ ft^2 \times 0.0991 = 1,986\ ft^3$
Design runoff volume (VR) $= 2,660\ ft^3$
Total drainage areas $= 20.037 + 4,785 = 24,822\ ft^2$

3. Settling basin design
The total settling basin volume:
$24,822\ ft^2 \times 4.5\ ft^3/100\ ft^2 = 1,117\ ft^3$
$1,117\ ft^3 \times 0.10 = 112\ ft^3$ (Safety factor)
Total design volume $= 1,229\ ft^3$
From Appendix J, calculate the settling basin dimensions after choosing 3-ft settling basin height (h), 12 ft width (b) and 15:1 slope.
$L_1 = 3 \times 15 = 45$ ft
$V_1 = (1/2)(12 \times 3 \times 45) = 810\ ft^3$
$'V_2 = 1,229–810 = 419\ ft^3$
$L_2 = 419/(12 \times 3) = 11$ ft–8 in. Round-off L_2 to 12 ft
Foundation drainage tiles are not needed as the soil survey indicated the groundwater table did not rise above 5-ft depth.
A 24-inch diameter riser pipe is provided and concrete is chosen as the settling basin construction material.

4. Field application area design
The field application area calculation:

$$\text{FAA} = \frac{2,660 \times 12}{(2 \times 2.0) - 1.69} = \frac{31,920}{231} = 13,818\ ft^3 \text{ minimum area needed}$$

Use Table in Appendix E to determine the dimensions of the field application area
Using the next larger sized area of $14,875\ ft^2$:
Slope = 1.0%
Length = 425 ft
Width = 35 ft
FAA $= 14,875\ ft^2 = 0.34$ acres.

5. Calculate flow onto field application area
Use Appendix G to determine the flow onto the field application area: Flow is approximately 40 gpm. A more accurate calculation can be made as follows:
$Q_f = (0.0026)(\text{FAA})$
$Q_f = (0.0026)(14,875\ ft^2) = 38.7$ gpm.

6. Effluent Transport System Design
The pipe to transport the settling basin effluent to the distribution manifold can be chosen using Appendix F. The smallest pipe available to handle 38.7 gpm is a 6-in. PVC pipe:
Slope = 0.5%
PVC = nonperforated pipe
Diameter of pipe = 6 in.

7. Junction box design
   A junction box will be constructed to the specifications provided in Appendix I.
   Adjustable slots are included in the drop boxes to compensate for frost heaving of the junction box.
8. Distribution manifold design
   The distribution manifolds are designed using the ½ pipe criteria at 150 gpm as provided in Appendix H.
   Length of each manifold = ½(35 − 2 ft) = 16.5 ft.
   An 8-in. diameter PVC pipe (17-ft long) would be purchased and cut in half down the pipe length to provide two manifolds each 4-in. deep. Each manifold will have 6 in. removed to provide the required length of 16.5 ft.

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Appendix A us Army Corps of Engineers Civil Works Construction Yearly Average Cost Index for Utilities (24)

Year	Index	Year	Index
1967	100	1989	383.14
1968	104.83	1990	386.75
1969	112.17	1991	392.35
1970	119.75	1992	399.07
1971	131.73	1993	410.63
1972	141.94	1994	424.91
1973	149.36	1995	439.72
1974	170.45	1996	445.58
1975	190.49	1997	454.99
1976	202.61	1998	459.40
1977	215.84	1999	460.16
1978	235.78	2000	468.05
1979	257.20	2001	472.18
1980	277.60	2002	484.41
1981	302.25	2003	495.72
1982	320.13	2004	506.13
1983	330.82	2005	516.75
1984	341.06	2006	528.12
1985	346.12	2007	539.74
1986	347.33	2008	552.16
1987	353.35	2009	570.38
1988	369.45		

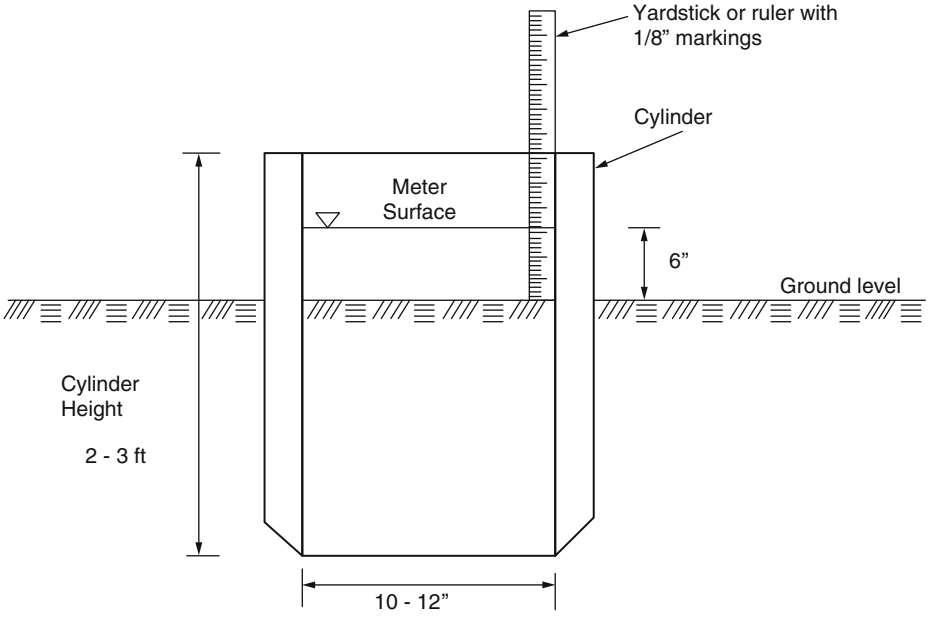
Appendix B Procedure to Estimate Volume of Feedlot Runoff (76) (Conversion factors: 1 $ft^2 = 0.0929\ m^2$; 1 $ft^3 = 0.0283\ m^3$)

	(A)	X	(B)	=	(C)
Type of Drainage Area	Area ft^2		Multiplication Factor (ft) *		Runoff Volume ft^3
Roof			0.1408		
Feedlot					
a. Paved or Concrete			0.0991		
b. Earthen			0.0748		

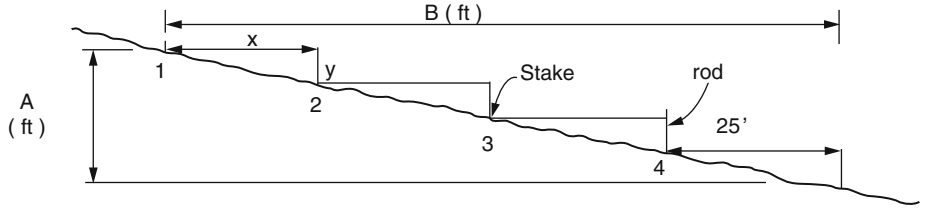
* Multiplication factor corresponds to Q of the U.S.D.A.-S.C.S. runoff equation.
Storm event (I) is 1-year, 2-hour storm of 1.69 inches.
Curve numbers (CN) are 100-roof; 95-paved; 91-earthern.
$S = (1000/CN) - 10$
$Q = (I - 0.2S)^2/(I + 0.8S)$

1. Feedlot Runoff Volume = Total of Column (C) (ft^3).
2. Milking Parlor Washwater = gallons x .936 (ft^3 per week) / day
3. Design Runoff Volume (VR) = 1 + 2 (ft^3). Use VR (ft^3) for designing field application area.
4. Total area (sum of column A in square feet) divided by 100 is used to design settling basin.
5. To convert Runoff Volume (VR) from units of ft^3 into equivalent units of gallons, multiply ft^3 by 7.481 gallons/ ft^3

Appendix C Cylinder Infiltrometer (76)
(Conversion factors: 1 ft = 0.3048 m; 1″ = 1 in = 2.54 cm)

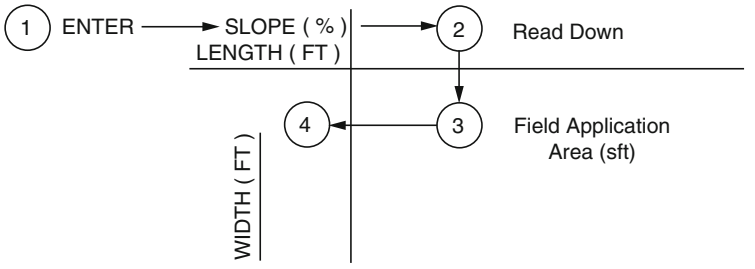


Appendix D Field Set-up for Determining Slope (76)
(Conversion factor : 1 ft = 1′ = 0.3048 m)



Appendix E Determination of Dimensions of Field Application Area (76) (Conversion factors: 1 FT = 1 ft = 0.3048 m; 1 sft = 1 ft^2 = 0.0929 m^2)

How to Use Table E-1



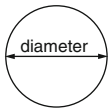
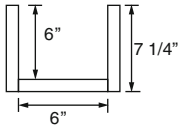
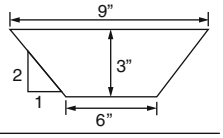
1. Enter at slope of field application area from Appendix D.
2. Read down column and find corresponding length of field application area.
3. Continue down column stopping at area closest to that previously calculated for your site.
4. Read left to find width of field application area.

Table E–1: Runoff Field Application Areas (ft^2)

Slope % / Length (ft)	0.5 / 300	0.7 / 375	1.0 / 425	1.5 / 525	2.0 / 600	3.0 / 750	4.0 / 800
20	6,000	7,500	8,500	10,500	12,000	15,000	17,000
25	7,500	9.375	10,625	13,125	15,000	18,750	21,250
30	9,000	11,250	12,750	15,750	18,000	22,500	25,500
35	10,500	13,125	14,875	18,375	21,000	26,250	29,750
40	12,000	15,000	17,000	21,000	24,000	30,000	34,000
45	13,500	16,875	19,125	23,625	27,000	33,750	38,250
50	15,000	18,750	21,250	26,350	30,000	37,500	42,500
55	16,500	20,625	32,375	28,875	33,000	41,250	46,750
60	18,000	22,500	25,500	31,500	36,000	45,000	51,000
65	19,500	24,375	27,625	24,125	39,000	48,750	55,250
70	21,000	26,250	29,750	36,750	42,000	52,500	59,500
75	22,500	28,125	31,875	39,375	45,000	56,250	63,750
80	24,000	30,000	34,000	42,000	48,000	60,000	68,000
85	25,500	31,875	36,125	44,625	51,000	63,750	72,250
90	27,000	33,750	38,250	47,250	54,000	67,500	76,500
95	28,500	35,625	40,375	49,875	57,000	71,250	80,750
100	30,000	37,500	42,500	52,500	60,000	75,000	85,000

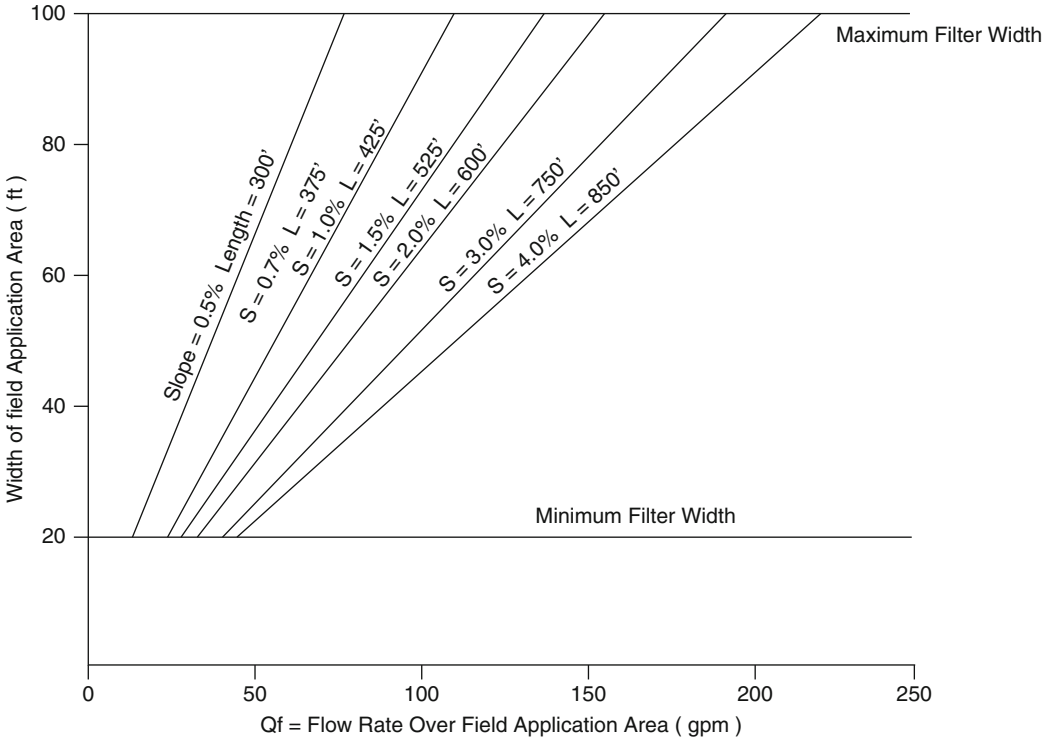
Conversion factors: 1 ft = 0.3048 m; 1 ft^2 = 0.0929 m^2

Appendix F Recommended Effluent Transport Systems Design (76) (Conversion factors: 1 gpm = 3.785 Lpm; 1″ = 1 in = 2.54 cm; 1 ft/s = 0.3048 m/s)

Type	Maximum Flow* Of (gpm)	Minimum Slope (%)	Design Velocity (ft/s)	Materials	Dimensions	Diagrams
Pipe	179	0.5	2	PVC	6” diam	
	332	0.4	2	PVC	8” diam	
Open Channel						
Rectangular	224	0.33	2	Concrete Wood, Asphalt, Aluminum	6” x 6”	
Trapezoidal	224	0.37	2	Concrete Wood, Asphalt, Aluminum	b = 6” s = 2 : 1 d = 3”	

*Mannings Equation with n = 0.013.

Appendix G Graph for Determining Flow Rate Over Field Application Area (76) (Conversion factors: 1 inch = 1″ = 2.54 cm; 1 ft = 0.3048 m; 1 gpm = 3.785 Lpm)



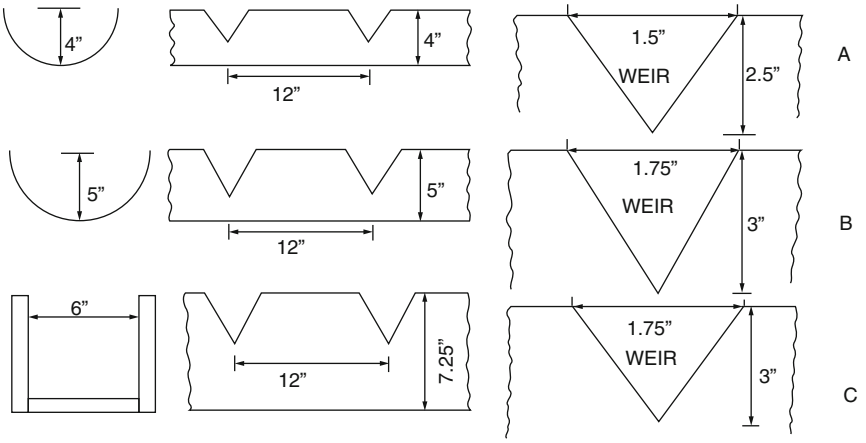
Assumptions : $Q = VA = \frac{(\text{Area x Depth of Flow})}{\text{Time}}$

Depth of Flow = 1/2 Inch = 0.042 ft

Contact Time = 2 hours = 7200 seconds

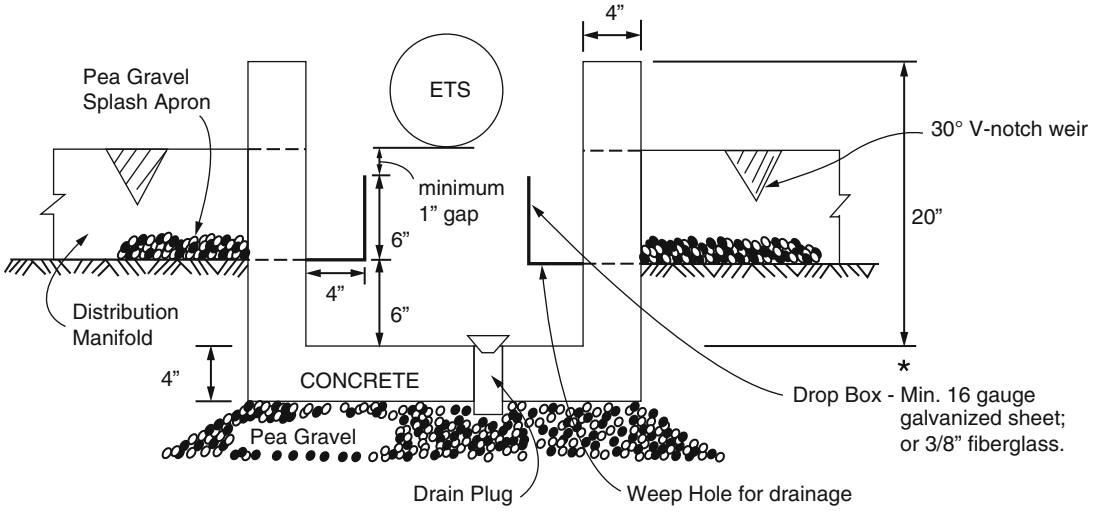
Appendix H Distribution Manifold Design (76)
(Conversion factors: 1 fps = 1 ft/s = 0.3048 m/s; 1 gpm = 3.785 Lpm; 1″ = 1 in = 2.54 cm; 1 feet = 1 ft = 0.3048 m)

Type[1]	Maximum Flow Qf (gpm)	Slope	Initial Velocity (fps)	Dimensions	Materials[2]	Weirs[3]	Diagram
1/2 Pipe	150 225	level level	1.3 1.3	8" diam 10"	PVC PVC	30° V–notch 30° V–notch	A B
Box Trough	225	level	1.0	6" x 6"	2" x 8" dimension 1umber	30° V–notch	C
Aluminum Guttering	(Size with the Box Trough Criteria Above)						

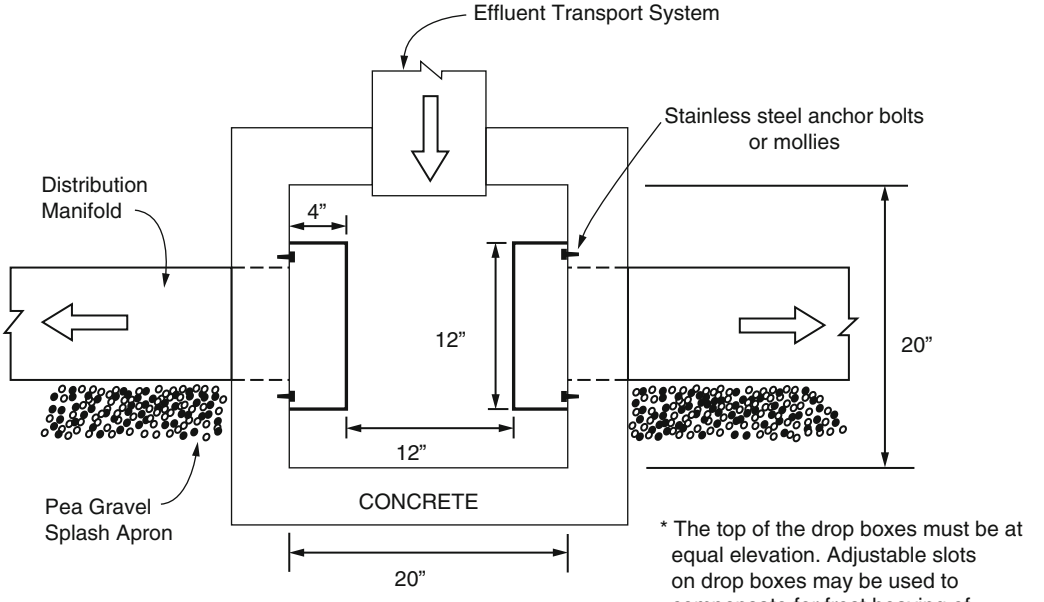


1 Anchor with 1/8" thick wire staples spaced 5 – 8 feet apart.
2 Manning's roughness coefficient n = 0.013.
3 One foot on center spacing; sharp crested weir on pipe, broad crested on box trough.

Appendix I Junction Box Design (76)
(Conversion factor: 1″ = 1 in = 2.54 cm)



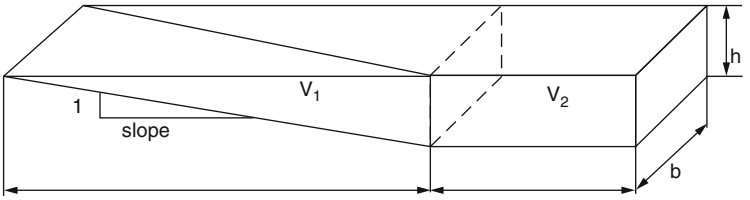
CROSS - SECTION VIEW



* The top of the drop boxes must be at equal elevation. Adjustable slots on drop boxes may be used to compensate for frost heaving of junction box.

TOP VIEW

Appendix J Diagram of Settling Basin Components (76)
(Conversion factors: 1″ = 1 inch = 2.54 cm; 1 ft = 0.3048 m; 1 ft^3 = 0.0283 m^3)



L_1 = h x slope

$L_2 = V_2/bh$

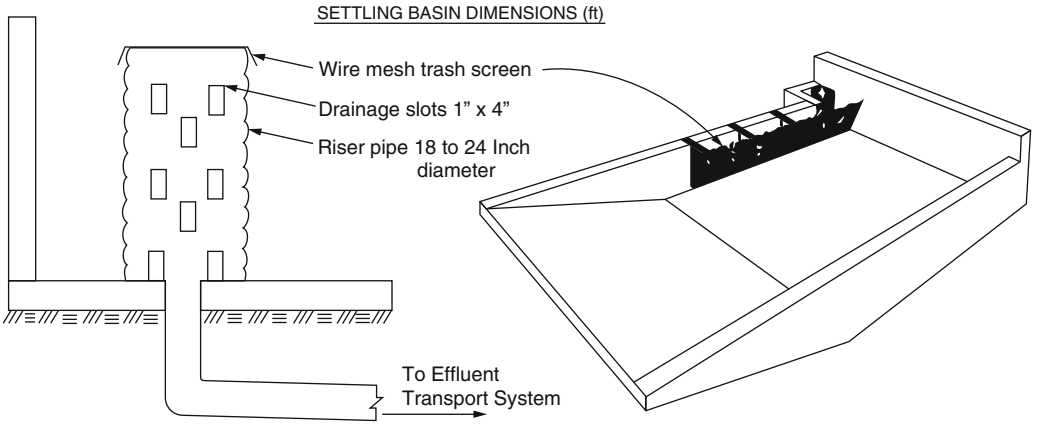
Total Volume = $V_1 + V_2$ (ft^3)

V_1 = 1/2 bhL_1

V_2 = Total Volume - V_1

Total Length = $L_1 + L_2$ (ft)

Pick: h - 2 to 4 (ft)
b - 8 to 15 (ft) recommended
slope - 12 to 15 recommended



13
Aerobic and Anoxic Suspended-Growth Biotechnologies

Nazih K. Shammas and Lawrence K. Wang

CONTENTS

Abstract Among the suspended-growth biological treatment processes covered in this chapter are conventional activated sludge, high rate activated sludge, pure oxygen activated sludge, contact stabilization, activated sludge with nitrification, separate stage nitrification, separate stage denitrification, extended aeration, oxidation ditch, Powdered Activated Carbon Treatment (PACT) process, carrier-activated sludge processes (CAPTOR and CAST systems), activated biofilter (ABF), vertical loop reactor (VLR), and phostrip process. This chapter describes the above processes and explains their practice, limitations, design criteria, performance, energy requirements, process equipment, performance, and costs.

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Key Words Suspended-growth • conventional activated sludge • high rate activated sludge • pure oxygen activated sludge • contact stabilization • nitrification • denitrification • carrier-activated sludge • ABF • VLR • CAPTOR • CAST • PACT.

1. CONVENTIONAL ACTIVATED SLUDGE

1.1. Description

Activated sludge is a continuous flow, biological treatment process characterized by a suspension of aerobic microorganisms, maintained in a relatively homogeneous state by the mixing and turbulence induced by aeration. The microorganisms are used to oxidize soluble and colloidal organics to CO_2 and H_2O in the presence of molecular oxygen (1). The process is generally preceded by primary sedimentation. The mixture of microorganisms and wastewater formed in the aeration basins, called mixed liquor, is transferred to gravity clarifiers for liquid–solids separation (Fig. 13.1). The major portion of the microorganisms settling out in the clarifiers is recycled to the aeration basins to be mixed with incoming wastewater, while the excess, which constitutes the waste biosolids (sludge), is sent to the biosolids handling facilities (2). The rate and concentration of activated sludge returned to the aeration basins determines the mixed liquor suspended solids (MLSS) level developed and maintained in the basins. During the oxidation process, a certain amount of the organic material is synthesized into new cells, some of which then undergo auto-oxidation (self-oxidation, or endogenous respiration) in the aeration basins, the remainder forming net growth or excess biosolids (3). Oxygen is required in the process to support the oxidation and synthesis reactions. Volatile compounds are driven off to a certain extent in the aeration process. Metals will also be partially removed, with accumulation in the sludge.

Activated sludge systems are classified as high rate, conventional, or extended aeration (low rate) based on the organic loading. In the conventional activated sludge plant, the wastewater is commonly aerated for a period of 4–8 h (based on average daily flow) in a plug flow hydraulic mode (Fig. 13.1). Either surface or submerged aeration systems can be employed to transfer oxygen from air to wastewater. Compressors are used to supply air to the submerged systems, normally through a network of diffusers, although newer submerged devices, which do not come under the general category of diffusers (e.g., static aerators and jet aerators) are applied. Diffused air systems may be classified as fine bubble or coarse bubble. Diffusers

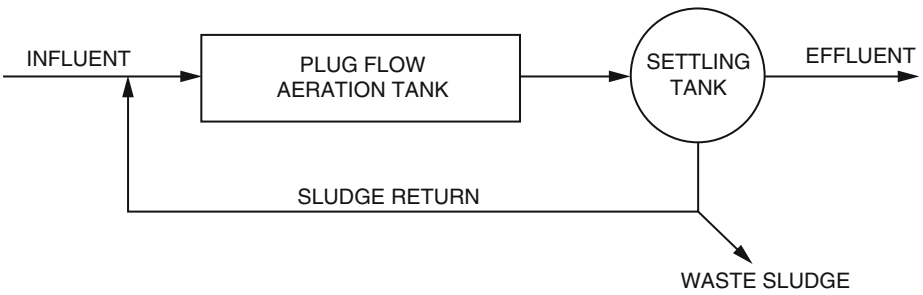

Fig. 13.1. Conventional activated sludge flow diagram (*Source:* U.S. EPA).

commonly used in activated sludge service include the following: porous ceramic plates laid in the basin bottom (fine bubble), porous ceramic domes or ceramic or plastic tubes connected to a pipe header and lateral system (fine bubble), tubes covered with synthetic fabric or wound filaments (fine or coarse bubble), and specially designed spargers with multiple openings (coarse bubble) (1).

Activated sludge is the most versatile and widely used biological process in wastewater treatment (4). Common process modifications include step aeration (Fig. 13.2); contact stabilization (Fig. 13.3); and complete mix flow regimes (Fig. 13.4). Alum or ferric chloride is sometimes added to the aeration tank for phosphorus removal (1).

The activated sludge process is used for the treatment of both domestic wastewater and biodegradable industrial wastewater. The main advantage of the conventional activated sludge system is the lower initial cost of the system, particularly when a high quality effluent is required. Industrial wastewater, which is amenable to biological treatment and degradation, may be jointly treated with domestic wastewater in a conventional activated sludge system.

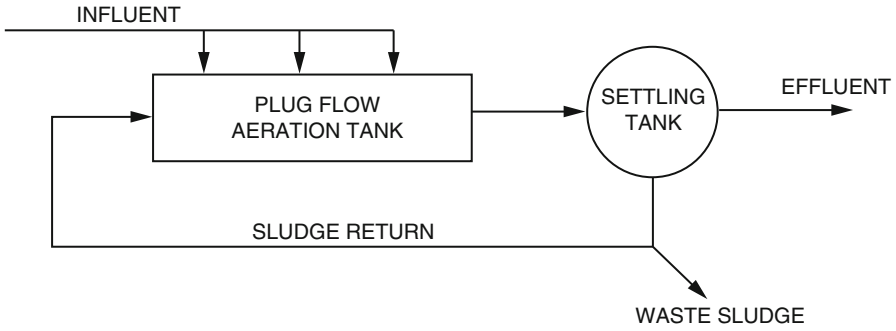

Fig. 13.2. Step aeration flow diagram (*Source:* U.S. EPA).

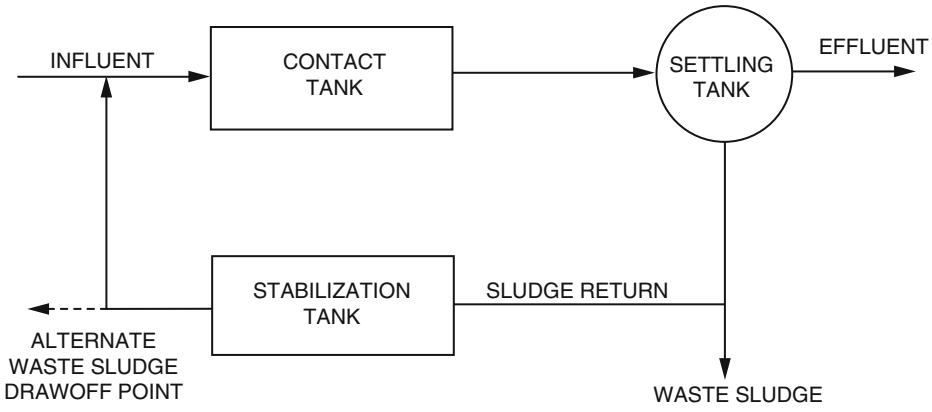

Fig. 13.3. Contact stabilization flow diagram (*Source:* U.S. EPA).

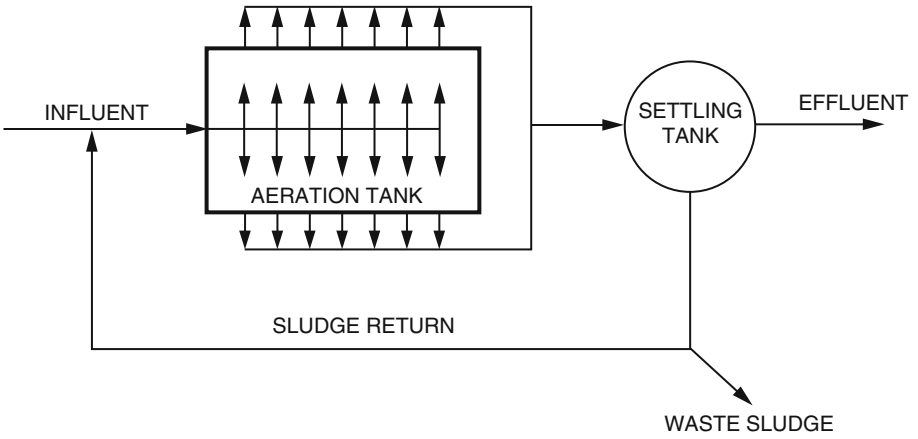

Fig. 13.4. Complete mix activated sludge flow diagram (*Source:* U.S. EPA).

Some of the process disadvantages are (5, 6):

1. Limited BOD_5 (5-day biochemical oxygen demand) loading capacity
2. Poor organic load distribution
3. Plant upset with extreme variations in hydraulic, organic, and toxic loadings
4. Operational complexity
5. High operating costs and energy consuming mechanical compressors
6. Diffuser maintenance

1.2. Performance and Design Criteria

BOD and ammonia-N removals are as follows (1, 7):

- BOD_5 removal (conventional activated sludge) 85–95%
- NH_4-N removal (nonnitrified systems) 10–20%

The residuals or biosolids increase (as measured by volatile suspended solids, VSS, production from the conventional activated sludge process) as food-to-microorganism (F/M) loadings increase (8, 9). For an F/M value of 0.3 lb BOD_5/d/lb MLVSS, the excess in VSS is 0.5 lb/lb BOD_5 removed; while for an F/M ratio of 0.5 lb BOD_5/d/lb MLVSS, the excess in VSS increases to 0.7 lb/lb BOD_5 removed. Here 1 lb/d/lb = 1 kg/d/kg; 1 lb/lb = 1 kg/kg.

Design criteria for the conventional activated sludge process are summarized as follows (1, 7):

- Volumetric loading = 25–50 lb BOD_5/d/1,000 ft^3 = 400.7−801.4 g BOD_5/d/m^3
- Aeration detention time (based on average daily flow) = 4–8 h
- MLSS = 1,500–3,000 mg/L
- F/M = 0.25−0.5 lb BOD_5/d/lb MLVSS = 0.25−0.5 kg BOD_5/d/kg MLVSS
- Air required = 800–1,500 std. ft^3/lb BOD_5 = 50−94 m^3/kg removed
- Biosolids retention time = 5–10 d

1.3. Mechanical Aeration

Mechanical aeration methods include the submerged turbine with compressed air spargers (agitator/sparger system) and the surface-type mechanical entrainment aerators (Fig. 13.5). The surface-type aerators entrain atmospheric air by producing a region of intense turbulence at the surface around their periphery. They are designed to pump large quantities of liquid, thus dispersing the entrained air and agitating and mixing the basin contents. The agitator/sparger system consists of a radial-flow turbine located below the mid-depth of the basin, with compressed air supplied to the turbine through a sparger (1).

The submerged turbine aeration system affords a convenient and relatively economical method for upgrading overloaded activated sludge plants. To attain optimum flexibility of oxygen input, the surface aerator can be combined with the submerged turbine aerator. Several manufacturers supply such equipment, with both aerators mounted on the same vertical shaft. Such an arrangement might be advantageous if space limitations require the use of deep

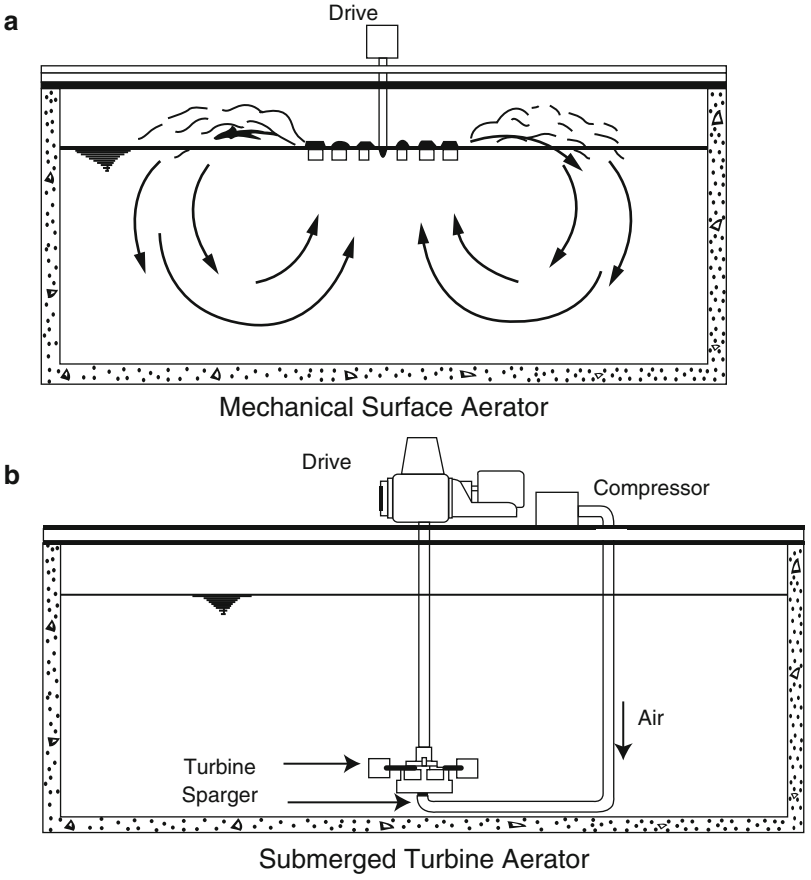

Fig. 13.5. Mechanical aeration (*Source:* U.S. EPA).

aeration basins. In addition, mechanical aerators may be either the floating or fixed installation type.

Mechanical aerators have been used primarily in industrial waste activated sludge treatment plants and are considered an attractive aeration system for very deep basins (with bottom mixers or spargers plus surface aerators), for activated sludges having high oxygen uptake rates, and for high concentrations of MLSS, as in aerobic digesters.

The mixing equipment for aeration or oxygen transfer must be sized to keep the solids in uniform suspension at all times. Depending on basin shape and depth, 4,000 mg/L of MLSS require about 0.75–1.0 HP/1, 000 ft^3 (0.02–0.03 kw/m^3) of basin volume to prevent settling if mechanical aerators are employed. However, the power required to transfer the necessary oxygen will usually equal or exceed this value (1).

2. HIGH RATE ACTIVATED SLUDGE

2.1. Description

Activated sludge systems have traditionally been classified as high rate, conventional, or extended aeration (low rate), based on organic loading. The term modified aeration has been adopted to apply to those high rate air activated sludge systems with design F/M loadings in the range of 0.75–1.5 lb BOD_5/d/lb MLVSS (0.75−1.5 kg BOD_5/d/kg MLVSS). Modified aeration systems are characterized by low MLSS concentrations, short aeration detention times, high volumetric loadings, low air usage rates, and intermediate levels of BOD_5 and suspended solids removal efficiencies (1). Before the enactment of nationwide secondary treatment regulations, modified aeration was utilized as an independent treatment system for plants where BOD_5 removals of 50–70% would suffice. With present-day treatment requirements, modified aeration no longer qualifies as a "stand-alone" activated sludge option.

Modified aeration basins are normally designed to operate in either complete mix (Fig. 13.4) or plug flow (Fig. 13.1) hydraulic configurations. Either surface or submerged aeration systems can be employed to transfer oxygen from air to wastewater, although submerged equipment is specified more frequently for this process. Compressors are used to supply air to submerged aeration systems.

Primarily due to rapidly escalating power costs, interest has been expressed in the development of high rate, diffused aeration systems, which would produce a high quality secondary effluent. As with modified aeration, aeration detention times would remain low and volumetric loadings high. In contrast to modified aeration systems, high MLSS concentrations would have to be utilized to permit F/M loadings to be maintained at reasonable levels. The key to development of efficient high rate air systems is the availability of submerged aeration equipment that could satisfy the high oxygen demand rates that accompany high MLSS levels and short aeration times. New innovations in fine bubble diffuser and jet aeration technology offered the technology for uniting high efficiency oxygen transfer with high rate air activated sludge flow regimes to achieve acceptable secondary treatment as independent "stand-alone" processes (10).

Since the early 1970s, modified aeration was employed generally as a pretreatment or roughing process in a two-stage activated sludge system, where the second stage is used for

biological nitrification (10, 11). Alum or one of the iron salts is sometimes added to modified aeration basins preceding second-stage nitrification units for phosphorus removal.

2.2. Performance and Design Criteria

BOD_5 removal for modified aeration is in the range of 50–70%; for high solids, high rate air system, a removal of 85–95% is obtainable. Ammonia-N removal is only 5–10% (1, 7).

A modified air aeration system produces, on the average, 1.1 lb excess VSS (secondary effluent plus waste sludge)/lb BOD_5 removed at an average F/M ratio loading of 1.2 lb BOD_5/d/lb MLVSS. Here 1 lb/lb = 1 kg/kg; 1 lb/d/lb = 1 kg/d/kg.

Design criteria for the two high rate air activated sludge process options are summarized as follows (1, 7):

(a) Modified aeration
- Volumetric loading = 50–100 lb BOD_5/d/1, 000 ft^3 = 801.4−1602.8 kg BOD_5/d/m^3
- MLSS = 800–2,000 mg/L
- Aeration detention time (based on influent flow) = 2–3 h
- F/M = 0.75–1.5 lb BOD_5/d/lb MLVSS = 0.75−1.5 kg BOD_5/d/kg MLVSS
- Air required = 400−800 Std. ft^3 air/lb BOD_5 removed = 25−50 m^3/kg BOD_5 removed
- Oxygen required = 0.4−0.7 lb O_2/lb BOD_5 removed = 0.4−0.7 kg O_2/kg BOD_5 removed
- Sludge retention time = 0.75–2 d
- Recycle ratio (R) = 0.25–1.0
- Volatile fraction of MLSS = 0.7–0.85

(b) High solids, high rate aeration
- Volumetric loading = 50–125 lb BOD_5/d/1, 000 ft^3 = 801.4−2003.5 kg BOD_5/d/m^3
- MLSS = 3,000–5,000 mg/L
- Aeration detention time (based on influent flow) = 2–4 h
- F/M = 0.4–0.8 lb BOD_5/d/lb MLVSS = 0.4−0.8 kg BOD_5/d/kg MLVSS
- Air required = 800–1,200 Std. ft^3 air/lb BOD_5 removed = 50−75 m^3/kg BOD_5 removed
- Oxygen required = 0.9–1.2 lb O_2/lb BOD_5 removed = 0.9−1.2 kg O_2/kg BOD_5 removed
- Sludge retention time = 2–5 d
- Recycle ratio (R) = 0.25–0.5
- Volatile fraction of MLSS = 0.7–0.8

3. PURE OXYGEN ACTIVATED SLUDGE, COVERED

3.1. Description

The use of pure oxygen for activated sludge treatment has become competitive with the use of air due to the development of efficient oxygen dissolution systems (10). The covered oxygen system is a high rate activated sludge system. The main benefits cited for the process include reduced power requirements for dissolving oxygen in the wastewater, reduced aeration tank volume requirements, and improved biokinetics of the activated sludge system (11). In the covered system, oxygenation is performed in a staged, covered reactor, in which

oxygen gas is recirculated within the system until it reaches a reduced level of purity and a decreased undissolved mass at which it can no longer be used and is vented to the atmosphere. High-purity oxygen gas (90–100% volume) enters the first stage of the system and flows concurrently with the wastewater being treated through the oxygenation basin. Pressure under the tank covers is essentially atmospheric, being held at 2–4 in. (5.1–10.2 cm) water column, sufficient to maintain oxygen gas feed control and prevent backmixing from stage to stage. Effluent mixed liquor is separated in conventional gravity clarifiers, and the thickened sludge is recycled to the first stage for contact with influent wastewater (Fig. 13.6).

Mass transfer and mixing within each stage are accomplished either with surface aerators or with a submerged turbine rotating-sparge system. In the first case, mass transfer occurs in the gas space; in the latter, oxygen is sparged into the mixed liquor where mass transfer occurs from the oxygen bubbles to the bulk liquid. In both cases, the mass-transfer process is enhanced by the high oxygen-partial pressure maintained under the tank covers in each stage (12).

Volatile compounds are driven off to a certain extent in the oxygenation process and removed in the vent gas. Metals may also be expected to be partially removed, with accumulation in the sludge. High purity oxygen may be produced on-site by cryogenic or PSA (Pressure Swing Adsorption) generators, or purchased as liquid oxygen produced off-site and stored at the treatment plant. Cost effectiveness of oxygen source depends upon plant size and process train.

Although flexibility is claimed to permit operation in any of the normally used flow regimes, i.e., plug flow, complete mix, step aeration, and contact stabilization, the method of oxygen contact employed favors the plug flow mode. Process may be designed to achieve: optimum carbonaceous oxidation only, combined carbonaceous and nitrogenous oxidation or optimum nitrogenous oxidation as a separate stage after secondary treatment (11).

The pure oxygen process can be applied to both domestic and biologically degradable industrial wastewaters; for upgrading existing air activated sludge plants; for new facilities – to reduce construction cost where effective odor control is required, where high effluent dissolved oxygen is required, where reduced quantity and higher concentration of waste sludge is required and where reduced aeration detention time is required.

3.2. Performance and Design Criteria

Performance data for pure oxygen are summarized below (11, 12)):

(a) Carbonaceous Oxidation:
- COD removal = 75–80%
- BOD_5 removal = 90–95%
- Suspended solids removal = 75–90%

(b) Nitrogenous Oxidation – NH_4-N removals:
- Single stage with carbonaceous oxidation = 20–90%
- Separate stage nitrification after carbonaceous oxidation = 80–98%

(c) Generated residuals 0.42–1.0 lb VSS/lb BOD_5 removed. $= 0.42-1.01\,\mathrm{kgVSS/kgBOD_5}$ removed

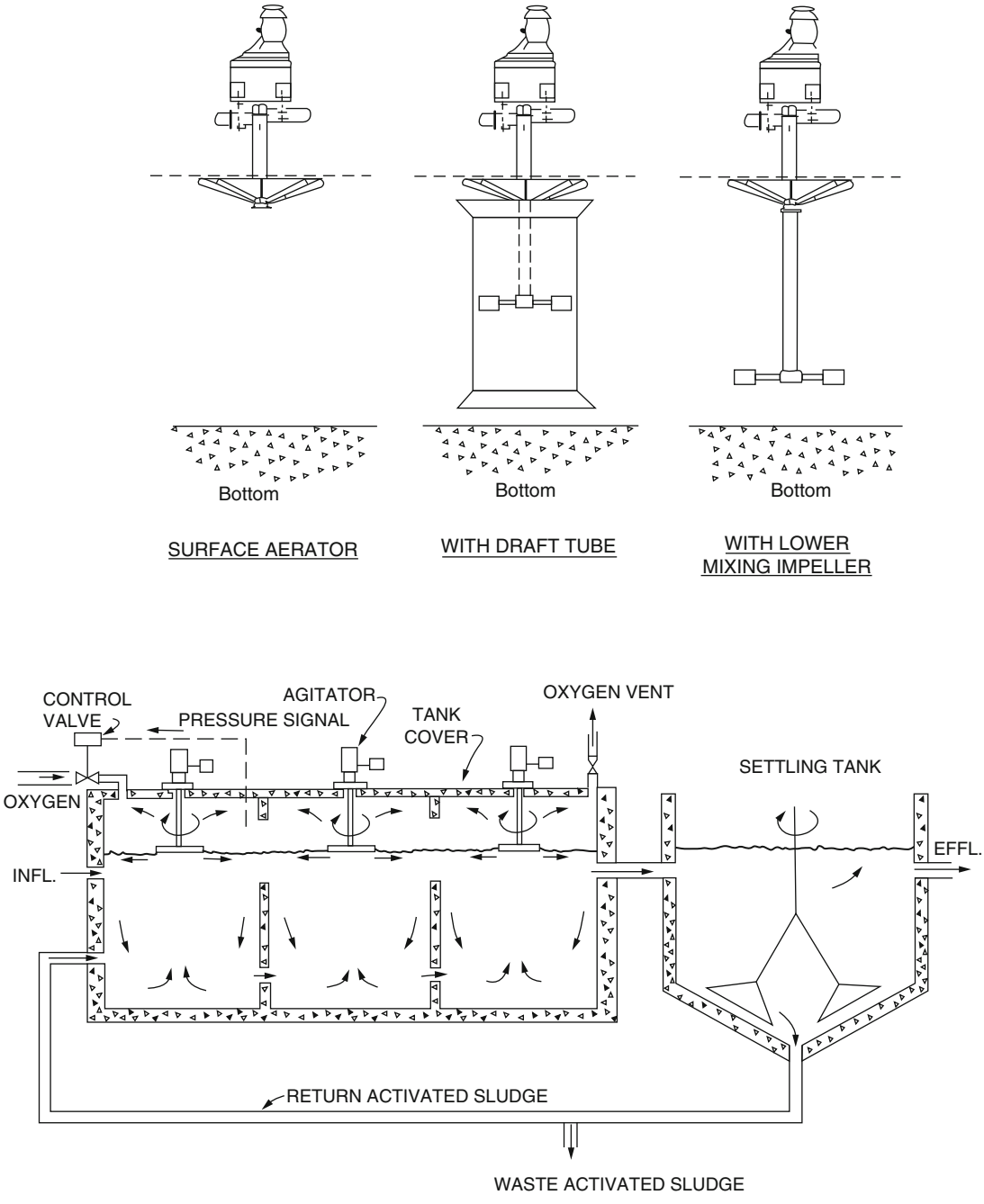

Fig. 13.6. Types of mechanical aerators and pure oxygen activated sludge (*Source:* U.S. EPA).

Design Criteria (Carbonaceous BOD Oxidation) (7, 11, 12):

- Volumetric loading = 100–200 lb BOD_5/d/1,000 ft^3 = 1601.6−3203.6gBOD_5/d/m^3
- F/M = 0.5–1.0 lb BOD_5/d/lb MLVSS = 0.5−1.0 kgBOD_5/d/kg MLVSS
- Oxygen required = 0.6–0.8 lb O_2/lb COD removed = 0.6−0.8 kg O_2/kg COD removed
- MLSS = 3,000–6,000 mg/L
- Aeration detention time = 1–3 h
- Mixed liquor dissolved oxygen = 4–8 mg/L
- Oxygen required = 0.9–1.3 lb O_2/lb BOD_5 removed = 0.9−1.3 kg O_2/kg BOD_5 removed

4. CONTACT STABILIZATION

4.1. Description

Contact stabilization is a modification of the activated sludge process (see Fig. 13.3). In this modification, the adsorptive capacity of the floc is utilized in the contact tank to adsorb suspended, colloidal, and some dissolved organics. The hydraulic detention time in the contact tank is only 30–60 min (based on average daily flow). After the biological solids are separated from the wastewater in the secondary clarifier, the concentrated biosolids are separately aerated in the stabilization tank with a detention time of 2–6 h (based on solids recycle flow). The adsorbed organics undergo oxidation in the stabilization tank and are synthesized into microbial cells. If the detention time is long enough in the stabilization tank, endogenous respiration will occur, along with a concomitant decrease in excess biosolids production. Following stabilization, the reaerated biosolids are mixed with incoming wastewater in the contact tank and the cycle starts anew (1, 13). Volatile compounds are driven off to a certain extent by aeration in the contact and stabilization tanks. Metals will also be partially removed, with accumulation in the sludge.

This process requires smaller total aeration volume than the conventional activated sludge process. It can also handle greater organic shock and toxic loadings because of the biological buffering capacity of the stabilization tank and the fact that, at any given time, the majority of the activated sludge is isolated from the main stream of the plant flow. Generally, the total aeration basin volume (contact plus stabilization basins) is only 50–75% of that required in the conventional activated sludge system.

4.2. Applications

Contact stabilization has evolved as an outgrowth of activated sludge technology since 1950 and seen common usage in package plants and some usage for on-site constructed plants.

Contact stabilization can be most advantageously applied in the following cases (1, 13):

1. Wastewaters that have an appreciable amount of BOD_5 in the form of suspended and colloidal solids
2. Upgrading of an existing, hydraulically overloaded conventional activated sludge plant
3. New installations, to take advantage of low aeration volume requirements
4. Where the plant might be subject to shock organic or toxic loadings
5. Where larger, more uniform flow conditions are anticipated (or if the flows to the plant have been equalized)

Some of the limitations or disadvantages associated with contact stabilization include the following:

1. It is unlikely that effluent standards can be met using contact stabilization in plants with flow rates $< 50,000$ gal/d (189, 250 L/d) without some prior flow equalization
2. Operational complexity
3. High operating costs
4. High energy consumption and high diffuser maintenance
5. As the fraction of soluble BOD_5 in the influent wastewater increases, the required total aeration volume of the contact stabilization process approaches that of the conventional process

4.3. Performance and Design Criteria

Contact stabilization can achieve the following BOD_5 and NN_4-N removals (1, 14, 15)):

- BOD_5 removal = 80–95%
- NN_4-N removal = 10–20%

Design criteria for the contact stabilization process are summarized as follows (1, 7, 14, 15):

- F/M = 0.2–0.6 lb BOD_5/d/lb MLVSS = 0.2−0.6kg BOD_5/d/kg MLVSS
- Volumetric loading = 30–50 lb BOD_5/d/1, 000 ft^3 (based on contact and stabilization volume) = 481−801 g/d/m^3
- MLSS = 1,000–2,500 mg/L, contact tank; 4,000–10,000 mg/L, stabilization tank
- Aeration time = 0.5–1.0 h, contact tank (based on average daily flow) 2–6 h, stabilization basin (based on sludge recycle flow)
- Sludge retention time = 5–10 d
- Recycle ratio (R) = 0.25–1.0
- Air supplied = 800–2,100 Std. ft^3 air/lb BOD_5 removed = 50−131 m^3 air/kg BOD_5 removed
- Oxygen required = 0.7–1.0 lb O_2/lb BOD_5 removed = 0.7−1.0 kg O_2/kg BOD_5 removed
- Volatile fraction of MLSS = 0.6–0.8

5. ACTIVATED SLUDGE WITH NITRIFICATION

5.1. Description

This process is also referred to as single-stage nitrification, because ammonia and carbonaceous materials are oxidized in the same aeration unit (the flow diagram is similar to Fig. 13.1). As in any aerobic biological process, carbonaceous materials are oxidized by heterotrophic aerobes. In addition, a special group of autotrophic aerobic organisms called nitrifiers oxidize ammonia in two stages: *Nitrosomonas* bacteria convert ammonia to nitrite and *Nitrobacter* convert nitrite to nitrate (1, 11).

The optimal conditions for nitrification, in genera are (1, 11, 16):

1. Temperature of about 30°C
2. pH of about 7.2–8.5
3. F/M of about 0.05–0.15
4. Relatively long aeration detention time as nitrifiers have a lower growth rate than other aerobe
5. Sludge retention time of about 20–30 d, depending upon temperature.

The degree of nitrification depends mainly on three factors (17):

1. SRT (sludge retention time), d
2. Mixed liquor DO concentration, mg/L
3. Wastewater temperature, °C

Of the above three factors, SRT is of primary importance because of the slow growth rate of nitrifiers. If the sludge is wasted at too high a rate, the nitrifiers will be eliminated from the system. Generally, nitrification begins at an SRT of about 5 d, but does not become appreciable until the SRT reaches about 15 d, depending upon temperature. The aeration system is designed to provide the additional oxygen needed to oxidize the ammonia nitrogen. Biological nitrification is very sensitive to temperature, resulting in poor reduction in colder months. In addition, heavy metals such as Cd, Cr, Cu, Ni, Pb and Zn, phenolic compounds, cyanide and halogenated compounds can inhibit nitrification reactions.

The conventional and high rate modifications of the activated sludge process do not provide the necessary hydraulic and sludge detention time. Besides, the F/M ratio is higher. As a result, single stage nitrification cannot be achieved in these configurations, although they effect a small reduction, about 20% in ammonia-N. Any low rate modification of the activated sludge process, such as the extended aeration and the oxidation ditch, can be used. In addition, the use of powdered activated carbon has the potential to enhance ammonia removal.

5.2. Performance and Design Criteria

A well-established extended aeration process will decrease ammonia-nitrogen to around 1 mg/L if the aerator temperature is about 55°F (1, 17).

This process produces no primary sludge. The secondary sludge is lesser in quantity and better stabilized than the high rate and conventional activated sludge process, which minimizes the magnitude of the disposal problem considerably.

The design criteria when using extended aeration modification are (5–7, 17):

- Volumetric loading = 5–10 lb BOD_5/d/1, 000 ft^3 = 80−160 gBOD_5/d/m^3
- MLSS = 3,000–6,000 mg/L
- F/M = 0.05–0.15 lb BOD_5/d/lb MLVSS = 0.05−0.15 kg BOD_5/d/kg MLVSS
- Aeration detention time (based on average daily flow) = 18–36 h
- Air supplied = 3,000–4,000 std. ft^3/lb BOD_5 applied = 187−250m^3/kg BOD_5 applied
- Oxygen required = 2.0–2.5 lb O_2/lb BOD_5 applied = 2.0−2.5 kg O_2/kg BOD_5 applied
- Sludge retention time = 20–30 d
- Recycle ratio = 0.7–1.5
- Volatile fraction of MLSS = 0.6–0.7

The design criteria when using oxidation ditch modification are:

- Volumetric loading = 10–15 lb BOD_5/d/1, 000 ft^3 = 160−240g BOD_5/d/m^3
- MLSS = 3,000–5,000 mg/L
- F/M = 0.03–0.10 lb BOD_5/d/lb MLVSS = 0.03−0.10 kg BOD_5/d/kg MLVSS
- Aeration detention time (based on average daily flow) = 24 h
- Oxygen required = 2.0–2.5 lb O_2/lb BOD_5 applied = 2.0−2.5 kg O_2/kg BOD_5 applied
- Sludge retention time = 20–30 d

- Recycle ratio = 0.25–0.75
- Volatile fraction of MLSS = 0.6–0.7 mg/L

6. SEPARATE STAGE NITRIFICATION

6.1. Description

The process by which ammonia is converted to nitrate in wastewater is referred to as nitrification. In the process, *Nitrosomonas* and *Nitrobacter* act sequentially to oxidize ammonia (and nitrite) to nitrate. The biological reactions involved in these conversions may take place during activated sludge treatment (as in previous section) or as a separate stage following removal of carbonaceous materials. Separate stage nitrification may be accomplished via suspended growth or attached growth unit processes. In either case, the nitrification step is preceded by a pretreatment sequence to reduce the carbonaceous demand. Possible pretreatment schemes include: activated sludge, trickling filter, roughing filter, primary treatment with chemical addition, and physical–chemical treatment. In general, if the pretreatment effluent has a BOD_5/TKN ratio of less than 3.0, sufficient carbonaceous removal has occurred such that the following nitrification process may be classified as a separate stage. Low BOD_5 is required to assure a high concentration of nitrifiers in the nitrification biomass (1, 11).

The most common separate stage nitrification process is the plug flow suspended growth configuration with clarification (Fig. 13.7). In this process, pretreatment effluent is pH adjusted (as required) and aerated in a plug flow mode. Because the carbonaceous demand is low, nitrifiers predominate. A clarifier follows aeration, and nitrification biosolids are returned to the aeration tank. A possible modification is the use of pure oxygen in place of conventional aeration during the plug flow operation.

Less prevalent are attached growth separate stage nitrification processes. These processes may be operated analogously to trickling filter, packed bed or rotating biological contactor systems. Since the biomass is attached to the reactor surface and solids synthesis is low, a clarifier may not be required. Final filtration is sometimes practiced to reduce effluent suspended solids, although this is often not required.

6.2. Performance and Design Criteria

Conversions of ammonia (and nitrite) to nitrate of up to 98% are achievable. Properly designed systems have effluent ammonia in the 1–3 mg/L range. BOD_5 reductions are generally 70–80% (influent BOD_5 assumed as approximately 50 mg/L) (18).

Design criteria – suspended growth systems (1, 5, 6, 17):

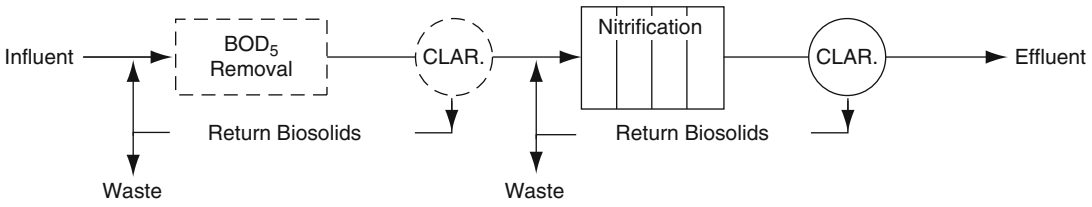

Fig. 13.7. Separate nitrification flow diagram (*Source:* U.S. EPA).

- Flow scheme: plug flow (preferable, but not mandatory)
- Optimum pH = 8.2–8.6
- MLVSS = 1,200–2,400 mg/L
- Min. aeration tank DO = 2.0 mg/L
- Clarifier surface loading rate = 400–600 gpd/ft^2 = 16.32−24.48 m^3/d/m^2
- Solids loading = 20–30 lb/d/ft^2 = 98−146 kg/d/m^2
- Return biosolids rate = 50–100%
- Detention time = 0.5–3 h
- Mean cell residence time (MCRT) = 10–20 d

7. SEPARATE STAGE DENITRIFICATION

7.1. Description

Denitrification involves the reduction of nitrates and nitrites to nitrogen gas through the action of facultative heterotrophic bacteria (19, 20). In suspended growth, separate stage denitrification processes, nitrified wastewater containing primarily nitrates, is passed through a mixed anoxic vessel containing denitrifying bacteria. Since the nitrified feedwater contains very little carbonaceous material, a supplemental source of carbon is required to maintain the denitrifying biomass. This supplemental energy is provided by feeding methanol to the biological reactor along with the nitrified wastewater. Mixing in the anoxic denitrification reaction vessel may be accomplished using low speed paddles analogous to standard flocculation equipment. Following the reactor, the denitrified effluent is aerated for a short period (5–10 min) to strip out gaseous nitrogen formed in the previous step, which might otherwise inhibit sludge settling. Clarification follows the stripping step with the collected sludge being either returned to the head end of the denitrification system, or wasted. The flow diagram of this process is illustrated in Fig. 13.8.

Common modifications include the use of alternate energy sources, such as sugars, acetic acid, ethanol or other compounds. Nitrogen deficient materials, such as brewery wastewater, may also be used. An intermediate aeration step for stabilization (about 50 min) between the denitrification reactor and the stripping step may be used to guard against carryover of carbonaceous materials. The denitrification reactor may be covered but not air tight to assure anoxic conditions by minimizing surface reaeration (19).

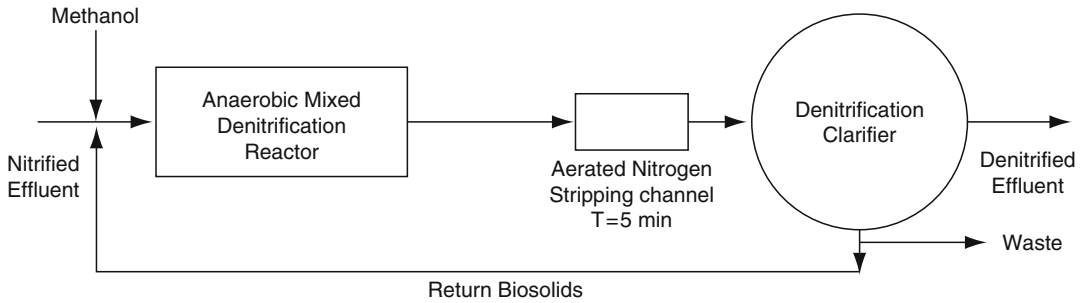

Fig. 13.8. Separate stage denitrification flow diagram (*Source:* U.S. EPA).

This process is used almost exclusively to denitrify municipal wastewaters that have undergone carbon oxidation and nitrification. It may also be used to reduce nitrate in industrial wastewaters.

7.2. Performance and Design Criteria

Separate stage denitrification is capable of reducing 50–98% of the nitrate and nitrite entering the system to gaseous nitrogen. Overall, nitrogen removals of 70–95% are achievable. Typical wastewater characteristics for NO_3–N: influent 20 mg/L, effluent 1 mg/L (18).

An energy source is needed which is usually supplied in the form of methanol. Methanol feed concentration may be estimated on the basis of 2.47 mg/L of methanol (CH_3OH) per mg/L of NO_3–N, 1.53 mg/L methanol/mg/L of NO_2–N and 0.87 mg/L methanol/mg/L of DO (1, 11).

If supplemental energy feed rates are controlled, very little excess biosolids are generated. Biosolids production is in the range of 0.6–0.8 lb/lb (0.6–0.8 kg/kg) NH_3–N reduced.

The design criteria for the denitrification process are listed below (5, 17):

- Flow scheme: plug flow (preferable, but not mandatory)
- Optimum pH = 6.5–7.5
- MLVSS = 1,000–3,000 mg/L
- Mixer power requirement = 0.25–0.50 HP/1, 000 ft^3 = 0.0066−0.0132 kW/m^3
- Clarifier depth = 12–15 ft = 3.66−4.57 m
- Clarifier surface loading rate = 400–600 gpd/ft^2 = 16.32−24.48 $m^3/d/m^2$
- Solids loading = 20–30 $lb/d/ft^2$ = 98−146 $kg/d/m^2$
- Return sludge rate = 50–100%
- Biosolids generation = 0.2 lb/lb CH_3OH or 0.7 lb/lb NH_3–N reduced (1 lb/lb = 1 kg/kg)
- Detention time = 0.2–2 h
- Cell residence time = 1–5 d

8. EXTENDED AERATION

8.1. Description

Extended aeration is the "low rate" modification of the activated sludge process with the same flow diagram that was shown in Fig. 13.1. The F/M loading is in the range of 0.05–0.15 lb BOD_5/d/lb MLVSS (0.05–0.15 kg BOD_5/d/kg MLVSS), and the detention time is about 24 h (1). Primary clarification is rarely used. The extended aeration system operates in the endogenous respiration phase of the bacterial growth cycle because of the low BOD_5 loading. The organisms are starved and forced to undergo partial auto-oxidation. Volatile compounds are driven-off to a certain extent in the aeration process. Metals will also be partially removed, with accumulation in the sludge.

In the complete mix version of the extended aeration process, all portions of the aeration basin are essentially homogeneous, resulting in a uniform oxygen demand throughout the aeration tank. This condition can be accomplished fairly simply in a symmetrical (square or circular) basin with a single mechanical aerator or by diffused aeration. The raw wastewater and return biosolids enter at a point (e.g., under a mechanical aerator) where they are quickly

dispersed throughout the basin. In rectangular basins with mechanical aerators or diffused air, the incoming waste end return biosolids are distributed along one side of the basin and the mixed liquor is withdrawn from the opposite side ().

Extended aeration plants have evolved since the latter part of the 1940s. Most common applications are for plants with flows of less then 50,000 gpd (189,250 L/d) as well as for emergency or temporary treatment needs. Preengineered, package plants have been widely utilized for this process.

Some of the limitations or disadvantages of the process are (7):

- High power costs
- Operation costs
- Capital costs (for barge permanent installations where the preengineered plants would not be appropriate)

8.2. Performance and Design Criteria

The carbonaceous and ammonia–N removal rates are expected to be high since long detention times are employed in the process. Actual BOD_5 removal is in the range of 85–95% and NH_3–N removal between 50 and 90%.

Because of the low F/M loadings end long hydraulic detention times employed, excess biosolids production for the extended aeration process (and the closely related oxidation ditch process) is the lowest of any of the activated sludge process modifications, generally in the range of 0.15–0.30 lb excess biosolids/lb BOD_5 removed (7, 21). Here 1 lb/lb = 1 kg/kg.

The design criteria for the extended aeration modification of the activated sludge process is summarized as follows (1, 7, 22, 23):

- Volumetric loading = 5–10 lb $BOD_5/d/1,000\ ft^3$ = 80−160 $g/d/m^3$
- MLSS = 3,000–6,000 mg/L
- F/M = 0.05–0.15 lb BOD_5/d/lb MLVSS = 0.05−0.15 kg BOD_5/d/kg MLVSS
- Aeration detention time = 18–36 h (based on average daily flow)
- Air required = 3,000–4,000 Std. ft^3 air/lb BOD_5 applied = 187−250 m^3 air/kg BOD_5 applied
- Oxygen required = 2.0–2.5 lb O_2/lb BOD_5 applied = (based on 1.5 lb O_2/lb BOD_5 removed + 4.6 lb O_2/lb NH_3–N removed) = 2.0−2.5 kg O_2/kg BOD_5 applied
- Biosolids retention time = 20–40 d
- Recycle ratio (R) = 0.75–1.5
- Volatile fraction of MLSS = 0.6–0.7

9. OXIDATION DITCH

9.1. Description

An oxidation ditch is an activated sludge biological treatment process; commonly operated in the extended aeration mode, although conventional activated sludge treatment is also possible. Typical oxidation ditch treatment systems consist of a single or closed loop channel 4–6 ft (1.22–1.83 m) deep, with 45° sloping sidewalls (Fig. 13.9).

Some form of preliminary treatment such as careering, comminution or grit removal normally precedes the process. After pretreatment (primary clarification is usually not practiced),

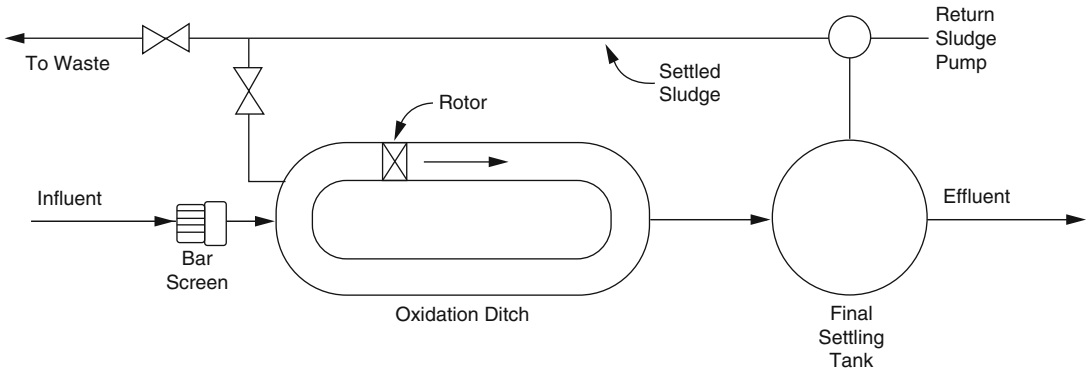

Fig. 13.9. Oxidation ditch (*Source:* U.S. EPA).

the wastewater is aerated in the ditch using mechanical aerators which are mounted across the channel. Horizontal brush, cage or disc-type aerators, specially designed for oxidation ditch applications, are normally used. The aerators provide mixing and circulation in the ditch, as well as sufficient oxygen transfer (24). Mixing in the channels is uniform, but zones of low, dissolved oxygen concentration can develop. Aerators operate in the 60–110 rpm range and provide sufficient velocity to maintain solids in suspension. A high degree of nitrification may occur in the process without special modification because of the long detention times and high solid retention times (10–50 d) utilized. Secondary Settling of the aeration ditch effluent is provided in a separate clarifier.

Ditches may be constructed of various materials, including concrete, gunite, asphalt, or impervious membranes. Concrete is the most common. Ditch loops may be oval or circular in shape. "Ell" and "horseshoe" configurations have been constructed to maximize land usage. Conventional activated sludge treatment, in contrast to extended aeration, may be practiced. Oxidation ditch systems with depths of 10 ft (3.05 m) or more with vertical sidewalls and vertical shaft aerators may also be used (25).

Oxidation ditch technology is applicable in any situation where activated sludge treatment (conventional or extended aeration) is appropriate. The process cost of treatment is generally less than other biological processes in the range of wastewater flows between 0.1 and 10 MGD (0.3785 and 3.785 MLD) (26).

9.2. Performance and Design Criteria

The average performance of shallow oxidation ditch plants is summarized below (24, 27–30):

- BOD_5 effluent = 10–15 mg/L
- BOD_5 removal = 90–95%
- Suspended solids effluent = 10–15 mg/L
- Suspended solids removal = 90–95%
- Ammonia–N removal = 40–80%

No primary biosolids are generated. Biosolids produced are less volatile due to higher oxidation efficiency and increased solids retention times.

Design criteria – extended aeration mode (24, 27–30):

- BOD_5 loading = 8.6–15 lb BOD_5/d/1, 000 ft^3 of aeration volume = 138−240 g $BOD_5/d/m^3$
- Biosolids retention time = 10–33 d
- Channel depth = 4–6 ft = 1.22−1.83 m
- Channel geometry = 45° or vertical sidewalls
- Aeration channel detention time = 1 d

10. POWDERED ACTIVATED CARBON TREATMENT

10.1. Types of PACT Systems

The powdered activated carbon (PAC) activated sludge system is a process modification of the activated sludge process. PAC is added to the aeration tank where it is mixed with the biological solids (Fig. 13.10). The mixed liquor solids are settled and separated from the treated effluent. In a gravity clarifier, polyelectrolyte will normally be added prior to the clarification step to enhance solids–liquid separation. If phosphorus removal is necessary, alum is often added at this point also. Even with polyelectrolyte addition, tertiary filtration is normally required to reduce the level of effluent suspended solids. The clarifier underflow solids are continuously returned to the aeration tank. A portion of the carbon-biomass mixture is wasted periodically to maintain the desired solids inventory in the system (31).

There are six types of combined biological and physicochemical PAC process systems (32–39):

(a) Continuous combined biological and physicochemical PAC process systems involving the use of sedimentation clarifiers

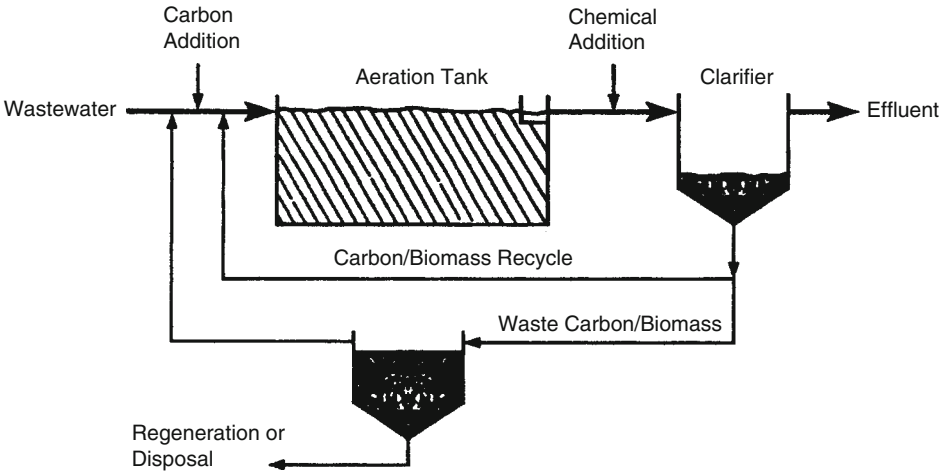

Fig. 13.10. Powdered activated carbon activated sludge process (PACT) (41, 45).

(b) Combined biological and physicochemical PAC sequencing batch reactor systems involving the use of sedimentation clarifiers.
(c) Continuous combined biological and physicochemical PAC process systems involving the use of dissolved air flotation (DAF) clarifiers
(d) Combined biological and physicochemical PAC sequencing batch reactor systems involving the use of DAF clarifiers
(e) Continuous combined biological and physicochemical PAC process systems involving the use of membrane filters (MF)
(f) Combined biological and physicochemical PAC sequencing batch reactor involving the use of membrane filters (MF)

When PAC is dosed into an activated sludge process for combined adsorption and biochemical reactions, the combined process is also called powdered activated carbon treatment (PACT) process, in which PAC still stands for powdered activated carbon, while ACT stands for activated sludge.

10.2. Applications and Performance

The addition of PAC to plug flow and complete mix suspended growth reactors is a more common process modification for industrial wastewater treatment than for municipal systems. Demonstrated advantages of PAC addition to suspended growth reactors include (39):

(a) Improved solids settling and dewatering characteristics
(b) The ability of PAC to adsorb biorefractory materials and inhibitory compounds
(c) Improving effluent quality and reducing the impact of organic shock loads
(d) Reduction in odor, foaming, and sludge bulking
(e) Improved color and 5-day BOD removal

Because PAC is wasted with excess biomass, virgin or regenerated PAC addition is required to maintain the desired concentration in the biological reactor. This can represent a significant cost factor for the system. When carbon addition requirements exceed 900–1,800 kg/d (2,400–4,000 lb/d), wet air oxidation/regeneration (WAR) is claimed to represent an economical approach to carbon recovery and waste biomass destruction (40). However, an ash separation step is needed in this case, affecting the economics of carbon regeneration and recovery (41). The economic analysis is further clouded by the inability to analytically differentiate powdered carbon from background refractory volatile materials, thus making it difficult to quantify the value of the volatile suspended material recovered after WAR. Although ash separation processes have been reported to be effective in at least two municipal PAC activated sludge plants, the economics of complete PAC/WAR systems relative to other activated sludge nitrification systems are unclear (38, 41, 42).

In the United States, PACT systems for nitrification have generally been applied at municipal treatment plants, where industrial sources contribute a significant fraction of the incoming wastewater. In all instances, PAC regeneration was included in the flowsheet (43). A summary of selected municipal PACT facilities is presented in Table 13.1.

The procedure to follow in designing PACT systems for nitrification involves a modification to those for complete mix or conventional plug flow systems in order to account for the effects of the addition of PAC (44). According to the major supplier of the technology (43, 45), most

Table 13.1
Summary of PACT process systems using wet air oxidation for PAC regeneration (41, 45)

Facility	Current design flow, m^3/s	PAC/WAR status	Reason[a] for PAC	Permit Limits		
				BOD_5, mg/L	TSS, mg/L	NH_4-N, mg/L
Vemon, CT	0.18/0.28	MA	C	10	20	–
Mt. Holly, NJ	0.11/0.22	MA	C,S	30	30	20
E. Burlington, NC	0.31/0.53	MA	C,N,T	12–24	30	4.0–8.0
S. Burlington, NC	0.30/0.42	AS	C,N,T	12–24	30	4.0–8.0
Kalamazoo, MI	1.1/2.4	MA	C,N,T	7–30	20–30	2.0–10.0
Bedford Hts., OH	0.15/0.15	NAC	N,S	10	12	5.1
Medina Co., OH	0.3l/0.44	MA	N	10	12	1.5–8.0
N. Olmsted,[a] OH	0.26/0.31	AS	N,S	30	30	2.3–6.9
Sauget, IL	0.70/1.2	AS	T	20	25	–
El Paso, TX	0.20/0.44	MA	N,O	Plan[b]	Plan	Plan

[a] *MA* modified operation and/or design for ash control; *AS* converted to conventional activated sludge; *NAC* converted to the use of nonactivated carbon without regeneration.
C color removal; *S* space; *N* nitrification; *T* toxics; *O* organics.
[b] Plan to convert to NAC without regeneration.

PAC process systems are designed at MLSS concentrations of approximately 15 g/L. The mixed liquor is composed of volatile activated carbon, biomass, nonvolatile PAC ash, biomass decay components, and influent inert material. The relative proportions of these materials are strongly influenced by whether carbon regeneration via wet air oxidation and a return of this material to the aerator is practiced. The intent is to maintain the PAC concentration at approximately 1.5 times the biomass level in nitrification PAC reactors (43, 45). The most appropriate PAC concentration will be dictated by the specific wastewater characteristics and often cannot be specified without bench or pilot scale studies. The PAC concentration to be added will depend on the design solids retention time, the hydraulic retention time, and the required PAC concentration in the reactor. According to the U.S. Environmental Protection Agency (45), for practical engineering design considering the loss, the PAC concentration to be added can be calculated from Eq. (1):

$$\text{PACI} = \text{PACE} + (\text{PACR})\,\text{HRT}/\text{SRT} \tag{1}$$

where PACI is the influent PAC concentration (mg/L), PACR is the mixed liquor PAC concentration in the reactor (mg/L), PACE is the effluent PAC concentration (mg/L), HRT is the hydraulic retention time (d), and SRT is the design solids retention time (d).

The value of PACE in Eq. (1) can be estimated by assuming that the carbon fraction in the effluent TSS (total suspended solids) is the same as the fraction of PAC in the MLSS (mixed liquor suspended solids).

PACT nitrification systems are normally selected when the municipal wastewater contains compounds originating from industrial operations, as stated previously. Nitrifiers are susceptible to a number of organic and inorganic inhibitors found in many industrial wastewaters

(45). Researchers have provided evidence that the addition of PAC to nitrifying activated sludge systems receiving industrial wastewaters improved nitrification rates (45–47). More recent studies have been completed with the goal of determining the mechanism of nitrification enhancement in PAC activated sludge systems in the presence of adsorbable and nonadsorbable inhibitors (48). The results indicated that the addition of the proper amount of PAC can completely nullify the toxic effects of an adsorbable nitrification inhibitor. A minor positive effect on nitrification rates was observed when PAC was added to a nitrifying activated sludge system receiving nonadsorbable inhibitors. The activated sludge used in these studies was not acclimated to the inhibiting compounds. Another possible contributing factor to the enhancement of nitrification could be attributed to the fact that the addition of PAC provides particulate matter for attachment of the nitrifying microorganisms, thereby promoting nitrification (49).

10.3. Process Equipment

PAC can be fed in the dry state using volumetric or gravimetric feeders or can be fed in slurry form. There are more than three major PAC producers, over 50 manufacturers of volumetric and gravimetric feeders, and over 50 manufacturers of slurry feeders (50–52). There are also many manufacturers of sequencing batch reactors (SBR) (33), dissolved air flotation (DAF) clarifiers (38), and membrane filtration (MF) reactors (37).

10.4. Process Limitations

The process limitations of PACT Process Systems are identical to that of the PAC physicochemical process. PACT process will increase the amount of generated sludge. Regeneration will be necessary at higher dosages in order to maintain reasonable costs. Most systems will require postfiltration to capture any residual carbon particles. Some sort of flocculating agent, such as an organic polyelectrolyte, is usually required to maintain efficient solids capture in the clarifier.

About one pound of dry sludge will be generated per pound of carbon added. If regeneration is practiced, carbon sludge is reactivated and reused with only a small portion removed to prevent buildup of inert material. PAC physicochemical process systems are reasonably reliable. In fact, PAC systems can be used to improve process reliability of existing systems.

Additional information on carbon adsorption and combined biological and physicochemical PACT process systems can be found in refs. (53–62).

11. CARRIER-ACTIVATED SLUDGE PROCESSES (CAPTOR AND CAST SYSTEMS)

There has been a substantial interest in recent years in the potential benefits of high biomass wastewater treatment. The major obstacle for achieving this has been the inability of biosolids separation in secondary clarifiers. For the most part, this has been overcome by using various forms of support media or carriers that have the ability to attach high concentrations of aerobic bacterial growth (63–65). The increase in immobilized biomass reduces the process dependence on secondary settling basins for clarification. In such hybrid systems, where

attached growth coexist with suspended growth, one gets more stable systems which possess the combined advantages of both fixed and suspended growth reactors.

11.1. Advantages of Biomass Carrier Systems

The performance of carrier systems is dependent on the amount of attached biomass, the characteristics of attached and suspended microorganisms, and the type of carriers. The advantages of such hybrid systems are (31):

(a) Heterogeneity of the microbial population. This is brought about by the differences in the microhabitat of organisms attached to the surface of a carrier and those in the bulk of the solution with respect to pH, ionic strength, and concentration of organics (66–70)
(b) Increased persistence in reactor. This leads to increase in biomass of organisms, reduction of hydraulic retention time and thus smaller reactor volumes (71–73)
(c) Higher growth rate (74–76)
(d) Increased metabolic activity. This leads to increase in respiration and substrate utilization, hence higher removal rates (77–80)
(e) Better resistance to toxicity (81–84)

11.2. The CAPTOR Process

One interesting concept of hybrid systems is the CAPTOR process developed jointly by the University of Manchester Institute of Science and Technology (UMIST) and Simon-Hartley, Ltd., in the United Kingdom. This high biomass approach uses small reticulated polyurethane pads as the bacterial growth medium (85). The pads are added to standard activated sludge aeration reactor, and the system is operated without sludge recycle, essentially combining suspended growth with a fixed film in one process. Excess growth is removed from the pads by periodically passing them through specially designed pressure rollers.

The British Water Research Centre (WRC) and Severn-Trent Water Authority conducted a full-scale evaluation of the CAPTOR process for upgrading the activated sludge plant at the Freehold Sewage Treatment Works, in the West Midlands area of England, to achieve year-round nitrification. This full scale study was jointly sponsored by the U.S. Environmental Protection Agency (86, 87).

11.3. Development of CAPTOR Process

As mentioned earlier, the CAPTOR process originated from research work on pure systems in the Chemical Engineering Department of UMIST. Single strands of stainless steel wire were woven into a knitted formation and then crushed into a sphere of about 6 mm (0.25 in.) diameter. These particles of known surface area were used for modeling liquid-fluidized bed systems. From this work derived the idea of using porous support pads for growing biomass at high concentrations that could be used in wastewater treatment systems. The idea was jointly developed and patented by UMIST and their industrial partner Simon-Hartley, Ltd. The present form of the CAPTOR process uses 25 mm × 25 mm × 12 mm (1 in. × 1 in. × 0.5 in.) reticulated polyether foam pads containing pores nominally of about 0.5–0.9 mm (0.02–0.035 in.) diameter and 94% free space (88–90).

11.4. Pilot-Plant Study

The conducted pilot-plant work indicated that it was possible to achieve the following (86, 87):

(a) Biomass concentrations of 7,000–10,000 mg/L
(b) Waste sludge concentrations of 4–6% dry solids using a special pad cleaner
(c) Improved oxygen transfer efficiencies, and
(d) High BOD volumetric removal rates

11.5. Full-Scale Study of CAPTOR and CAST

The full-scale evaluation of the CAPTOR process was undertaken at the Freehold Sewage Treatment Works near Stourbridge, West Midlands. The Freehold plant did not achieve any nitrification in the winter and only partial nitrification in the summer. Freehold's activated sludge system consisted of five trains equipped with tapered fine bubble dome diffusers arranged in a grid configuration. The system was modified as shown in Fig. 13.11 to split the wastewater flow into two equal volumes. Half went to two trains that were modified by adding CAPTOR pads to the first quarter of two aeration basins, and the other half went to two trains that remained unaltered and served as a control. The CAPTOR modified trains were each equipped with a CAPTOR pad cleaner (Fig. 13.12), and the CAPTOR pads were prevented from escaping into the remainder of the experimental system aeration basins by screens placed at the effluent ends of the CAPTOR zones.

The Simon-Hartley design predicted that, with a concentration of 40 pads/L, an annual average removal of 75% of the BOD_5 coming into the plant could be achieved in the CAPTOR zones, resulting in a reduced food-to-microorganism (F/M) loading on the follow-on activated sludge stage of 0.08 kg BOD_5/d/kg MLSS. With the reduced load, it was predicted that the modified system would achieve year-round nitrification with an effluent ammonia nitrogen concentration of 5 mg/L or less (87).

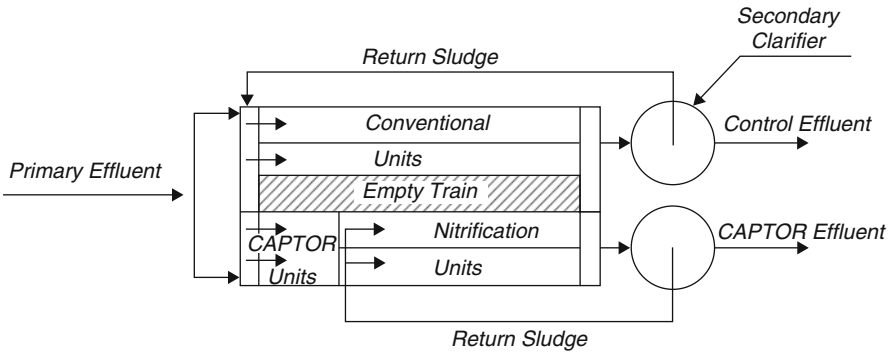

Fig. 13.11. Schematic of treatment plant showing incorporation of CAPTOR (87).

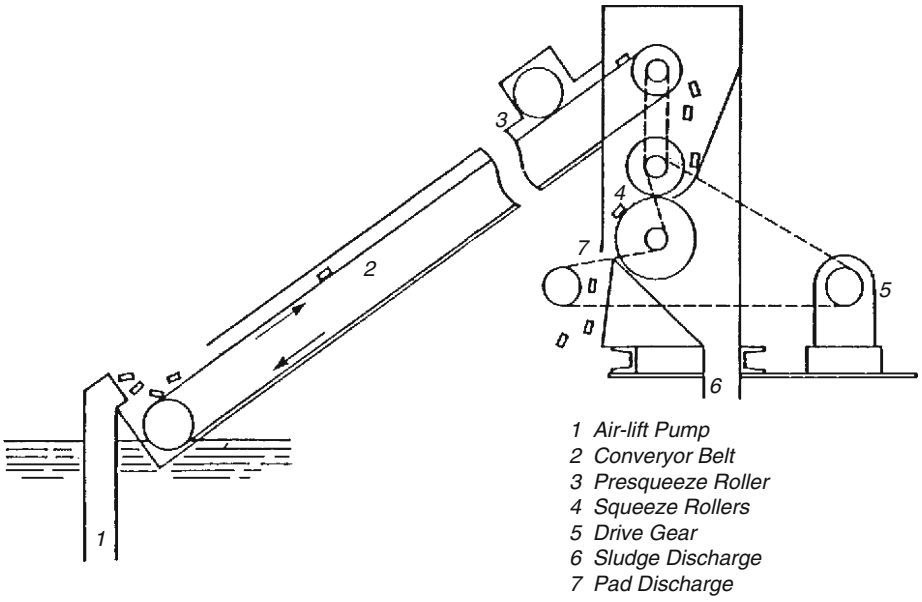

Fig. 13.12. CAPTOR pad cleaner (87).

11.5.1. Full-Scale Plant Initial Results

The Freehold modified CAPTOR activated sludge system was put in operation and immediately encountered a major problem. The CAPTOR pads floated on the surface of the tanks and would not become incorporated into the tank liquor. A solution was found by removing three of the seven longitudinal rows of fine bubble diffusers in the CAPTOR aeration basins. This was done to create a spiral roll in the tanks, which leads to areas of rising and falling liquid with quite large channels down, where the pads can fall. The spiral roll modification provided the necessary falling zone and produced complete mixing of the CAPTOR pads.

Another problem that occurred was mal distribution of the pads. The flow of wastewater tended to push the CAPTOR pads to the outlet of their zones, resulting in a concentration of 50–60 pads/L at the outlet and only 10–20 pads/L at the inlet end.

An other disturbing feature was the rapid deterioration in the CAPTOR pads. The CAPTOR pads used initially were black and were wearing at such a rate that they would not have lasted for more than 3 years, rendering the process uneconomical.

It had also become evident by this time that with the Freehold wastewater it would be possible to achieve the concentration of 200 mg biomass/pad predicted in the design. However, it was found that if the biomass was allowed to grow beyond 180 mg/pad, the biomass in the center of the pad became anaerobic. The control of pad biomass was difficult because the pad cleaners provided were not reliable and were situated at the CAPTOR zone inlets, while most of the pads gravitated to the outlet ends of the zones.

During this early period, while the above problems were being tackled on the full-scale plant, there were some occasions when the effluent from the CAPTOR units was reasonable

(BOD removals of 40–50%), but BOD removal never approached the average of 75% predicted based on the earlier pilot-plant results. Poor BOD removals were being experienced because the suspended solids concentration in the effluent was always high ($> 80\,mg/L$).

Consequently more pilot-scale studies were used to find solutions to the operating problems described above before attempting further full-scale evaluation at Freehold.

11.5.2. Pilot-Scale Studies for Project Development

It was decided to evaluate two variations of the CAPTOR process. The new variation differed from the original CAPTOR, in that the pads were placed directly into the mixed liquor of the activated sludge aeration tank rather than in a separate stage before the activated sludge tank. WRC named this process variation CAST (CAPTOR in activated sludge treatment). The CAST system had been applied to upgrade several overloaded wastewater treatment plants in Germany and France, and was found to be useful in improving the treatment efficiency and plants performance (91–93).

In addition, a single aeration tank filled with 40 CAPTOR pads/L was fed effluent from the above activated sludge control unit to assess the potential of CAPTOR as a second-stage nitrification process. Neither pad cleaning nor final clarification was necessary with this process variation because of the low sludge yields characteristic of nitrifier growth.

Studies were conducted using two well-mixed CAPTOR tanks in series. A range of loading and pad cleaning rates were used to evaluate process removal capabilities for CAPTOR. The intermediate effluent was used as a measure of process efficiency of the primary reactor and the final effluent for the entire system. This permitted plotting (Fig. 13.12) of % BOD_5 removal (total and soluble) vs. volumetric organic loading rate over the range of 1–3.5 kg $BOD_5/d/m^3$ (62–218 $lb/d/1,000\,ft^3$). High and low pad cleaning rates are differentiated in Fig. 13.13 as $\geq 16\%$ and $< 16\%$ of the total pad inventory/d, respectively (87).

Total BOD_5 removal efficiency was less than soluble BOD_5 removal efficiency because of the oxygen demand exerted by the biomass solids lost in the process effluent. The higher pad cleaning rates are believed to have contributed to the improved total and soluble BOD removals shown in Fig. 13.13, although low bulk liquid DO's may have adversely affected removals on some of the low cleaning runs. Low cleaning rates ($< 16\%/d$) were detrimental to soluble BOD_5 removal efficiency because of a gradual decline in activity of the biomass remaining in the pad. Cleaning rates greater than 24%/day, however, resulted in reduced biomass levels in the pads and a reduction in performance.

The problem of mal distribution of CAPTOR pads in the aeration tank (i.e., crowding of pads into the effluent end of the tank when operated in plug flow fashion as at Freehold) was solved by modifying the flow pattern to transverse flow (across the width of the tank rather than down the length). When implemented later at Freehold, this pattern resulted in a fourfold decrease in flow velocity.

Several mixing intensities and diffuser arrangements were tried to decrease biomass shedding into the process effluent. It became obvious, however, that production of effluent biomass solids was not significantly affected by changes in mixing intensity or diffuser arrangement.

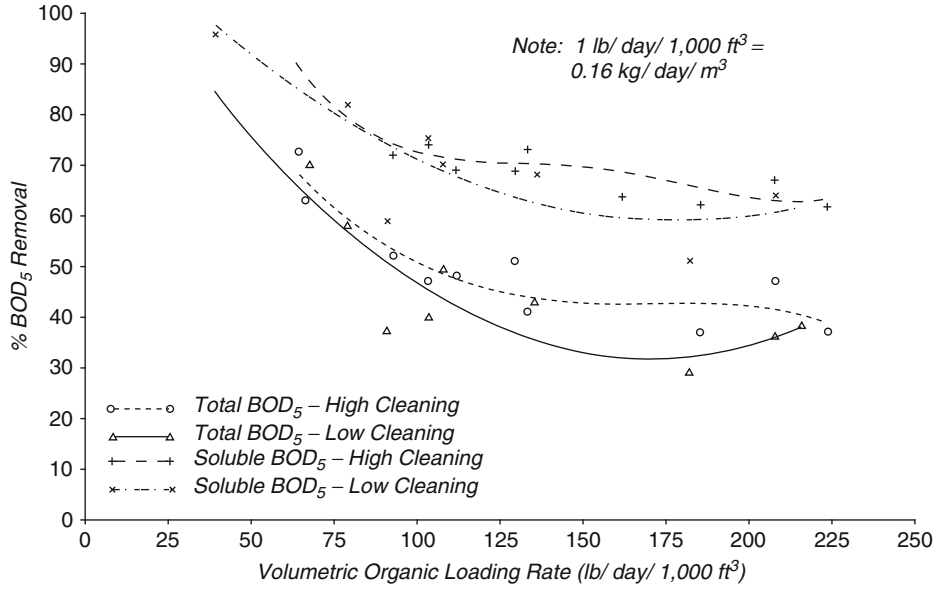

Fig. 13.13. Pilot-scale CAPTOR BOD_5 removals as a function of organic loading rate (87).

High effluent suspended solids proved to be far more dependent on pad cleaning rate, biochemical activity of the biomass, and biomass growth directly in the liquor.

Using the transverse flow scheme and a regular pad cleaning regimen, CAPTOR process performance was similar to that experienced in the small tanks. Operating parameters and process performance are summarized in Table 13.2 for two different volumetric loading rates (87).

Respiration studies conducted on pads indicated that biomass held within the pads respires at up to 40–50% less than equivalent biomass in free suspension. Any increase in net biomass concentration achieved in a CAPTOR reactor above that in a conventional activated sludge reactor may not produce noticeable benefits, therefore, due to the lower specific activity. These observations suggest that diffusion limitations were occurring in the CAPTOR pads.

The CAST variation of CAPTOR was operated in conjunction with a final clarifier to settle the mixed liquor solids component of the total biomass inventory and return it to the aeration tank. CAPTOR pads and biomass retained therein were kept in the reactor by screens. Operating and performance data are compared in Table 13.3 for the CAST unit and the parallel activated sludge control unit for a 25-day period when the volumetric loadings and hydraulic residence times (HRTs) for both units were identical.

In the nitrification experiments conducted on the CAPTOR process, the biomass concentrations per pad ranged from 99 to 124 mg. This is within the range of 100–150 mg/L reported by other researchers (94). With a pad concentration of 40/L, equivalent MLSS levels varied from 3,960 to 4,960 mg/L. Liquor DO concentrations were maintained between 6.4 and 8.4 mg/L, and liquor temperature ranged from 11.50 to 6.5°C.

Table 13.2
Pilot-scale operating conditions and process performance (87)

Parameter	Period			
	1		2	
Volumetric loading ($lbBOD_5/d/1,000\,ft^3$)[a]	113		213	
HRT(h)	2.32		1.52	
Pads/L	40		40	
Biomass/pad(mg)	121		126	
Equivalent MLSS (mg/L)	4, 840		5, 040	
F/M loading (kg BOD_5/d/kg MLSS)	0.37		0.68	
SRT (days)	3.23		1.72	
DO (mg/L)	4.2		4.7	
	In	Out	In	Out
Total BOD_5 (mg/L)	175	93	216	129
Soluble BOD_5 (mg/L)	86	24	85	33
SS (mg/L)	116	120	178	160
Total BOD_5 removal (%)	47		40	
Soluble BOD_5 removal (%)	72		61	
SS removal (%)	−3		10	

[a] $1\,lb/d/1{,}000\,ft^3 = 0.016\,kg/d/m^3$

Secondary effluent from the control activated sludge pilot unit used in the CAST experiments was applied to the nitrification reactor over a range of loading conditions. Essentially, complete nitrification was achieved at TKN and ammonia nitrogen loadings of approximately $0.25\,kg/d/m^3$ ($15.6\,lb/d/1,000\,ft^3$) and $0.20\,kg/d/m^3$ ($12.5\,lb/d/1,000\,ft^3$), respectively.

11.5.3. Full-Scale Plant Results After Modifications

Following the successful testing of the transverse mixing arrangement in the pilot-scale study, the two Freehold CAPTOR trains were modified. The modifications involved the following (87):

(a) Splitting each of the CAPTOR trains, C1 and C2, into two compartments, C1A and C1B and C2A and C2B, as shown in Fig. 13.14
(b) Feeding influent flow along long weirs at the side of the trains instead of at the narrow inlet ends
(c) Modifying the aeration pipe work to place all three rows of dome diffusers directly below the outlet screens (covering about 25% of the width of the tanks), thereby creating a spiral roll of pads and liquid counter-current to the flow of wastewater entering along the weirs on the sidewalls
(d) Installing two extra pad cleaners so that each CAPTOR subunit was provided with a cleaner, and

Table 13.3
Pilot-scale CAST and activated sludge operating conditions and performance (87)

Parameter	System			
	CAST		Activated sludge	
Volumetric loading (lb BOD_5/d/1,000 ft^3)[a]	148		148	
HRT (h)	1.8		1.8	
Pads/L	34		–	
Biomass/pad (mg)	116		–	
Equivalent MLSS in pads (mg/L)	3,930		–	
MLSS in suspension (mg/L)	3,720		6,030	
Total MLSS (mg/L)	7,650		6,030	
F/M loading (kg BOD_5/d/kg total MLSS)	0.31		0.39	
SRT, based on total MLSS (days)	3.6		3.0	
DO (mg/L)	2.5		3.0	
	In	Out	In	Out
Total BOD_5 (mg/L)	178	12	178	20
Soluble BOD_5 (mg/L)	101	5	101	4
SS (mg/L)	121	15	121	23
Total BOD_5 removal (%)	93		89	
Soluble BOD_5 removal (%)	95		96	
SS removal (%)	88		81	

[a] 1 lb/d/1,000 ft^3 = 0.016 kg/d/m^3

(e) Installing fine screens at the outlet from the primary clarifiers to reduce the quantity of floating plastic material entering the CAPTOR units that created problems with the cleaners

The objective of the first three modifications was to achieve uniform mixing of the pads in the CAPTOR units and prevent the situation that had occurred previously where high concentrations of pads (50–60 pads/L) collected at the outlet end and very low concentrations (10–20 pads/L) at the inlet end. Pads were removed from the tanks during the modifications. After the modifications were completed, the number of pads in each compartment was equalized at about 35/L.

The changes were completely successful in obtaining uniform distribution and complete mixing of the CAPTOR pads. A lithium chloride tracer test conducted on the modified tanks indicated that no dead zone was occurring in the "eye" of the roll. Formation of floating pad rafts (which had occurred at the outlet end of the tank with the original arrangement) was completely eliminated. The modifications, however, had no effect on the high level of suspended solids present in the liquor. The modified CAPTOR system was operated at an

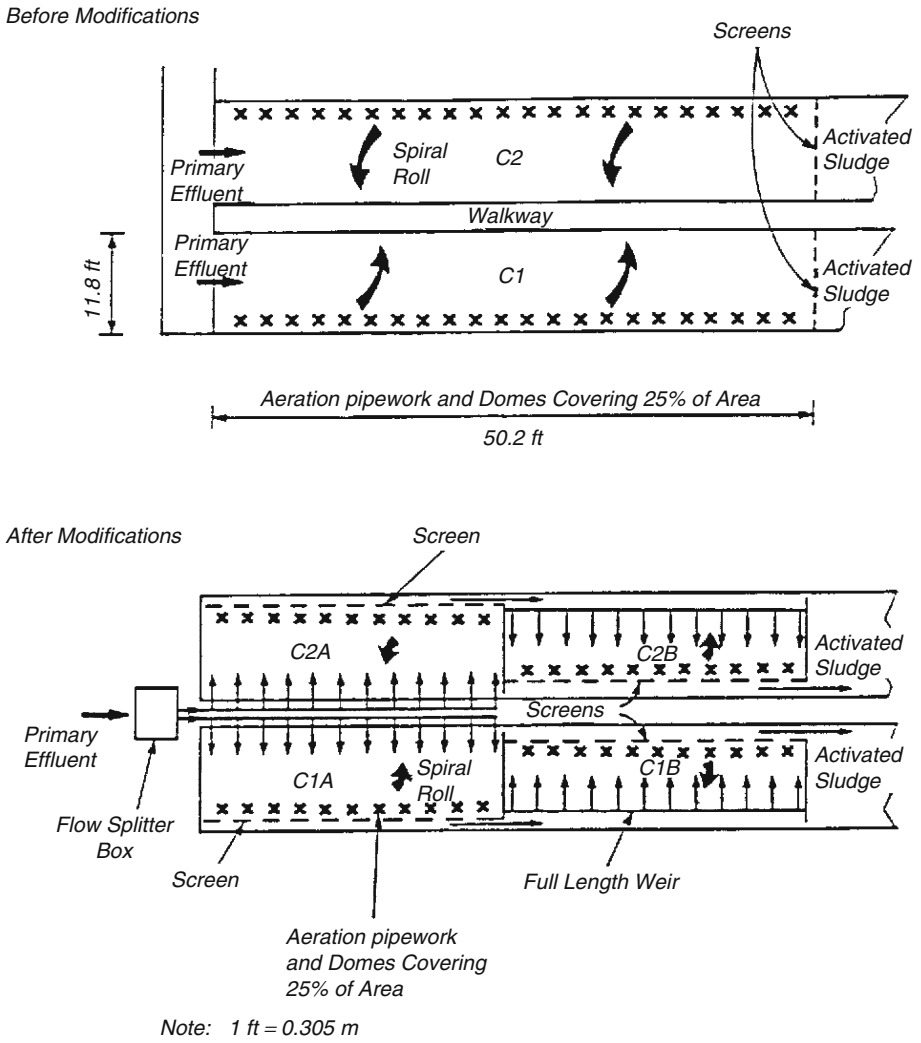

Fig. 13.14. Modifications to full-scale CAPTOR system flow pattern (87).

average volumetric loading rate of 1.24 kg $BOD_5/d/m^3$ (77 lb/d/1, 000 ft^3), an average HRT (excluding sludge recycle) of 2.55 h and an overall biomass concentration of 4,830 mg/L.

The CAST variation of the CAPTOR process, which had exhibited somewhat better performance than conventional activated sludge in the small tank experiments, was also field evaluated at Freehold. The CAPTOR trains were further modified so that return sludge could be introduced to the CAPTOR zones (35 pads/L), providing an activated sludge component throughout the entire aeration tanks, not just in the nitrification stage. The average volumetric organic loadings and HRTs (excluding sludge recycle) were 1.11 kg $BOD_5/d/m^3$ (69 lb/d/1,000 ft^3) and 3.40 h, respectively.

Table 13.4
Full-scale modified CAPTOR performance results (87)

Parameter	Influent, mg/L	Effluent, mg/L	Removal, %
Total BOD_5	128	22	83
Soluble BOD_5	40	4	90
SS	138	32	77
NH_4-N	24	24.4	0

Table 13.5
Full-scale modified CAST performance results (87)

Parameter	Influent, mg/L	Effluent, mg/L	Removal, %
Total BOD_5	138	16	88
Soluble BOD_5	56	2	96
SS	120	27	78
NH_4-N	26.7	17.2	36

Performance data summarized in Tables 13.4 and 13.5 indicate that the CAST system exhibits somewhat better performance than the CAPTOR version. In the CAST process, the removal of soluble BOD_5 is 96% compared to 90% in CAPTOR; the removal of total BOD_5 is 88% compared to 83%; and the removal of SS is about the same at about 78%.

11.5.4. Overall Conclusions

The US EPA conclusions and recommendations for the CAPTOR/CAST treatment systems are as follows (86, 87, 95):

(a) In the initial phase, when the CAPTOR process was installed at the Freehold Sewage Treatment Works, several problems were immediately evident. There were major problems with respect to pad mixing, suspension, and distribution, and the process performance was adversely affected by the high-level of suspended solids in the CAPTOR stage effluent. The problems of pad mixing and distribution were solved by pilot- and full-scale development work
(b) The performance of the CAPTOR process was still adversely affected by the high level of suspended solids in the CAPTOR stage effluent after correction of the pad mixing, suspension, and distribution problems. This prevented the achievement of nitrification in the follow-on activated sludge stage
(c) The presence of CAPTOR pads in the tank liquid did not improve oxygen transfer efficiency
(d) The durability of the CAPTOR pads was solved by switching to different pads
(e) The peak biomass concentration in the pads is unpredictable. It does not appear to be related to the BOD concentration of the wastewater. There were indications in the various studies, however, that the frequency of pad cleaning (and, hence, the biomass/pad concentration) was critical to the performance of the process. Regular pad cleaning is essential to prevent anaerobic conditions from developing in the pads
(f) It is possible to raise the biomass concentration in a CAPTOR stage to 6,000–8,000 mg/L, but the respiration rate of the biomass in the pads is lower than the respiration of the same biomass if freely suspended and less than that of normal activated sludge. These data suggest that the

geometry of the CAPTOR pads results in diffusion limitations, which demands further pad design improvement to enhance the potential for economic utilization of the CAPTOR process in wastewater treatment

(g) The CAST variation of the CAPTOR process performs well
(h) CAPTOR has the potential as an add-on package for tertiary nitrification
(i) The CAPTOR option was projected to be more cost effective than extending the activated sludge plant for upgrading Freehold to complete year-round nitrification.
(j) For CAPTOR and CAST to achieve their full potential, as predicted by the pilot-scale studies, further design development and improvements are needed.

12. ACTIVATED BIO-FILTER

12.1. Description

Activated bio-filters (ABF) are a recent innovation in the biological treatment field. This process consists of the series combination of an aerobic tower (biocell) with wood or other packing material, followed by an activated sludge aeration tank and secondary clarifier. Settled sludge from the clarifier is recycled to the top of the tower. In addition, the mixture of wastewater and recycle sludge passing through the tower is also recycled around the tower, in a similar manner to a high rate trickling filter (31). No intermediate clarifier is utilized. Forward flow passes directly from the tower discharge to the aeration tank (Fig. 13.15). The use of the two forms of biological treatment combines the effects of both fixed and suspended growth processes in one system. The microorganisms formed in the fixed growth phase are passed along to the suspended growth unit, whereas the suspended growth microorganisms are recycled to the top of the fixed media unit (96). This combination of the two processes results in the formation of a highly stable system that has excellent performance and good settling biological floc when treating wastewaters that have variable loads (97).

The biomedia in the biocell consists of individual racks made of wooden laths fixed to supporting rails. The wooden laths are placed in the horizontal direction, permitting wastewater to pass downward, and air horizontally and vertically. The horizontal surfaces reduce premature

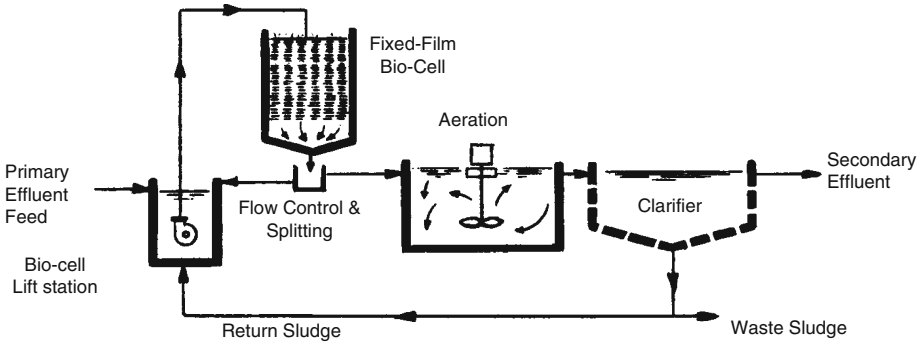

Fig. 13.15. ABF process flow diagram (96).

sloughing of biota. Droplet formation and breakup induced by wastewater dripping from lath to lath enhances oxygen transfer. Other types of material for the biomedia have also been reported by other researchers and equipment manufacturers (98–101). The aeration basin is a short detention unit that can be designed for either plug flow or complete mix operation. The effluent from the aeration basin passes to a secondary clarifier where the activated sludge is collected and recycled to the top of the biocell tower and to waste.

ABF units can be used for the removal of either carbonaceous material or for carbonaceous removal plus nitrification by appropriately modifying the detention time of the aeration basin. When nitrification is desired, the biocell acts as a first-stage roughing unit and the aeration basin as a second-stage nitrification unit (102, 103). ABF biocells can be either rectangular or round. Various types of aeration equipment can be used in the aeration system, including both surface and diffused aerators. The detention time of the aeration tank can be modified, depending on influent quality and desired effluent quality. ABF units can be supplied with mixed media effluent filters for enhanced treatment.

12.2. Applications

Activated bio-filters can be used for treating municipal wastewater and biodegradable industrial wastewater. ABF systems are especially useful where (96, 97):

(a) Both BOD_5 removal and nitrification are required
(b) Land availability is low
(c) Raw wastewater organic loadings fluctuate greatly, due to its ability to handle shock conditions
(d) Existing trickling filter facilities and overloaded existing secondary plants need to be upgraded at reduced cost

A typical ABF application is the Burwood Beach Wastewater Treatment Works in Australia (104). The plant was upgraded in the 1990s using ABF at a cost of $48M. The facility currently serves a population of 180,000 with a flow of 43ML a day, and has the capacity to treat 53 ML/day for a population of 220,000 in the year 2020. The Biofilter is 30 m in diameter and has a design organic loading of 3.2 kg $BOD_5/m^3/d$. The aeration tank is designed for 1.5 h of hydraulic detention time. The plant has been in operation for around 10 years producing an effluent that is consistently within the required US EPA set limits.

12.3. Design Criteria

The design criteria for the ABF system are reported to be as follows (96, 105, 106):

(a) Biocell organic load: 100–200 lb $BOD_5/d/1,000\,ft^3 = 1.6-3.2$ kg $BOD_5/d/m^3$
(b) Return sludge rate: 25–100%
(c) Biocell recycle rate: 0–100%
(d) Biocell hydraulic load: 1–5.5 $gpm/ft^2 = 40.7-224$ Lpm/m^2
(e) Aeration basin detention time: 0.5–3.0 h for BOD_5 removal only, 5.8–7.5 h for two-stage nitrification
(f) System F/M: 0.25–1.5 lb $BOD_5/d/lb$ MLVSS for BOD removal, 0.18 lb $BOD_5/d/lb$ MLVSS for two-stage nitrification. Here 1 lb/d/lb = 1 kg/d/kg.

Table 13.6
Performance of BAF systems (96)

Parameter	Influent, mg/L	Effluent, mg/L	Removal, %
BOD_5	153	14	91
COD	330	58	82
TSS	222	20	91
NH_4-N[a]	20	1	90

[a] When used for nitrification.

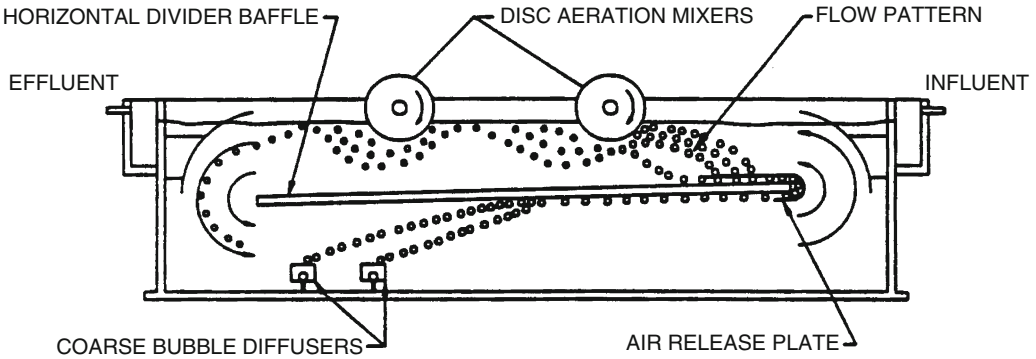

Fig. 13.16. Diagram of the vertical loop reactor (108, 109).

12.4. Performance

ABF systems are quite stable and highly reliable. They can treat standard municipal, combined municipal/industrial, or industrial wastewaters to BOD_5 and suspended solids levels of 20 mg/L or less. Test study on a package system showed at least 90% removal of BOD_5, TSS and NH_4–N (96). The detailed results are shown in Table 13.6.

Sludge production was reported at 0.25–1.0 lb of waste VSS per lb of BOD_5 removed. The mean yield, over the course of the study, was 0.60 lb VSS per lb of BOD removed. Here 1 lb/lb = 1 kg/kg.

13. VERTICAL LOOP REACTOR

13.1. Description

A vertical loop reactor (VLR) is an activated sludge biological treatment process similar to an oxidation ditch (107, 108). The wastewater in an oxidation ditch circulates in a horizontal loop; the water in a VLR circulates in a vertical loop around a horizontal baffle, as shown in Fig. 13.16 (109). A typical VLR consists of an 18 ft deep concrete or steel basin, with a horizontal baffle extending the entire width of the reactor and most of its length. Operating basins are reported to have side-wall depths which range from approximately 10–22 ft (3.05–6.71 m) (110). The length and width of the VLR are determined by the required capacity but, as a rule, the length is at least twice the width. The baffle is generally 5–11 ft (1.52–3.35 m)

below the surface of the water. Because a VLR is typically deeper than an oxidation ditch, the VLR requires less land area (31).

Aeration in a VLR is provided by coarse bubble diffusers, which are located below the horizontal baffle and by disc aeration mixers. The disc mixers also circulate the wastewater around the baffle at a velocity of 1–1.5 ft/s (0.30–0.46 m/s) (111). Because the diffusers are positioned below the baffle, the air bubble residence time in a VLR is as much as six times longer than the bubble residence time in a conventional aeration system. This extended bubble contact time increases the process aeration efficiency. Denitrification in an anoxic zone also reduces oxygen requirements.

The VLR process is usually preceded by preliminary treatment such as screening, comminution or grit removal. Secondary settling of the VLR effluent is typically provided by a separate clarifier. An intrachannel clarifier may be used for secondary settling in place of a separate clarifier.

Vertical loop reactors may be operated in parallel or series. When a series of VLRs are used, the dissolved oxygen profile can be controlled to provide nitrification, denitrification, and biological phosphorus removal at hydraulic detention times of 10–15 h.

13.2. Applications

VLR technology is applicable in any situation where conventional or extended aeration activated sludge treatment is appropriate. The technology is applicable for nitrification and denitrification. Biological phosphorus removal may be incorporated in the system design. Power costs may be lower for a VLR system than for other aerated biological treatment systems, due to improved oxygen transfer efficiency. There are currently more than ten municipal wastewater treatment facilities in the United States with VLRs. One such example is the City of Willard, OH waste water treatment plant (112). The facility is designed for an average daily flow of 4.5 MGD (17.03 MLD), and is capable of handling a peak flow of 7.2 MGD (27.25 MLD).

The following advantages have been reported for VLR systems (113):

(a) Land area required for VLRs is about 40% less than for oxidation Ditches
(b) The VLR aeration basin cost is about 30% less than for oxidation ditches
(c) The multiple tank basin series arrangement is an advantage for facilities with highly variable flow
(d) VLRs are useful for retrofitting existing basins for plant upgrade to suit increased flows or more stringent effluent requirements

13.3. Design Criteria

The design criteria for the VLR process are reported to be as follows (107):

- BOD loading: 14–22 lb $BOD_5/1,000\,ft^3/d = 224-353$ g $BOD_5/d/m^3$
- SRT: 17–36 d
- Detention time: 12–24 h

13.4. Performance

The average effluent BOD_5 and TSS concentrations for the five studied operating VLR facilities are 4.2 and 7.1 mg/L, respectively. The average effluent ammonia concentration is 0.8 mg/L. Only one of the VLRs studied was designed for biological phosphorus removal; the average effluent phosphorus concentration for this plant was 1.45 mg/L, and alum was added in the final clarifiers. A second VLR facility was not designed for biological phosphorus removal but was required to monitor phosphorus. This plant had an average effluent phosphorus concentration of 2.19 without any chemical addition.

The VLR system is quite reliable. Table 13.7 indicates the percent of time the monthly average effluent concentration of the given pollutants was less than the concentration given in the first column. No significant difference in results was observed between winter and summer data.

13.5. EPA Evaluation of VLR

The following summarizes the major findings and conclusions of US EPA evaluation of VLRs (108). The information is based on analysis of available information from site visits, a detailed design of a full scale VLR system, and information from consultants and manufacturers.

(a) The VLR is a modification of the conventional activated sludge process. The unique features of the process are circulating mixed liquor around a horizontal baffle with a dual aeration system, bubble diffused air beneath the horizontal baffle, and disc aerators at the surface of the aeration tank. The process operates as a plug flow reactor with capability for varying dissolved oxygen profiles to achieve biological, phosphorus and nitrogen removal. The VLR process also features a stormwater by-pass design for treatment of high peak to average flows

(b) There are currently over ten operating VLRs in the U.S. ranging in size from 0.22 to 5.0 MGD (1.06–219 L/s)

(c) Performance data from operating VLRs show that this process is capable of achieving effluent carbonaceous biochemical oxygen demand levels of less than 10 mg/L; effluent total suspended

Table 13.7
Reliability of the VLR treatment process (107)

Concentration, mg/L	BOD[a]	NH_3-N[a]	TSS[a]	P[a]
0.2	0	30	0	2
0.5	0	63	1	10
1.0	0	83	1	24
2.0	20	88	5	63
3.0	71	95	43	93
10.0	97	96	75	100
20.0	100	100	96	100
Number of plants	5	5	5	1

[a] Percentage of time the monthly average concentration of the pollutant was less than the stated value in the first column.

solids levels of less than 10 mg/L; and effluent ammonia-nitrogen levels of less than 1.0 mg/L. The process is further capable of achieving total nitrogen and phosphorus removals of 60–80%

(d) The VLR process is applicable for flows ranging from 0.05 to over 10 MGD (2.19 to over 438 L/s)

(e) The claimed advantages of this process by the manufacturer include the following:
- Higher dissolved oxygen transfer than conventional equivalent technology
- Improved response to peak flows due to a stormwater bypass feature
- A credit for oxygen release due to denitrification with the credit based on 80% denitrification
- Increased mixed liquor settleability and process stability

(f) The design criteria for the existing VLRs are conservative. HRTs range from 11.9 to 24 h. Volumetric loading ranged from 13.6 to 23.1 lb CBOD/1,000 ft^3 (218 to 370 g CBOD/d/m^3). This loading is similar to that used for extended aeration systems and is about 1/3 to 1/2 of that normally used for conventional activated sludge designs

(g) The VLR technology has been designated as Innovative Technology by the US EPA for three plants due to a 20% claimed energy savings

(h) Based on this assessment, the 20% energy savings over competing technology could not be verified

(i) The VLR was compared to oxidation ditches as "Equivalent Technology." The results of this comparison indicated:
- The VLR technology produces comparable to slightly improved effluent levels of BOD, TSS and NH_3-N than oxidation ditch plants
- Total removal of phosphorus and total nitrogen are equivalent to oxidation ditches designed for the same level of treatment
- The energy requirements for aeration were found to be similar to 10% less than for oxidation ditches
- The land area required for VLRs was found to be approximately 40% less than for oxidation ditches based on equivalent aeration tank loadings
- The VLR aeration basin cost was found to be approximately 30% less than for oxidation ditches for situations where rock excavation is not required for the deeper VLR basin
- A definitive comparison of total VLR plant costs to total oxidation plant costs could not be made. Data submitted from both manufacturers indicated a comparable cost for plants in the 0–2 MGD (0–87.6 L/s) range. The reported VLR costs at plants ranging from 2 to 10 MGD (87.6 to 438 L/s) were significantly less than oxidation ditch plant costs. This would be expected because of the modular design and common wall construction of the VLR compared to oxidation ditches
- The total operation and maintenance costs of the two technologies were found to be similar

13.6. Energy Requirements

The VLR energy requirements are shown in Fig. 13.17. The requirements are based on the following assumptions (107):

(a) Water Quality
Influent BOD_5 = 200 mg/L, Effluent = 20 mg/L
Influent TKN = 35 mg/L, Effluent = 1 mg/L

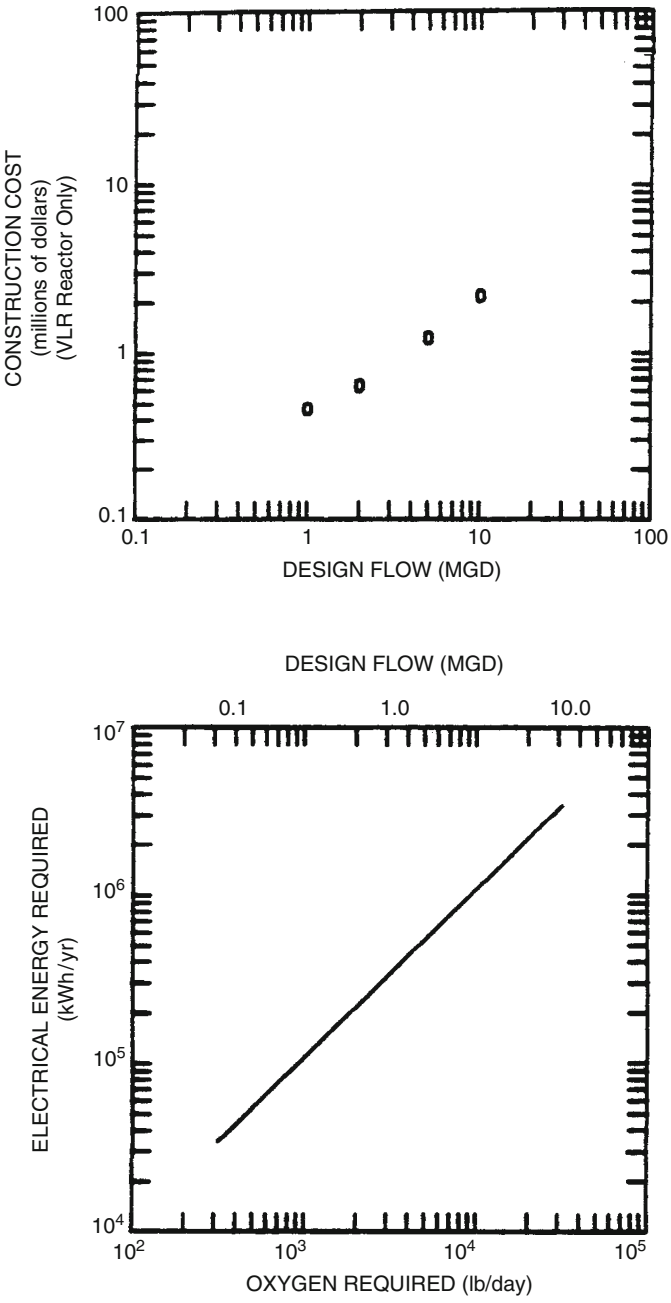

Fig. 13.17. VLR energy requirements and construction cost (107, 108). 1 MGD = 3.785 MLD = 43.8 L/s; 1 lb = 453.6 g

(b) Design Basis
Oxygen transfer efficiency = 2.5 lb O_2/HP hour = 1.52 kg/kWh
Nitrification occurs
(c) Operating Parameters
Oxygen Requirement = 1.5 lb O_2/lb BOD_5 removed, 4.57 lb O_2/lb TKN removed (1 lb/lb = 1 kg/kg)
(d) Type of energy: Electrical

13.7. Costs

Construction costs (1991 Dollars, Utilities Index = 392.35) for VLR are shown in Fig. 13.17. To obtain the values in terms of the present 2009 U.S. Dollars, using the Cost Index for Utilities (Appendix), multiply the costs by a factor of 570.38/392.35 = 1.45 (114). The operation costs are similar to oxidation ditch type treatment plant.

14. PHOSTRIP PROCESS

14.1. Description

"PhoStrip" is a combined biological–chemical precipitation process based on the use of activated sludge microorganisms to transfer phosphorus from incoming wastewater to a small concentrated substream for precipitation. As illustrated in Fig. 13.18, the activated sludge is subjected to anoxic conditions to induce phosphorus release into the substream and to provide phosphorus uptake capacity when the sludge is returned to the aeration tank. Settled wastewater is mixed with return activated sludge in the aeration tank. Under aeration, sludge microorganisms can be induced to take up dissolved phosphorus in excess of the amount required for growth. The mixed liquor then flows to the secondary clarifier where liquid effluent, now largely free of phosphorus, is separated from the sludge and discharged. A portion of the phosphorus-rich sludge is transferred from the bottom of the clarifier to a thickener-type holding tank: the phosphate stripper. The settling sludge quickly becomes anoxic and, thereupon, the organisms surrender phosphorus, which is mixed into the supernatant. The

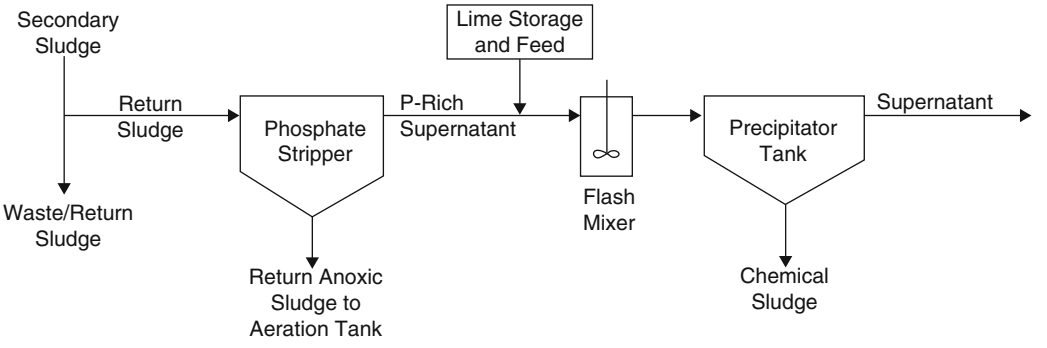

Fig. 13.18. PhoStrip process flow diagram (96).

phosphorus-rich supernatant, a low volume, high concentration substream, is removed from the stripper and treated with lime for phosphorus precipitation. The thickened sludge, now depleted in phosphorus, is returned to the aeration tank for a new cycle (96).

The PhoStrip process has demonstrated a compatibility with the conventional activated sludge process and is compatible with its modifications. The process can operate in various flow schemes, including full or Split flow of return activated sludge through the phosphate stripper, use of an elutriate to aid in the release of phosphorus from the anoxic zone of the stripper, or returning lime-treated stripper supernatant to the primary clarifier for removal of chemical sludge.

This technique is a new development in municipal wastewater treatment and has been demonstrated in pilot plant and full-scale studies. Notable large scale evaluations have been conducted at Seneca Falls, New York and, more recently, Reno/Sparks, Nevada. Nearly a dozen commercial installations are reported to be in the operational phase (31).

14.2. Applications

This method, which involves a modification of the activated sludge process, can be used in removing phosphorus from municipal wastewaters to comply with most effluent standards. Direct chemical treatment is simple and reliable, but it has the two disadvantages of significant sludge production and high operating costs. The PhoStrip system reduces the volume of the substream to be treated, thereby reducing the chemical dosage required, the amount of chemical sludge produced, and associated costs. Lime is used to remove phosphorus from the stripper supernatant at lower pH levels (8.5–9.0) than normally required. The cycling of sludge through an anoxic phase may also assist in the control of bulking by the destruction of filamentous organisms to which bulking is generally attributed (96).

On the negative side, it should be pointed out that more equipment and automation, along with a greater capital investment, are normally required than for conventional chemical addition systems. Since this method relies on activated sludge microorganisms for phosphorus removal, any biological upset that hinders uptake ability will also affect effluent concentrations. It has been found that sludge in the stripper tank is very sensitive to the presence of oxygen. Anoxic conditions must be maintained for phosphorus release to occur.

14.3. Design Criteria

The fraction of the total sludge flow that must be processed through the stripper tank is determined by the phosphorus concentration in the influent wastewater to the treatment plant and the level required in the treated effluent. Required detention time in the stripper tank ranges from 5 to 15 h. Typical phosphorus concentrations produced in the stripper are in the range of 40–70 mg/L. The volume of the phosphorus-rich supernatant stream to be lime treated is 10–20% of the total flow (96). Typical design criteria for the PhoStrip process are shown in Table 13.8 (105)

Table 13.8
Typical design criteria for the PhoStrip process (96)

Design parameter	Unit	Value
Food-to-microorganisms ratio (F/M)*	lb BOD/lb MLSS/d	0.3–0.5
Solids retention time (SRT)	d	10–30
Mixed liquor suspended solids (MLSS)	mg/L	600–5,000
Hydraulic retention time in stripper (t)	h	8–12
Hydraulic retention time in aeration tank (t)	h	4–10
Return activated sludge (RAS)	% of influent	20–50
Internal recycle (stripper underflow)	% of influent	10–20

* 1 lb/lb/d = 1 kg/kg/d

14.4. Performance

Pilot and full-scale studies of the process have shown it to be capable of reducing the total phosphorus concentration of typical municipal wastewaters to 1.5 mg/L (105), or even to 0.5 mg/L or less (106). A plant-scale evaluation of the method treating 6 MGD (262.8 L/s) of municipal wastewater at the Reno/Sparks Joint Water Pollution Control Plant in Nevada demonstrated satisfactory performance for achieving greater than 90% phosphorus removal. Results showed that the process enhanced the overall operation and performance of the activated sludge process, since it produced a more stable, better settling sludge. Regular maintenance of mechanical equipment, including pumps and mixers, is necessary to ensure proper functioning of entire system.

14.5. Cost

14.5.1. Construction Cost

Construction costs (1980 Dollars, Utilities Index = 277.60) for PhoStrip are shown in Fig. 13.19. To obtain the values in terms of the present 2009 U.S. Dollars, using the Cost Index for Utilities (Appendix), multiply the costs by a factor of 570.38/277.60 = 2.05 (114). Construction costs include: stripper (10 h detention time at 50% of return sludge); flash mixer; flocculator/clarifier; thickeners; lime feed and storage facilities (96).

14.5.2. Operation and Maintenance Cost

The electrical energy required for operation of pumps, lime mixing equipment, and clarifiers, is shown in Fig. 13.11. Operation and maintenance costs (1980 Dollars, Utilities Index = 277.60) for PhoStrip are shown in Fig. 13.20. To obtain the values in terms of the present 2009 U.S. Dollars, using the Cost Index for Utilities (Appendix), multiply the costs by a factor of 570.38/277.60 = 2.05 (114). Operation and maintenance costs include: labor for operation, preventive maintenance, and minor repairs; materials to include replacement parts and major repair work; lime and power costs based on the electrical energy requirement shown in Fig. 13.21 (96).

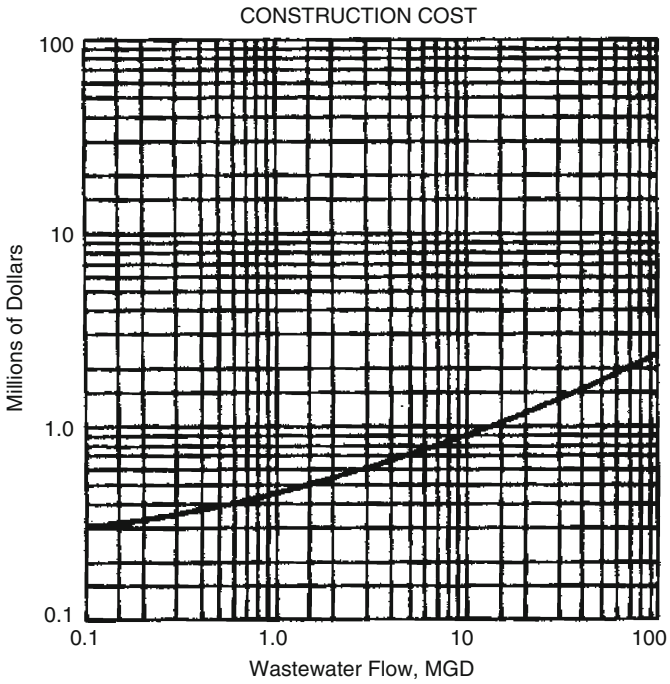

Fig. 13.19. PhoStrip construction cost (96). 1 Mgal/d = 1 MGD = 3.785 MLD = 43.8 L/s

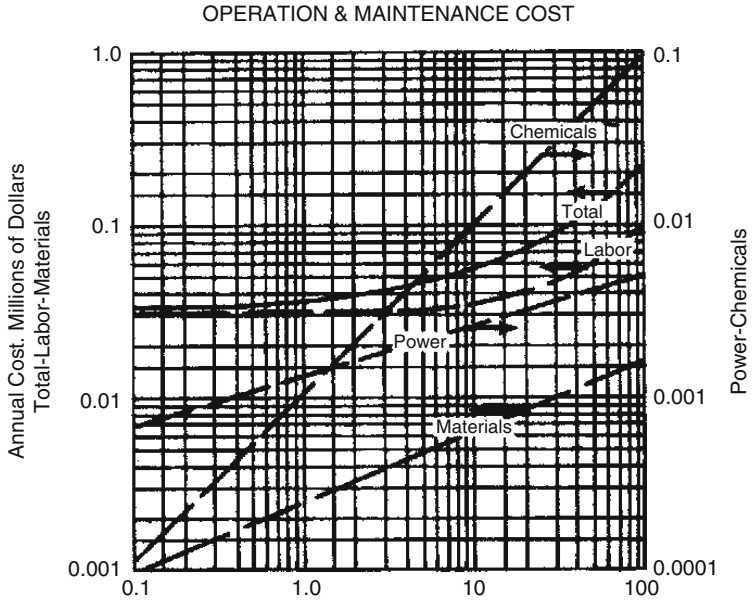

Fig. 13.20. PhoStrip operation and maintenance cost (96). 1 Mgal/d = 1 MGD = 3.785 MLD = 43.8 L/s

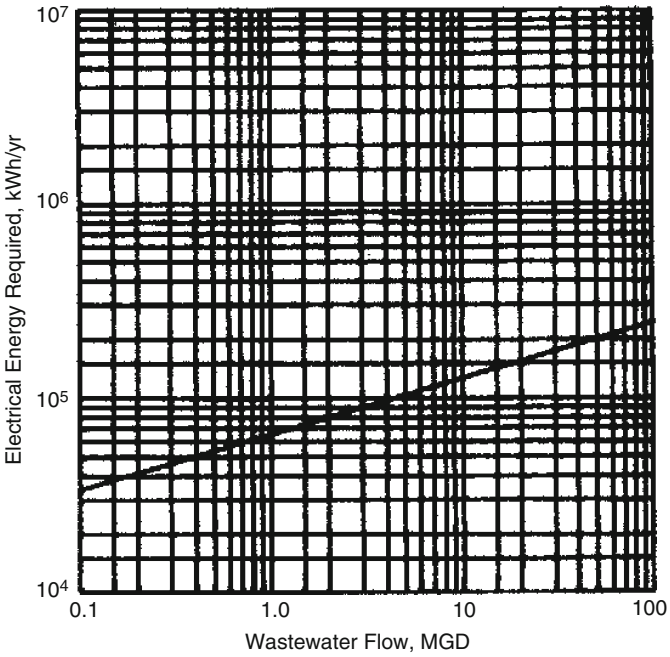

Fig. 13.21. PhoStrip electrical energy requirement (96). 1 Mgal/d = 1 MGD = 3.785 MLD = 43.8 L/s

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APPENDIX

U.S. Army Corps of Engineers Civil Works Construction Yearly Average Cost Index for Utilities (114))

Year	Index	Year	Index
1967	100	1989	383.14
1968	104.83	1990	386.75
1969	112.17	1991	392.35
1970	119.75	1992	399.07
1971	131.73	1993	410.63
1972	141.94	1994	424.91
1973	149.36	1995	439.72
1974	170.45	1996	445.58
1975	190.49	1997	454.99
1976	202.61	1998	459.40
1977	215.84	1999	460.16
1978	235.78	2000	468.05
1979	257.20	2001	472.18
1980	277.60	2002	484.41
1981	302.25	2003	495.72
1982	320.13	2004	506.13
1983	330.82	2005	516.75
1984	341.06	2006	528.12
1985	346.12	2007	539.74
1986	347.33	2008	552.16
1987	353.35	2009	570.38
1988	369.45		

14
Aerobic and Anaerobic Attached Growth Biotechnologies

Nazih K. Shammas and Lawrence K. Wang

CONTENTS

Abstract Among the attached growth biological treatment processes covered in this chapter are trickling filter, denitrification filter, rotating biological contactor (RBC), fluidized bed reactor (FBR), packed bed reactor (PBR), biological aerated filter (BAF), and hybrid biological-activated carbon systems including downflow conventional biological GAC systems and upflow fluidized bed biological GAC systems (FBB-GAC). This chapter describes the above processes and explains their practice, limitations, process design, performance, energy requirements, process equipment, costs, and case studies.

Key Words Attached growth • trickling filter • denitrification filter • rotating biological contactor • biocontactor • fluidized bed reactor • packed bed reactor • biological aerated filter • hybrid systems • downflow conventional biological GAC systems; upflow fluidized bed biological GAC system.

1. TRICKLING FILTER

The trickling filter consists of a fixed bed of rock or plastic media over which wastewater is applied for aerobic biological treatment. Zoogleal slimes form on the media, which assimilate

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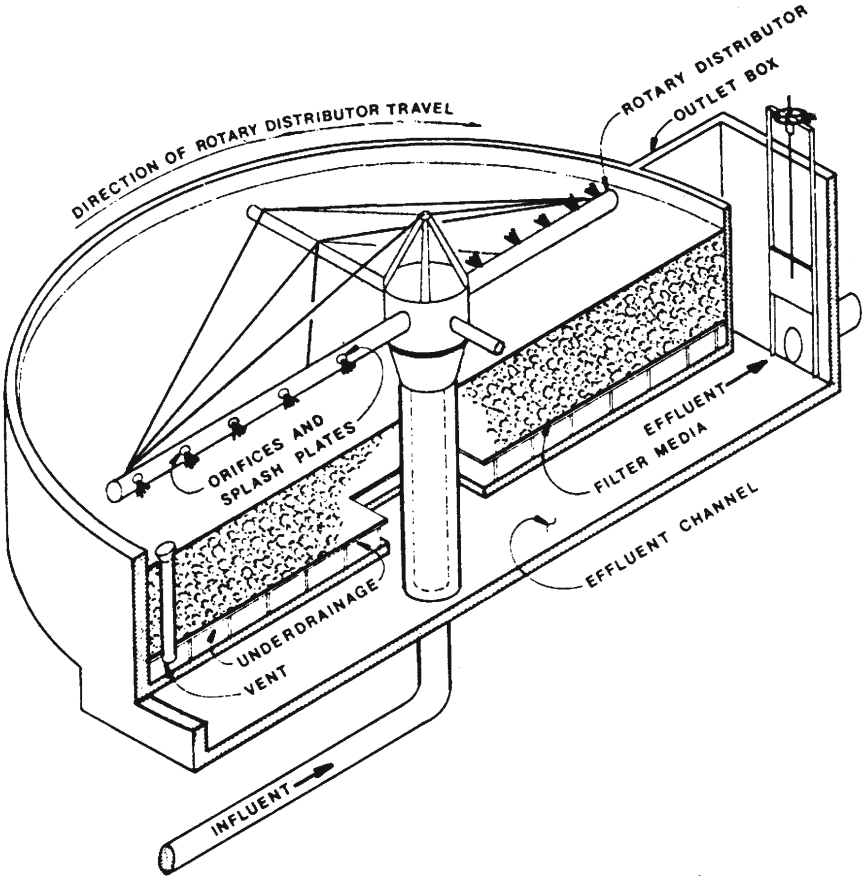

Fig. 14.1. Trickling filter with rotary distribution system (U.S. EPA).

and oxidize substances in the wastewater. The bed is dosed by a distributor system, and the treated wastewater is collected by an underdrain system. Primary treatment is normally required to optimize trickling filter performance.

Containment structures are normally made of reinforced concrete and installed in the ground to support the weight of the media. The rotary distributor has become the standard because of its reliability and ease of maintenance (Fig. 14.1). It consists of two or more arms that are mounted on a pivot in the center of the filter. Nozzles distribute the wastewater as the arms rotate as a result of the dynamic action of the incoming primary effluent. Underdrains are manufactured from specially designed vitrifiedclay blocks that support the filter media and pass the treated wastewater to a collection sump for transfer to the final clarifier (1, 2).

The organic material present in the wastewater is degraded by a population of microorganisms attached to the filter media. As the microorganisms grow, the thickness of the slime layer increases. Periodically, wastewater washes the slime off the media, and a new slime layer

starts to grow. This phenomenon of losing the slime layer is called sloughing and is primarily a function of the organic and hydraulic loadings on the filter.

Some advantages and disadvantages of trickling filters are listed below (3, 4).

(a) Advantages:
1. Simple, reliable, biological process.
2. Suitable in areas where large tracts of land are not available for land-intensive treatment systems.
3. May qualify for equivalent secondary discharge standards.
4. Effective in treating high concentrations of organics depending on the type of medium used.
5. Appropriate for small and medium-sized communities.
6. Rapidly reduce soluble BOD_5 in applied wastewater.
7. Efficient nitrification units.
8. Durable process elements.
9. Low power requirements.
10. Moderate level of skill and technical expertise needed to manage and operate the system.

(b) Disadvantages
1. Additional treatment may be needed to meet more stringent discharge standards.
2. Possible accumulation of excess biomass that cannot retain an aerobic condition and can impair performance (maximum biomass thickness is controlled by hydraulic dosage rate, type of media, type of organic matter, temperature, and nature of the biological growth).
3. Requires regular operator attention.
4. Incidence of clogging is relatively high.
5. Requires low loadings depending on the medium.
6. Flexibility and control are limited in comparison with activated sludge processes.
7. Vector and odor problems.
8. Snail problems.

1.1. Low-Rate Trickling Filter, Rock Media

The filter media for the low-rate trickling filter consists of 1- to 5-in (2.54 to 12.7 cm) stone. In contrast to the high-rate trickling filter that uses continuous recirculation of filter effluent to maintain a constant hydraulic loading to the distributor arms, either a suction-level controlled pump or a dosing siphon is employed for that purpose with a low-rate filter. Nevertheless, programmed rest periods may be necessary at times because of inadequate influent flow.

The low-rate trickling filter media bed is generally circular in plan, with a depth of 5–10 ft (1.52–3.04 m). Although filter effluent recirculation is generally not utilized, it can be provided as a standby tool to keep filter media wet during low flow periods (5).

The process is widely used and is highly dependable in moderate climates. Use of after-treatment or multistaging has frequently been found necessary to ensure uniform compliance with effluent limitations in colder regions. The trend in new installations is to replace the rock media with plastic media systems.

1.1.1. Applications

Slow trickling filters are used for the treatment of domestic and compatible industrial wastewaters amenable to aerobic biological treatment in conjunction with suitable pretreatment. This process is good for removal of suspended or colloidal materials and is somewhat

less effective in removal of soluble organics. This type of filter can be used for nitrification following prior (first-stage) biological treatment or as a stand-alone process in warm climates if the organic loading is low enough.

1.1.2. Limitations

- Slow rate trickling filters are vulnerable to climate changes and low temperatures.
- Filter flies and odors are common.
- Periods of inadequate moisture for slimes can be common.
- Less effective in treatment of wastewater containing high concentrations of soluble organics.
- Limited flexibility and process control in comparison with competing processes.
- High land and capital cost requirements, and
- Recovery times of several weeks with upsets.

1.1.3. Performance

Single-stage configuration with primary and secondary clarification and no recirculation are expected to have the following percent removals:

- 5-day Biochemical Oxygen Demand (BOD_5): 75–90%
- Phosphorus: 10–30%
- NH_4-N: 20–40%
- Suspended Solids (SS): 75–90%

Generated residual of biosolids is withdrawn from the secondary clarifier at a rate of 3,000–4,000 gal/MG (4,000 L/ML) of wastewater, containing 500–700 lb (226.8–317.5 kg) dry solids.

1.1.4. Design Criteria

Design criteria for low-rate trickling filters include the following (1, 3, 4):

- Hydraulic loading: 1–4 MG/acre/d (25–90 $gal/d/ft^2$) = 1.02–3.67 $m^3/d/m^2$
- Organic loading: 200–900 lb $BOD_5/d/acre\,ft$ (5–20 lb $BOD_5/d/1,000\,ft^3$) = 80–320 $g/d/m^3$
- Dosing interval: Continuous for majority of daily operating schedule, but may become intermittent (not more than 5 min) during low flow periods
- Effluent channel minimum velocity: 2 ft/s at average daily flow
- Media: Rock, 1–5 in (2.54–12.7 cm), must meet sodium sulfate soundness test
- Recirculation ratio: 0
- Depth: 5–10 ft, or 1.52–3.04 m
- Sloughing: Intermittent
- Underdrain minimum slope = 1%

1.2. High-Rate Trickling Filter, Rock Media

The high-rate filter media consists of 1- to 5-in (2.54 to 12.7 cm) stone similar to slow-rate filters. Continuous recirculation of filter effluent is used to maintain a constant hydraulic loading to the distributor arms. The high-rate trickling filter media bed is generally circular in plan, with a depth of 3–6 ft (0.91–1.82 m) (1–4).

The organic material present in the wastewater is degraded by a population of microorganisms attached to the filter media. As the slime layer increases in thickness, the absorbed organic matter is metabolized before it can reach the microorganisms near the media face. As

a result, the microorganisms near the media face enter into an endogenous phase of growth. In this phase, the microorganisms lose their ability to cling to the media surface. The liquid then washes the slime off the media, and a new slime layer starts to grow. Filters effluent recirculation is vital with high-rate trickling filters to promote the flushing action necessary for effective sloughing control, without which media clogging and anaerobic conditions could develop because of the high organic leading rates employed.

1.2.1. Applications

High-rate trickling filters are used in the treatment of domestic and compatible industrial wastewaters amenable to aerobic biological treatment in conjunction with suitable pre- and posttreatment. Industrial and joint wastewater treatment facilities may use the process as a roughing filter prior to activated sludge or other unit processes. The process is effective for removal of suspended or colloidal materials and is less effective removal of soluble organics. When used for secondary treatment, the media bed is generally circular in plan and dosed by a rotary distributor (Fig. 14.1). Roughing applications often utilize rectangular media beds with fixed nozzles for distribution (Fig. 14.2).

1.2.2. Limitations

1. Vulnerable to below freezing weather
2. Recirculation may be restricted during cold weather due to cooling effects
3. Marginal treatment capability in single-stage operation

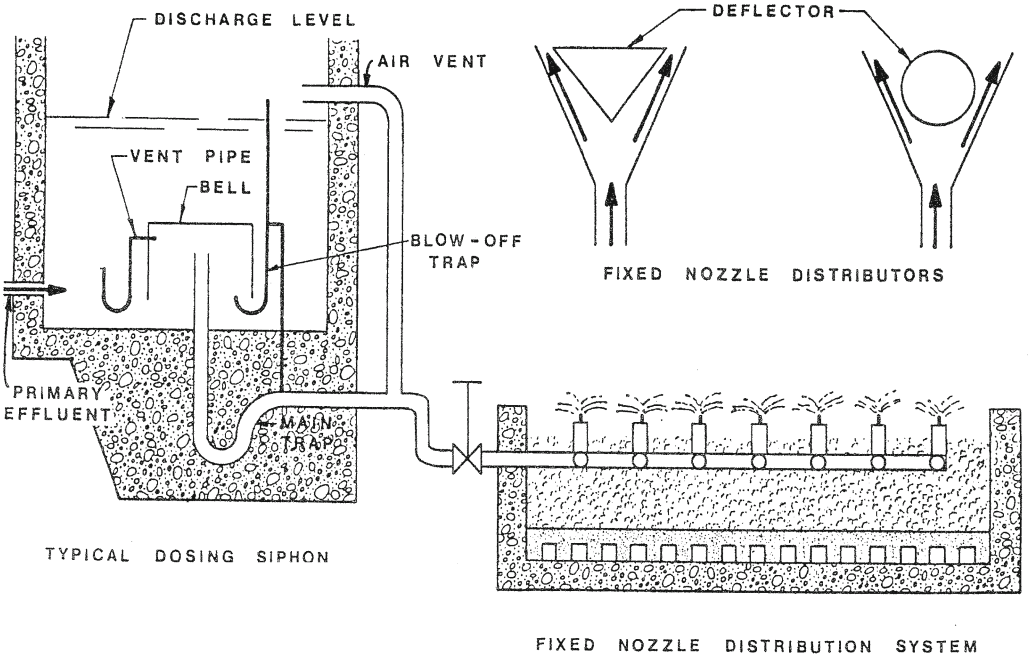

Fig. 14.2. Trickling filter with fixed nozzle distribution system (U.S. EPA).

4. Is less effective in treatment of wastewater containing high concentrations of soluble organics
5. Has limited flexibility and control in comparison with competing processes
6. Has potential for vector and odor problems, although they are not as prevalent as with low-rate trickling filters
7. Long recovery times with upsets
8. Limited to 60–80% BOD_5 removal

1.2.3. Performance

Single-stage configuration with any pattern of filter effluent recirculation and primary and secondary clarification (see Fig. 14.3) has the following percent removal (1–4):

- BOD_5: 60–80%
- Phosphorus: 10–30%
- NN_4-N: 20–30%
- SS: 60–80%

The generated residual of biosolids is withdrawn from the secondary clarifier at a rate of 2,500–3,000 gal/MG (2,500–3,000 L/ML) wastewater containing 400–500 lb (226.8–317.5 kg) dry solids.

1.2.4. Design Criteria

Design criteria for high-rate trickling filters include the following (1, 3, 4):

- Hydraulic loading (with recirculation): 10–50 MG/acre/d (230–1, 150 $gal/d/ft^2$) = 9.4–46.9 $m^3/d/m^2$
- Organic loading: 900–2,600 lb BOD_5/d/acre ft (20–60 lb $BOD_5/d/1,000\,ft^3$) = 331.2–956.8 $g/d/m^3$
- Recirculation ratio: 0.5:1–4:1
- Bed depth: 3–6 ft = 0.91–1.82 m
- Dosing interval: Not more than 15 s (continuous)
- Power requirements: 10–50 hp/MG = 1.97–9.85 kW/ML
- Sloughing: continuous
- Underdrain minimum slope: 1%
- Media: Rock, 1–5 in (2.54–12.7 cm), must meet sodium sulfate soundness test

Process and mechanical reliability: The process can be expected to have a high degree of reliability if operating conditions minimize variability and the installation is in a climate where, wastewater temperatures do not fall below 13°C for prolonged periods. The process is simple to operate, and its mechanical reliability is high.

1.3. Trickling Filter, Plastic Media

Plastic media is comparatively light with a specific weight 10–30 times less than rock media. Its high void space (approximately 95%) promotes better oxygen transfer during passage through the filter than rock media with its approximate 50% void space (5–7). Because of its light weight, plastic media containment structures are normally constructed as elevated towers 20–30 ft high. Excavated containment structures for rock media can sometimes serve as a foundation for elevated towers for converting an existing facility to plastic media.

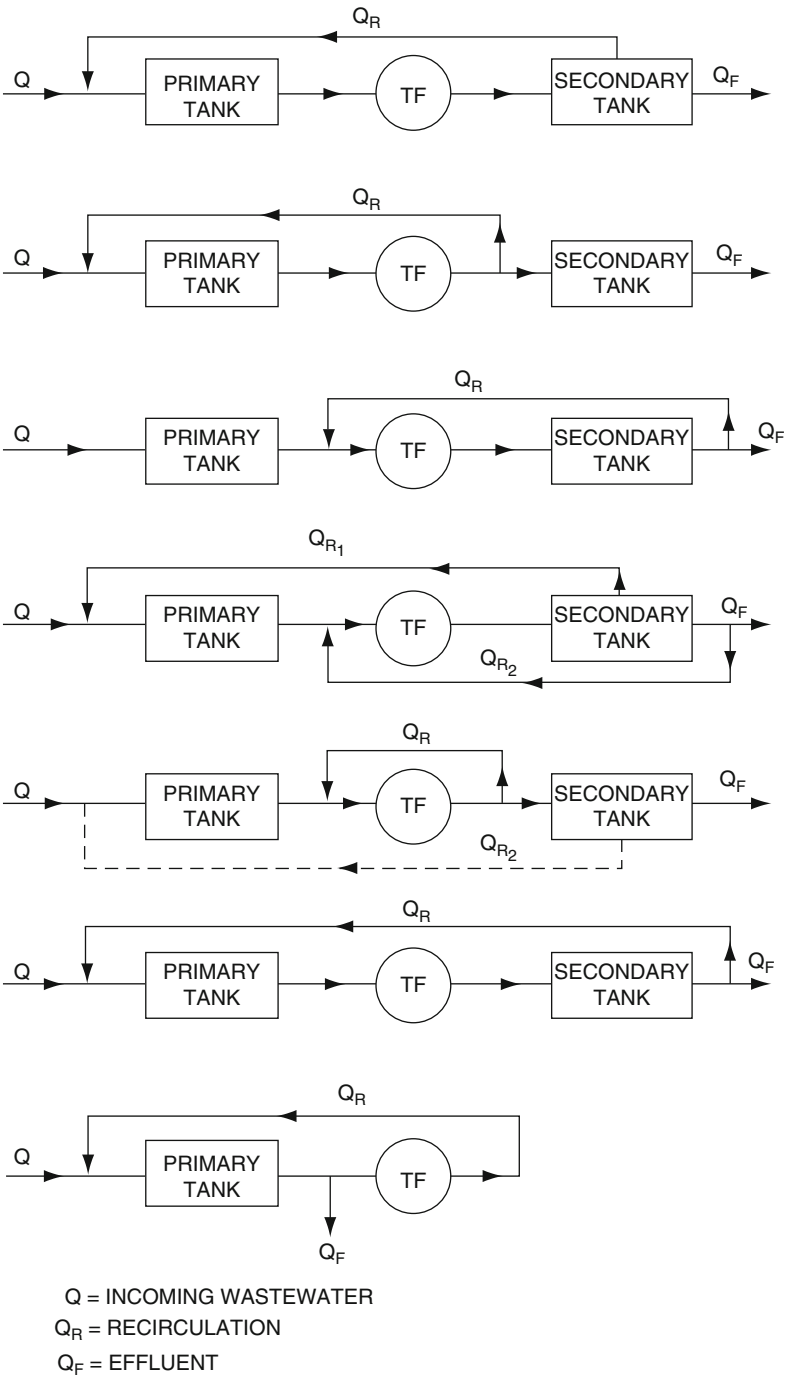

Fig. 14.3. Flow diagrams of trickling filters with various recirculation patterns (U.S. EPA).

Plastic media trickling filters can be employed to provide independent secondary treatment or roughing ahead of a second-stage biological process. When used for secondary treatment, the media bed is generally circular in plan and dosed by a rotary distributor. Roughing applications often utilize rectangular media beds with fixed nozzles for distribution.

Filter effluent recirculation is vital with plastic media trickling filters to ensure proper wetting of the media and to promote effective sloughing control compatible with the high organic loadings employed. The plastic media filters can also be used as a roughing filter at flow rates above 1, 400 gal/d/ft^2 (57.12 m^3/d/m^2) or as a separate stage nitrification process.

1.3.1. Applications

High-rate plastic media trickling filters are used in the treatment of domestic and compatible industrial wastewaters amenable to aerobic biological treatment. Industrial and joint wastewater treatment facilities may use the process as a roughing filter prior to activated sludge or other unit processes. Existing rock filter facilities can be upgraded via elevation of the containment structure and conversion to plastic media (8). The plastic media filters can also be used for nitrification following prior (first-stage) biological treatment (9).

1.3.2. Limitations

1. Vulnerable to below freezing weather
2. Recirculation may be restricted during cold weather due to cooling effects
3. Marginal treatment capability in single-stage operation
4. Is less effective in treatment of wastewater containing high concentrations of soluble organics
5. Has limited flexibility and control in comparison with competing processes
6. Has potential for vector and odor problems, although they are not as prevalent as with low rate rock media trickling filters
7. Long recovery times with upsets

1.3.3. Performance

Employing the loadings listed below for secondary treatment and using a single-stage configuration with filter effluent recirculation and primary and secondary clarification, the plastic media trickling filters can attain the following percent removal (1, 3, 4):

- BOD_5: 80–90%
- Phosphorus: 10–30%
- NH_4-N: 20–30%
- SS: 80–90%

The generated residual of biosolids is withdrawn from the secondary clarifier at a rate of 3,000–4,000 gal/MG (3,000–4,000 L/ML) of wastewater, containing 500–700 lb (226.8–317.5 kg) dry solids.

1.3.4. Design Criteria

Design criteria for plastic media trickling filters include the following (1, 3, 4, 8, 9):

- Hydraulic loading (with recirculation):
  Secondary treatment: 15–90 MG/acre/d (350–2, 050 gal/d/ft^2) = 14.3–83.6 m^3/d/m^2
  Roughing: 60–200 MG/acre/d (1, 400–4, 600 gal/d/ft^2) = 57.1–187.7 m^3/d/m^2

- Organic loading
  Secondary treatment: 450–1,750 lb BOD_5/d/acre/ft (10–40 lb BOD_5/d/1,000 ft^3) = 165–644 g/d/m^3
  Roughing treatment: 4,500–22,000 lb BOD_5/d/acre ft (100–500 lb BOD_5/d/1,000 ft^3) = 1650–8096 g/d/m^3
- Recirculation ratio: 0.5:1–5:1
- Bed depth: 20–30 ft = 6.1–9.1 m
- Dosing interval: Not more than 15 s (continuous)
- Underdrain minimum slope: 1%
- Sloughing: continuous
- Power requirements: 10–50 hp/MG = 1.97–9.85 kW/ML

Process and mechanical reliability: The process can be expected to have a high degree of reliability if operating conditions minimize variability, and the installation is in a climate where wastewater temperatures do not fall below 13°C for prolonged periods. The process is simple to operate, and its mechanical reliability is high.

2. DENITRIFICATION FILTER

2.1. Denitrification Filter, Fine Media

In the denitrification process, nitrates and nitrites in nitrified wastewater are converted to nitrogen gas by the action of facultative heterotrophic bacteria. The fine media denitrification filter is an attached growth biological process in which nitrified wastewater is passed through a pressurized submerged bed of sand or other fine filter media (up to about 15 mm in diameter) in which anoxic conditions are maintained. The nitrified wastewater contains very little carbonaceous material, and consequently requires a supplemental energy source (usually methanol) to maintain the attached denitrifying slime (1, 2). Because of the relatively fine media used, physical filtration analogous to that occurring in a pressure filter takes place. As a result, a clear effluent is produced, eliminating the need for final clarification. Backwashing is required to maintain an acceptable pressure drop. Surface loading rates may be somewhat lower than those common for pressure filtration. Development of the denitrifying slime and consequent denitrification efficiency are a function of the specific surface area of the filter, and in practice, fine media denitrification filters convert nitrates to nitrogen gas at a much higher rate than suspended growth systems. The coarser the media, the less frequent the backwashing, although the effluent may be more turbid. (See coarse media denitrification filters.)

Common modifications include the use of various media, such as garnet sand, silica sand, or anthracite coal with varying size distributions. Multimedia systems have also been used. Alternate energy sources, such as sugars, volatile acids, ethanol, or other organic compounds, as well as nitrogen-deficient materials such as brewery wastewater, may be used. An air scour may be incorporated into the backwashing cycle; however, temporary inhibition of denitrification may result. Various types of underdrains may be used. A bumping procedure (short periodic flow reversals) has been used to remove entrapped nitrogen gas bubbles produced during denitrification. Denitrification may be combined with refractory organic removal. Upflow systems utilizing fine media (sand or activated carbon) have been operated as fluidized bed reactors.

Denitrification filters are used almost exclusively to denitrify municipal wastewaters that have undergone carbon oxidation and nitrification. They may also be used to reduce nitrate in industrial wastewater.

Compared to suspended growth systems, denitrification filters have:

1. High nitrogen removal efficiency
2. Smaller structures (land use)

2.1.1. Performance

Denitrification filters are capable of converting nearly all nitrate and nitrite in a nitrified secondary effluent to gaseous nitrogen. Overall nitrogen removals of 75–90% are achievable. Suspended solid removals of up to 93% have been achieved (10).

An energy source is commonly supplied in the form of methanol. Methanol feed concentrations may be estimated using the following values per mg/L of the material at the inlet to the process.

mg/L CH_3OH per mg/L of

2.47	NO_3-N
1.53	NO_2-N
0.87	DO (Dissolved oxygen)

If supplemental energy feed rates are controlled, little excess biosolids are generated.

2.1.2. Design Criteria

- Flow scheme: Downflow (although upflow systems with different design criteria have been utilized
- Optimum pH: 6.5–7.5
- Surface loading rate: 0.5–7.0 gal/min/ft^2 = 1.22–17.08 g m^3/m^2/hr
- Media diameter (d_{50}): 2–15 mm
- Column depth: 3–20 ft (function of specific surface ft^2/ft^3 and contact time) = 0.9–6.1 m
- Backwash rate: 8–25 gal/min/ft^2 = 19.52–61 m^3/m^2/h
- Backwash cycle frequency: 0.5–4.0 d
- Specific surface: 85–300 ft^2/ft^3 = 278.8–984 m^2/m^3
- Voids: 40–50%

2.2. Denitrification Filter, Coarse Media

During denitrification, nitrates and nitrites are reduced to nitrogen gas through the action of facultative heterotrophic bacteria. Coarse media denitrification filters are attached growth biological processes in which nitrified wastewater is passed through submerged beds containing natural (gravel or stone) or synthetic (plastic) media. The process system may be a pressure system or a gravity system. Minimum media diameter is about 15 mm (1, 2).

Anaerobic or near anaerobic conditions are maintained in the submerged bed, and since the nitrified wastewater is usually deficient in carbonaceous materials, a supplemental carbon source (usually methanol) is required to maintain the attached denitrifying biomass. Because of the high void percent and low specific surface area characteristic of high porosity coarse

denitrification filters, biomass (attached slime) continuously sloughs off. As a result, the coarse media column effluent is usually moderately high in suspended solids (20–40 mg/L) requiring a final polishing step.

A wide variety of media types may be used as long as high void volume and low specific volume are maintained. Both dumped plastic media and corrugated sheet media have been used. Backwashing is infrequent and is usually done to control effluent suspended solids rather than pressure drop. Alternate energy sources, such as sugars, volatile acids, ethanol, or other organic compounds, as well as nitrogen-deficient materials, such as brewery wastes, may be used. Nitrogen gas-filled coarse media denitrification filters are a possible modification.

These filters are used almost exclusively to denitrify municipal wastewater that has undergone carbon oxidation and nitrification. They may also be used to reduce nitrate in industrial wastewater.

2.2.1. Performance

Denitrification coarse media filters are capable of converting nearly all nitrates in a nitrified secondary effluent to gaseous nitrogen. Overall nitrogen removals of 70–90% are achievable. Generally, less operator attention is required than with fine media systems (11–14).

The required amount of the most common energy source, methanol, may be estimated using the following values per mg/L of the material in the inlet to the process.

mg/L CH_3OH per mg/L of

2.47	NO_3-N
1.53	NO_2-N
0.87	DO

If supplemental carbon feed rates are controlled, little excess biosolids are generated. Biosolid production is in the range of 0.6–0.8 lb/lb NH_3-N reduced (0.6–0.8 kg/kg NH_3-N reduced).

2.2.2. Design Criteria

- Optimum pH: 6.5–7.5
- Voids: 70–96%
- Specific surface: 65–274 $ft^2/ft^3 = 213.2–898.7\ m^2/m^3$
- Nitrate loading rate: lb NO_3-N/ft^2 packing surface/d,
  Up to 0.5×10^{-4} at 5°C
  $0.2–0.8 \times 10^{-4}$ at 15°C
  $0.8–1.3 \times 10^{-4}$ at 25°C (Note: 1 lb/ft^2/d = 4.8824 kg/d/m^2)
- Surface loading rate: 2.5 and 4.1 gal/ft^2/d for a flow of 0.3 and 0.5 MGD respectively, or 0.1 and 0.167 m^3/m^2/d for a flow of 13.1 and 21.9 L/s, respectively.

3. ROTATING BIOLOGICAL CONTACTOR

The rotating biological contactor (RBC) was put into commercial use in Europe in the 1960s, and about a decade later, it was introduced USA (15). The RBC process is a fixed film biological reactor consisting of plastic media mounted on a horizontal shaft and placed

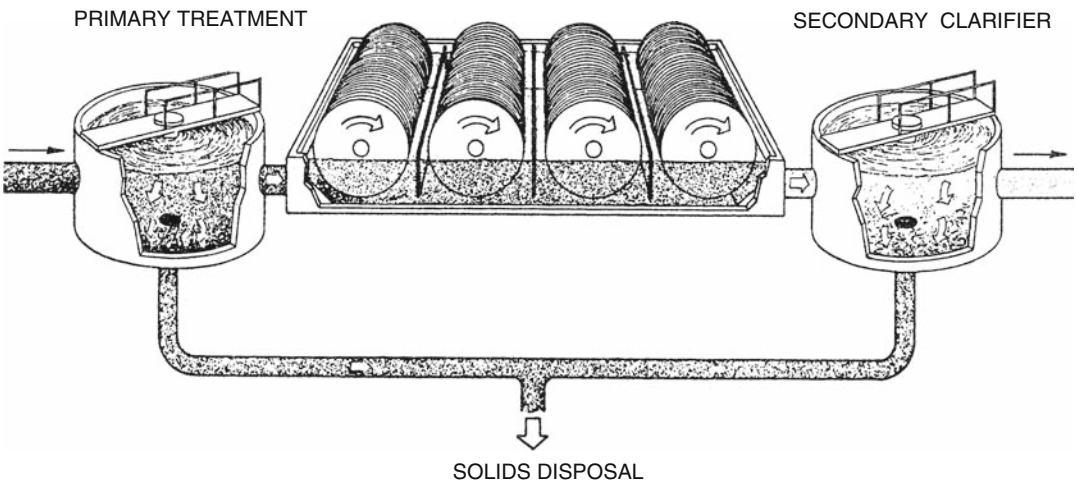

Fig. 14.4. Typical configuration of an RBC system (U.S. EPA).

in a tank. Common media forms are disc type made of Styrofoam, and a denser lattice type made of polyethylene. While wastewater flows through the tank, the media are slowly rotated, about 40% immersed, for contact with the wastewater for removal of organic matter by the biological film that develops on the media. Rotation results in exposure of the film to the atmosphere as a means of aeration. Excess biomass on the media is stripped oft by rotational shear forces, and the stripped solids are maintained in suspension by the mixing action of the rotating media (1,2,16). Multiple staging of RBCs increases treatment efficiency and aids in achieving nitrification year-round. A complete system could consist of two or more parallel trains, with each train consisting of multiple stages in series (see Fig. 14.4).

Shammas (17) has shown that the RBC process can be assumed to behave as a plug flow reactor in which first-order BOD removal and nitrification kinetics prevail (see **Eq. (1)**). The reaction rate constant, k, was reported to be 0.77/h for BOD removal and 0.5/h for nitrification (17).

$$C = C_o\, 10^{-kt} \tag{1}$$

where C is the substrate concentration (mg/L), C_o is the initial substrate concentration (mg/L), t is the time (h), and k is the reaction rate constant (1/h).

RBCs are used in the treatment of domestic and compatible industrial wastewater amenable to aerobic biological treatment in conjunction with suitable pre- and posttreatment. They can be used for nitrification, roughing, secondary treatment and polishing. There are several advantages for the system including high treatment efficiency, economy, simplicity of operation, and low O & M costs (18).

3.1. Operating Characteristics

3.1.1. Effect of Hydraulic Loading and Staging

Generally, BOD removal and nitrification increase with increasing number of stages and decreasing hydraulic loading as can be seen in Figs. 14.5–14.7. According to Shammas (19), employing six stages BOD removals of 90, 94, and 98% can be obtained for hydraulic loadings of 0.47, 0.31, and 0.16 $m^3/d/m^2$, respectively, while 61, 73, and 95% removals could be achieved for the same hydraulic loadings with three stages.

As illustrated in Fig. 14.5, BOD removals differ substantially for the early stages at various hydraulic loadings; however, they approach to closer values at the end of the sixth stage. Thus, it can be deduced that higher hydraulic loadings have a distinct dampening effect on BOD removal, especially in the early stages.

As shown in Fig. 14.6, the rate of decrease in BOD removal for a corresponding increase in hydraulic loading from 0.16 to 0.31 $m^3/d/m^2$ is sharper than the rate of decrease from 0.31 to 0.47 $m^3/d/m^2$. However, rates of decrease in BOD removals, for both ranges, decrease with increasing number of stages since the readily oxidizable BOD is already considerably removed when wastewater reaches the latter stages. Therefore, it can be concluded that an

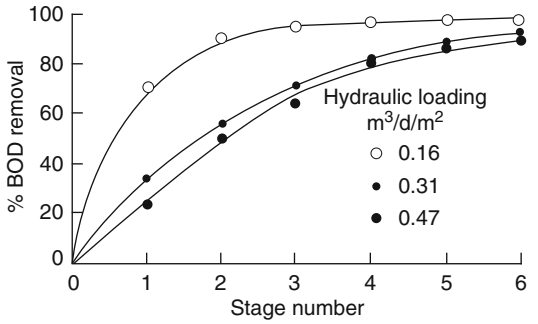

Fig. 14.5. BOD removal as a function of staging (19).

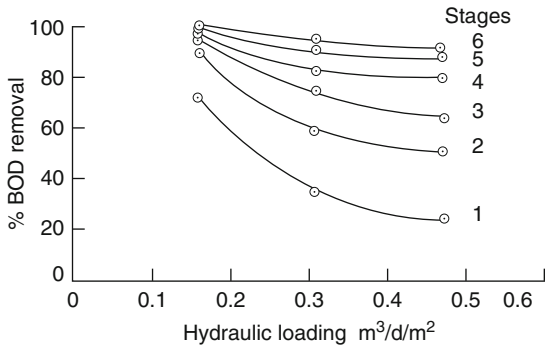

Fig. 14.6. BOD removal as a function of hydraulic loading (19).

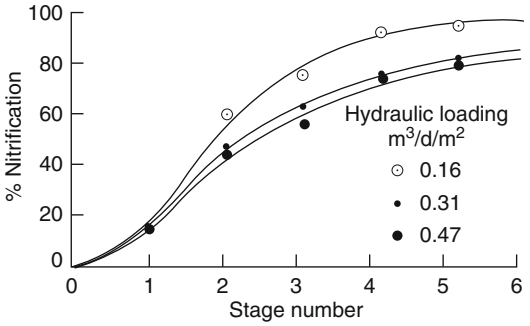

Fig. 14.7. Nitrification as a function of staging (19).

increase in number of stages will dampen the effect of the hydraulic loading, and that the effect of a shock load can be minimized by increasing the number of stages (19).

The biocontactors process is well suited for nitrification because of the natural development of nitrifying organisms in the latter stages of multistage biocontactors installations. Figure 14.7 shows that the percent decrease in ammonia-N concentration increases with increasing number of stages (19). A hydraulic loading of $0.16\,m^3/d/m^2$ could produce the highest percent decrease in ammonia-N concentration (97 and 92% at stages 6 and 4, respectively). Hydraulic loadings of 0.31 and $0.47\,m^3/d/m^2$ results in lower and somewhat equal decrease in ammonia-N concentration (an average of 85 and 75% for both at stages 6 and 4, respectively).

It is clear that the early stages are not sufficient for good nitrification. According to Shammas (19), there is a distinct change in the pattern of nitrification at stage 4 (see Fig. 14.7, indicating that a minimum of four stages will be necessary to obtain good nitrification in the system. Moreover, it is important to notice that over 90% nitrification can be achieved utilizing four stages with a hydraulic loading of $0.16\,m^3/d/m^2$, which is triple the loading recommended by Antonie (20).

3.1.2. Effect of Residence Time

Figure 14.8 shows the BOD removal efficiency as a function of residence time for different hydraulic loadings and number of stages (19). Here, the various combinations of hydraulic loadings and stages give approximately equivalent performance when compared at the same residence time. For example, 90% BOD removal is obtained for hydraulic loadings of 0.16 and $0.47\,m^3/d/m^2$ utilizing two and six stages at the same residence period of 65 min. Similarly, 95% BOD removal can be produced at 0.16 and $0.31\,m^3/d/m^2$ with three and six stages in 100 min.

Based on the results of these observations, it is concluded that in order to obtain a high BOD removal, it is essential to consider the residence time as the prime design factor (19). The performance is independent of the hydraulic loading or the number of stages as long as the combination of the two parameters produces the minimum residence time required. This optimum time varies from 75 to 100 min for 90 and 95% efficiencies, respectively.

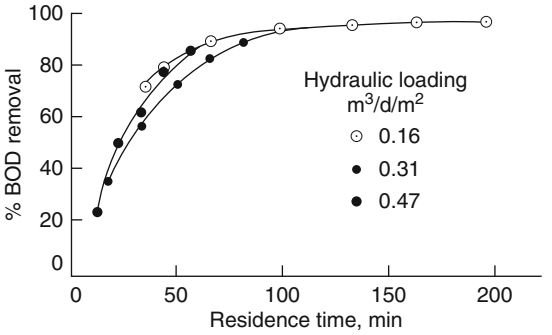

Fig. 14.8. BOD Removal as a function of residence time (19).

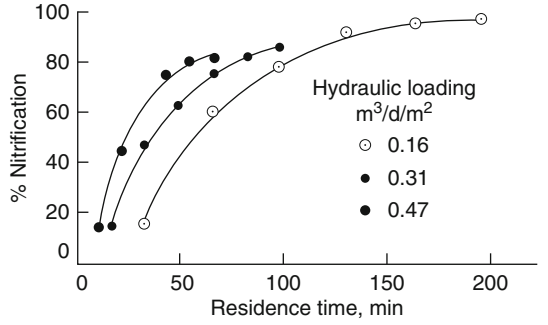

Fig. 14.9. Nitrification as a function of residence time (19).

In a similar fashion, the efficiency of nitrification as a function of residence time and for different hydraulic loadings and number of stages is given in Fig. 14.9 (19). In contrast to BOD removal, higher hydraulic loadings can result in a significantly better nitrification at the same residence time. This fact is illustrated by considering the ammonia-N removal at a residence time of 60 min. Hydraulic loadings of 0.16, 0.31, and 0.47 $m^3/d/m^2$ produces ammonia-N removals of 60, 75 and 90% utilizing two, four, and six stages, respectively. The number of stages appears to be extremely important in the case of nitrification in contrast to BOD removal where the residence time is the prime factor (19). This can be explained by the fact that higher hydraulic loadings need a greater number of stages for a given residence time, thus producing contact for the wastewater with the nitrifying biomass, which cannot compete with the bulk of other microorganism in the first few stages, while they would develop and flourish in the latter stages where the BOD has already been reduced.

3.1.3. Effect of Influent BOD Concentration

The effect of influent BOD concentrations of 270 and 700 mg/L was investigated at the same hydraulic loading and disc speed. As shown in Fig. 14.10, identical BOD removals greater than 95% are obtained for the last four stages.

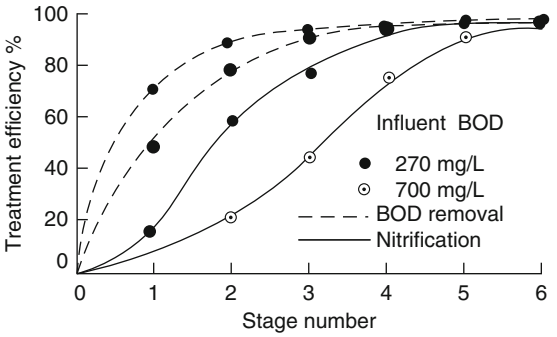

Fig. 14.10. Effect of influent BOD on treatment efficiency (19).

Figure 14.10 also depicts the effect of influent BOD concentration on nitrification. It is obvious that nitrification is much better at lower BOD concentration. However at stage 6 when the BOD drops to 6 and 16 mg/L (for BOD influents of 270 and 700 mg/L), ammonia oxidation is almost complete. These results reinforce the previous findings on the importance of staging in achieving high nitrification efficiencies (19).

3.1.4. Effect of Disc Speed

The disc speed is usually maintained near 3.5 rpm (peripheral velocity of 0.092 m/s). Lower speeds decrease the sloughing of biomass and cause clogging; while higher speeds do not produce any improvement in efficiency (19). Further information on contribution factors can be found in other references (21–23).

3.2. Performance

The common four-stage system configuration with final clarifier and preceded by primary treatment can attain the following percent removals:

- BOD_5: 60–90%
- SS: 80–90%
- Phosphorus: 10–30%
- NH_4-N: up to 95%

The rate of biosolids production, which is removed in the secondary clarifier is 3,000–4,000 gal/MG (3000–4000 L/ML) of wastewater, 500–700 lb dry solids/MG (60–84 g dry solids/m^3) wastewater.

3.3. Design Criteria

The organic loadings for RBCs, as recommended by South Dakota Department of Environmental & Natural Resources (24), are as follows:

- The organic loading to the first stage should be in the range of 3.5–6.0 lb total $BOD_5/1,000\ ft^2/d$ or 1.5–2.5 lb soluble $BOD_5/1,000\ ft^2/d$ = 17087–29292 kg total $BOD_5/km^2/d$ or 7323–12205 kg soluble $BOD_5/km^2/d$.

- For average conditions, the design loading should not exceed 2.5 lb soluble $BOD_5/1,000\,ft^2/d$ (12205 kg soluble $BOD_5/km^2/d$) on the first stage shaft(s) of any treatment train.
- For peak conditions, the design loading should not exceed 2.0 lb of soluble $BOD_5/1,000\,ft^2/d$ (9764 kg soluble $BOD_5/km^2/d$) for the third shaft(s) in a treatment train.
- For average conditions, the overall system loading shall not exceed 0.6 lb of soluble $BOD_5/1,000\,ft^2/d$ (2929 kg soluble $BOD_5/km^2/d$). This soluble BOD_5 loading to all shafts should be used to determine the total number of shafts required.

Other design criteria as reported in the literature (17–23) are given below:

- BOD loading:
  - Without nitrification: 30–60 lb $BOD_5/d/1,000\,ft^3$ media (480.5–961.0 $g/d/m^3$)
  - With nitrification: 15–20 lb $BOD_5/d/1,000\,ft^3$ media (240.2–320.4 $g/d/m^3$)
- Hydraulic loading:
  - Without nitrification: 0.75–1.5 $gal/d/ft^2$ media surface area (0.03–0.06 $m^3/d/m^2$)
  - With nitrification: 0.3–0.6 $gal/d/ft^2$ media surface area (0.012–0.024 $m^3/d/m^2$)
- Number of stages per train: 1–4 depending on treatment objectives
- Number of parallel trains: Recommended at least 2
- Peripheral velocity:
  - 60 ft/min for mechanically driven (18.3 m/min)
  - 30–60 ft/min for air driven (9.1–18.3 m/min)
- Media surface area:
  - 20–25 ft^2/ft^3 for typical discs (65.6–82 m^2/m^3)
  - 30–40 ft^2/ft^3 for standard lattice discs (98.4–131.2 m^2/m^3)
  - 50–60 ft^2/ft^3 for high-density lattice discs (164–196.8 m^2/m^3)
- Percent media submerged: 40%
- Tank volume: 0.12 gal/ft^2 of disc area (0.004896 m^3/m^2)
- Detention time
  - 40–120 min without nitrification
  - 90–250 min with nitrification
- Secondary clarifier overflow rate: 500–800 $gal/d/ft^2$
- Power:
  - 3.0–5.0 HP consumed/25 ft shaft (2.2–3.7 kW/7.6 m shaft)
  - 5.0–7.5 HP connected/25 ft shaft (3.7–5.6 kW/7.6 m shaft)

4. FLUIDIZED BED REACTOR

Fluidized bed reactors (FBR), Packed bed reactors (PBR), and biological aerated filters (BAF) represent attached growth processes that have been utilized to some extent for nitrification of municipal wastewaters. Unlike trickling filters, the hydraulic design of these systems is such that the media are submerged in the reactor liquid. In packed bed reactors and biological aerated filters, the media are stationary during normal operation, held in place by gravity. In the fluidized bed reactor, the media are expanded or fluidized as the incoming flow passes upward through the reactor.

4.1. FBR Process Description

In the conventional biological fluidized bed reactor, often referred to as an expanded bed reactor, wastewater or wastewater plus recycled effluent is introduced at the bottom of the reactor at a hydraulic loading rate or upflow velocity sufficient to expand the bed media, resulting in a fluidized state. The fluidized media particles provide a vast surface area for biological growth, in part leading to the development of a biomass concentration approximately five to ten times greater than that normally maintained in a conventional suspended growth reactor (25). To date, the media employed in most full-scale fluidized bed reactors have either been silica sand or granular activated carbon.

The mechanical components and subsystems critical to the development of fluidized bed commercial systems are (26):

(a) The device or method to distribute the influent flow to the reactor
(b) The device or method to transfer oxygen in a controlled fashion to the fluidized bed reactor in aerobic applications of the technology. The oxygenation system is particularly critical in the treatment of wastewaters containing medium to high concentrations of oxygen demanding material (i.e., O_2 requirements greater than 25 mg/L)
(c) The device or method to control the expansion of the fluidized bed due to biofilm growth. The bed height control system is particularly critical in treatment applications where the net yield of biomass is significant. Further details concerning the critical components have been presented elsewhere (27).

Although the development of water and wastewater systems using a fluidized bed of biomass can be traced back to the 1940s in England (28), media-based fluidized bed reactors were not developed until the early 1970s. Researchers at Manhattan College in New York, at the EPA Municipal Environmental Research Laboratory in Cincinnati, OH, and at the Water Research Centre in Medmenham, England, can be credited for the initial application of media-based fluidized bed reactors to water and wastewater treatment. The Manhattan College researchers were granted a U.S. patent in 1974 (assigned to Ecolotrol, Inc.) for the application of the fluidized bed process configuration to "denitrifying wastewater" (29). In a paper published in 1970 by researchers from the University of Michigan, biological activity was observed in expanded-bed activated carbon reactors and was believed to be the reason for the observed nitrate reduction (30).

The ability of the biological fluidized bed process configuration to intensify biological reaction rates through accumulation of high concentrations of active biomass has attracted attention for many years (31). The results from laboratory and field pilot scale studies have consistently illustrated the technical advantages of the fluidized bed over most other suspended and attached growth reactor configurations in many wastewater treatment applications. In 1981, a comprehensive account of ongoing fluidized bed process development activities was published based on a 1980 seminar held in Manchester, England (32). Although hailed at that time as the most significant development in the wastewater treatment field in the last 50 years, it also was claimed that no full-scale plants were yet in operation. Since that time, even though more than 70 commercial, fluidized bed reactors have been installed in North America and Europe, wider use of the technology has been hampered by such factors as (33):

(a) Mechanical scale-up issues
(b) Slow development of economically attractive system configurations, and
(c) Proprietary constraints

According to a 1991 state-of-the-art review of fluidized beds for water and wastewater treatment, the technology was being applied largely for industrial versus municipal wastewater treatment at current operating full-scale installations in North America and Europe (33). Although full-scale fluidized bed industrial systems are operating under conditions that result in nitrification (34), few, if any, systems have been installed for nitrification of municipal wastewaters on a full scale. A limited number of reactors have been installed for denitrification of municipal wastewater (35).

4.2. Process Design

Information useful for the process design of full-scale systems for nitrification of municipal wastewater derived from the results of fluidized bed pilot plant studies (36–48) is summarized as follows:

(a) A half-order model appears appropriate to describe the kinetics of ammonium oxidation in fluidized bed reactors under nonlimiting DO conditions
(b) The volumetric removal rate and the specific ammonium oxidation rate decrease significantly at low reactor ammonium concentrations.
(c) The fluidized bed hydraulic retention time required to achieve nitrification down to ammonium levels of 2 mg/L or less ranges from 10 to 40 min. This HRT is for treatment of municipal wastewaters containing less than 50 mg/L of $CBOD_5$ and approximately 20 mg/L of oxidizable nitrogen compounds, and providing that the reactor is designed to promote the buildup of at least 8.5 g/L of volatile attached solids and that nonlimiting DO conditions are achieved. The actual HRT required will depend on such factors as the concentration of carbonaceous BOD in the wastewater, the system hydraulics (i.e., plug flow versus complete mixing conditions), and the reactor temperature and pH conditions.

If the use of the fluidized bed for nitrification is being considered, onsite piloting is recommended given the limited amount of full-scale operating and performance information on this application.

4.3. Applications

The fluidized bed reactor is more commonly used for industrial wastewater rather than municipal wastewater. Concerns over municipal applications have included mechanical scale-up factors, proprietary constraints, and economically unattractive system appurtenances (49). However, there are successful municipal applications; Table 14.1 lists the design parameters and loadings of four industrial and municipal installations with fluidized bed reactors operating in the denitrification mode (25).

The principal commercial suppliers of fluidized bed systems are Dorr-Oliver, Envirex, and Ecolotrol. Both Dorr-Oliver and Envirex systems were developed on the basis of Ecolotrol process patents. Currently, Envirex is the only manufacturer actively marketing the fluidized bed reactor for denitrification applications in the United States. Table 14.2 summarizes the types of reactors in use (25).

Table 14.1
Design parameters and loadings of denitrification FBR plants[a] (26)

Parameter	Facility			
	Pensacola[b,c]	Reno-Sparks	Rancho, CA[c]	IBM[c]
Mean wastewater flow, L/s	1,052	1,883	263	113
Mean wastewater flow, mgd	24	43	6[d]	1[d]
Maximum wastewater flow, L/s	1,490	2,400		
Maximum wastewater flow, mgd	34	55		
Influent NO_3^--N, mg/L	20	18	21	54
Effluent NO_3^--N, mg/L	< 6	2	2.5	8
Design wastewater temperature, °C	18	13	22	10
Estimated reactor biomass, mg/L VSS	NA	18,000	28,000	NA
Hydraulic retention time,[e] min	8.5	13.8	10	26
Hydraulic loading rate,[f] $m^3/m^2/d$	672	550	336	578
Hydraulic loading rate,[f] gpd/sq ft	11.4	9.3	0.8	1.3
Estimated settled sand depth, m	1.8	2.4	1.2	1.5
Estimated settled sand depth, ft	6	8	4	5
Fluidized bed height, m	4	4.9	2.4	2.7
Fluidized bed height, ft	13	16	8	9

[a] Source: US EPA
[b] Modified design as developed by Dorr-Oliver, Inc.
[c] No longer operated for denitrification.
[d] Equalization provided to achieve a constant wastewater flow rate.
[e] Based on mean wastewater flow and fluidized bed/empty bed volume.
[f] Based on total flow to the reactor (plant flow plus recycle).

Table 14.2
Types of FBRs in use (26)

Oxitron system
– Developed by Dorr-Oliver
– System based on Ecolotrol process patents
– Uncertain regarding system marketing in North America
– Dorr-Oliver Europe marketing systems in Europe
Rex aerobic fluidized bed process, anaerobic and biological denitrification configuration
– Developed by Envirex/Ecolotrol based on Ecolotrol process patents
– Sold in North America by Envirex
Custom engineered systems
– Developed by consulting engineering firms
– Normally designed and operated under conditions falling outside the limits of Ecolotrol patents

The principle of the fluidized bed reactor is the same, regardless of the application. Examples of applications to the remediation of groundwater to remove various organic contaminants and produce cleaner and safer water supplies can be found in the literature (50–55).

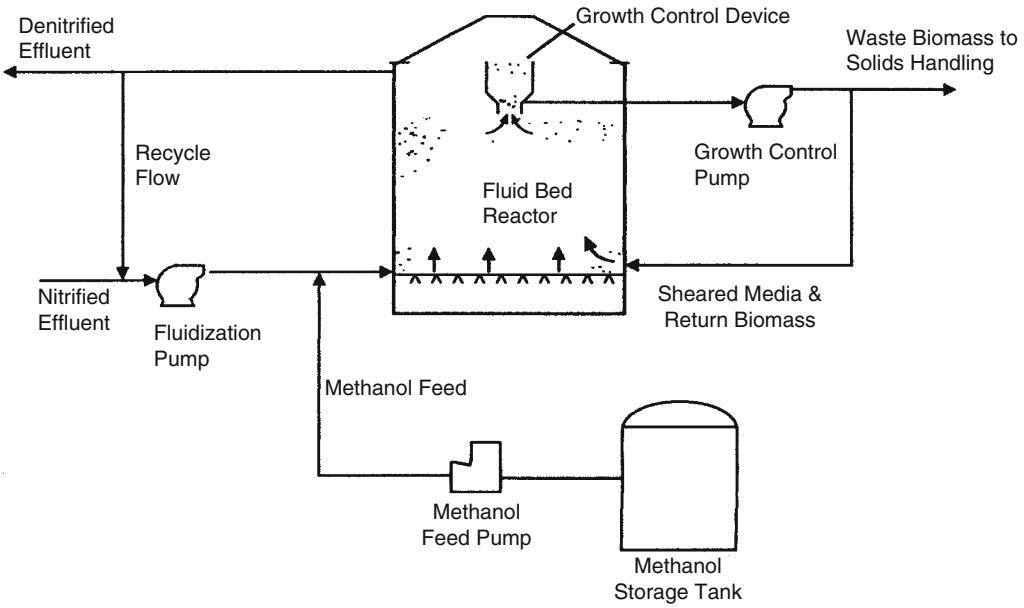

Fig. 14.11. Flow diagram of upflow fluidized bed system (26).

4.4. Design Considerations

The upflow fluidized bed system usually consists of a reactor vessel in the form of an above-ground steel and fiberglass tower or in-ground concrete reactors. The flow rate and strength of waste determines the size of the reactor vessel. The reactor size is dependent on temperature; at 15°C(59° F), the design loading rate is 6,420 kg NO_3^--N/1, 000 m^3/d (400 lb/ 1, 000 ft^3/d) (51).

When the fluidized bed system is operated for denitrification, methanol is fed to the nitrified influent by injection into the recycle line (see Fig. 14.11). The reactor operates as a plug flow process; however, the high recycle ratio of reactor effluent to plant flow (10:1–20:1 for high strength waste treatment and 2:1–5:1 for municipal denitrification) emulates a complete mix system. The high recycle ratio also helps protect the reactor from shock loads and is required to achieve bed fluidization. The amount of recycle is dictated by a maximum allowable fluid-bed height, structural considerations often control bed height (57–60).

4.5. Case Study: Reno-Sparks WWTP

A flow diagram for the 1,753-L/s (40-MGD) Reno-Sparks Wastewater Treatment Plant is shown in Fig. 14.12. The treatment plant, which serves the cities of Reno and Sparks in Nevada, consists of preliminary treatment, primary treatment, phosphorus and BOD removal in a sidestream phosphorous-removal system, nitrification biotowers, denitrification upflow fluidized bed reactors, postaeration, effluent filtration and disinfection. The solids handling system consists of thickening, anaerobic digestion, and dewatering.

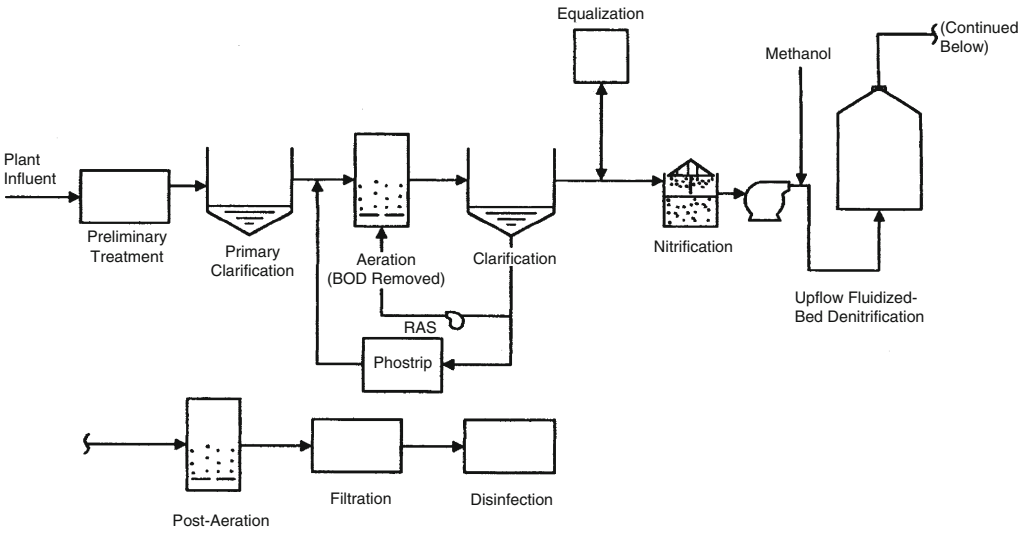

Fig. 14.12. Flow diagram of Reno-Sparks wastewater treatment plant (26).

The denitrification system consists of four upflow fluidized bed towers measuring approximately 8.2 m (27 ft) in diameter by 6.2 m (20.5 ft) high. The hydraulic residence time at average daily flow is 13.8 min, and the solids residence time (SRT) is 8.5 d. The denitrification system, manufactured by Envirex, was designed to produce effluent with a nitrate level of 2 mg/L. A summary of monthly plant operating data is provided in Table 14.3. The data indicate that the Reno-Sparks plant has consistently met its effluent requirements, with an average effluent ammonia level of 0.16 mg/L and a NO_x-N level of 0.29 mg/L. The plant's efficiency in removing total nitrogen has been 94%. The removal rate of the fluidized bed reactors has been 6.4 kg NO_x-N/m^2/d (1.3 lb/ft^2/d), and the plant has regularly produced an effluent TN of less than 3 mg/L and an average effluent TN of 1.78 mg/L. The one event over 3 mg/L TN was 3.55 mg/L.

5. PACKED BED REACTOR

5.1. Aerobic PBR

A packed bed reactor, often referred to as a submerged filter, contains a stationary bed of media, which provides support for biological growth. The influent wastewater (or wastewater plus recycled effluent) is normally introduced at the bottom of the reactor through a flow distribution system. Methods utilized to supply the necessary oxygen to support biomass growth have included direct introduction of air (61) or high-purity oxygen (62) into the bottom of the reactor through a gas distribution system or injection of air or oxygen into the feed line entering the reactor. Alternatively high-purity oxygen has been dissolved in the feed stream in an oxygenation device prior to the feed entering the reactor (61).

Table 14.3
Reno-Sparks wastewater treatment plant: monthly performance data (26)

Month	Plant fluent					Denitrification process influent		Denitrification process effluent		Final plant effluent				
	Flow		BOD_5	NH_4^+-N	TN	NH_4^+-N	NO_x-N	TSS	NO_x-N	BOD	TSS	NH_4^+-N	NO_x-N	TN
	L/s	MGD	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
JAN 90	1,133	25.86	167			0.37	14.44		2.12	5	5	0.13	0.79	2.53
FEB 90	1,153	26.30	171			0.53	14.58		3.91	8	5	0.24	0.43	2.25
MAR 90	1,174	26.79	178			0.31	14.80		0.80	5	4	0.27	0.88	2.79
APR 90	1,156	26.37	177			1.37	14.21		0.82	6	2	0.29	0.90	3.35
MAY 90	1,169	26.66	183			0.67	12.84		0.19	5	3	0.08	0.15	1.38
JUN 90	1,221	27.87	175			0.35	14.02		0.06	3	2	0.11	0.16	1.43
JUL 90	1,202	27.42	152			0.30	14.07		0.04	3	2	0.10	0.06	1.11
AUG 90	1,233	28.14	164			0.91	13.70		0.29	6	10	0.32	0.14	1.82
SEP 90	1,211	27.64	168			0.56	14.18		0.12	7	18	0.41	0.11	2.50
OCT 90	1,175	26.81	149			0.15	11.03		0.03	3	2	0.05	0.01	1.01
NOV 90	1,144	26.10	162			0.37	13.17		0.10	4	3	0.19	0.07	1.26
DEC 90	1,192	27.20	150			1.13	13.92		0.22	4	6	0.33	0.37	2.00
JAN 91	1,168	26.64	132	21.1	32.3	0.45	14.04	22	0.24	3	4	0.17	0.35	1.64
FEB 91	1,122	25.60	130	22.2	32.3	0.46	14.26	20	0.16	3	3	0.18	0.28	1.58
MAR 91	1,149	26.21	135	22.5	32.2	0.35	14.88	18	0.39	3	4	0.10	0.42	1.70
APR 91	1,103	25.17	142	22.7	32.2	0.16	15.56	20	0.31	4	4	0.02	0.28	1.53
MAY 91	1,137	25.95	186	22.0	31.9	0.59	15.07	21	0.17	4	4	0.03	0.14	1.47
JUN 91	1,171	26.72	167	21.0	31.9	0.08	13.72	17	0.03	2	4	0.02	0.03	1.18
JUL 91	1,210	27.36	160	20.1	31.6	0.06	13.85	13	0.08	3	4	0.03	0.03	1.01
Average	1,169	26.67	160	21.7	32.1	0.48	14.02	19	0.53	4	5	0.16	0.29	1.78

In 1975, the EPA Process Design Manual for Nitrogen Control noted that packed bed reactors for nitrification were a comparatively recent development, having progressed from laboratory and pilot status to the point of commercial availability (63). Since that time, packed bed reactors have been widely applied for commercial treatment of industrial wastewaters and contaminated ground waters. Despite continuing interest in packed bed reactors for nitrification of municipal wastewaters (61–66) and additional pilot studies, packed bed reactors have not been widely applied on a full scale. The lack of information clearly demonstrating significant advantages of the technology relative to alternatives for this application has limited the acceptance of packed bed reactors at the full-scale level for municipal wastewater treatment.

Several types of media including stones, gravel, anthracite, and random plastic media, had been successfully utilized in pilot plant studies of packed bed reactors. In more recent studies, the media utilized have normally been either random or corrugated plastic structures with high void volume (61–66). The use of such media may eliminate the need for backwashing to control the buildup of reactor SS. If solids buildup is not prevented or controlled, the hydraulic integrity of the reactor will be compromised. Design and operating strategies that minimize the buildup of reactor SS include:

(a) The use of media with a high void volume (greater than 90%)
(b) The supply of oxygen by the direct introduction of air into the bottom of the reactor

5.2. Anaerobic Denitrification PBR

5.2.1. Coarse Media Beds

When PBRs are used for denitrification, nitrates and nitrites are reduced to nitrogen gas through the action of facultative heterotrophic bacteria. Coarse media denitrification filters are attached growth biological processes in which nitrified wastewater is passed through submerged beds containing natural (gravel or stone), granular activated carbon (GAC), or synthetic (plastic) media. The systems may be pressure or gravity. Minimum bed media size is about 15 mm. Anaerobic or near anaerobic condition is maintained in the submerged bed, and since the nitrified wastewater is usually deficient in carbonaceous materials, a supplemental carbon source (usually methanol) is required (Fig. 14.13) to maintain the attached denitrifying slime (67). Because of the high void percent and low specific surface area characteristic of high porosity coarse denitrification filters, biomass (attached slime) continuously sloughs off. As a result, the coarse media column effluent is usually moderately high in suspended solids (20–40 mg/L), requiring a final polishing step.

A wide variety of media types may be used as long as high void volume and low specific volume are maintained. Both dumped plastic media (Fig. 14.14) and corrugated sheet media have been used. Backwashing is infrequent and is usually done to control effluent suspended solids rather than pressure drop. Alternate energy sources, such as sugars, volatile acids, ethanol, or other organic compounds, as well as nitrogen-deficient materials such as brewery wastes, may be used. Nitrogen gas-filled coarse media denitrification filters are a possible modification.

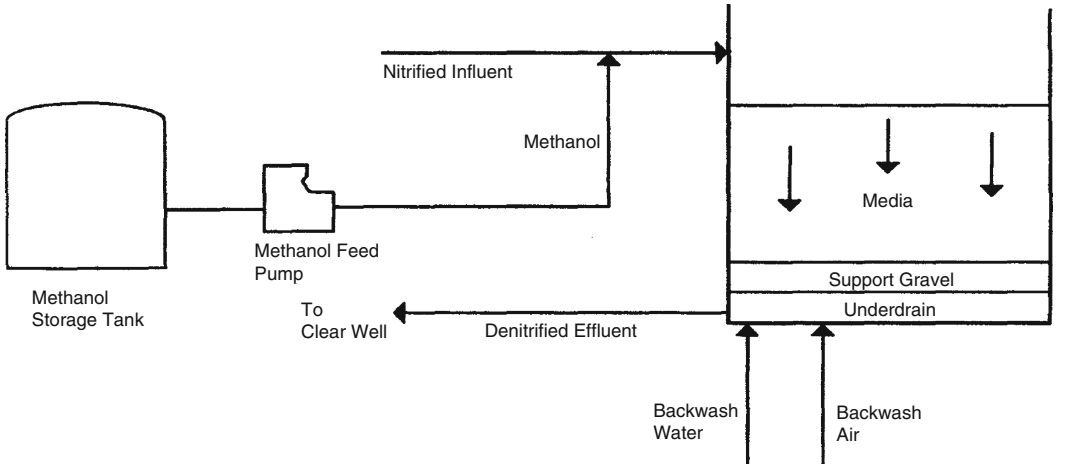

Fig. 14.13. Flow diagram of packed bed reactor system (26).

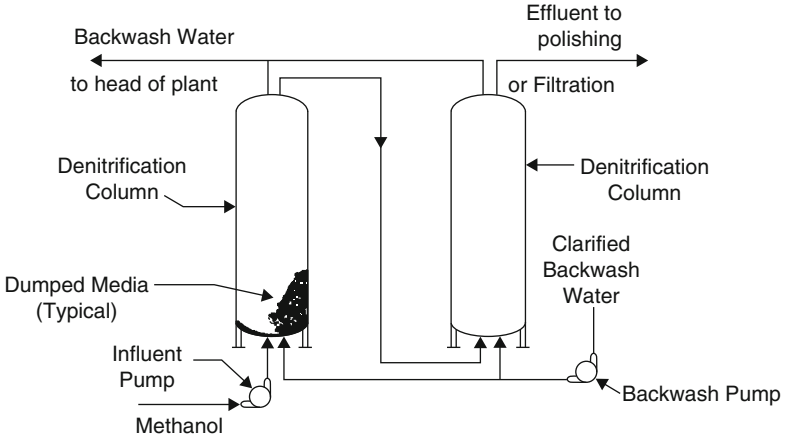

Fig. 14.14. PBR system with coarse media denitrification columns (67).

5.2.2. Fine Media Beds

The fine media denitrification filter is an attached growth biological process in which nitrified wastewater is passed through a pressurized submerged bed of sand or other fine filter media (up to about 15 mm in diameter) in which anoxic conditions are maintained. Because of the relatively fine media used, physical filtration analogous to that occurring in a pressure filter takes place. As a result, a clear effluent is produced, eliminating the need for final clarification (67). Backwashing is required to maintain an acceptable pressure drop. Surface loading rates may be somewhat lower than those common for pressure filtration. Development of the denitrifying slime and consequent denitrification efficiency are a function of the specific surface area of the filter, and in practice fine media denitrification filters convert nitrates to

nitrogen gas at a much higher rate than suspended growth systems. The coarser the media, the less frequent the backwashing, although the effluent may be more turbid.

Common modifications include the use of various media such as garnet sand, silica sand, anthracite coal, or activated carbon with varying size distributions. Multimedia systems have also been used. Alternate energy sources, such as sugars, volatile acids, ethanol, or other organic compounds, as well as nitrogen-deficient materials such as brewery wastewater, may be used. An air scour may be incorporated into the backwashing cycle; however, temporary inhibition of denitrification may result. Various types of underdrains may be used. A bumping procedure (short periodic flow reversals) has been used to remove entrapped nitrogen gas bubbles produced during denitrification. Denitrification may be combined with refractory organic removal. Upflow systems utilizing fine media (sand or activated carbon) have been operated as fluidized bed reactors.

5.3. Applications

PBRs are used mostly for nitrogen removal by biological nitrification–denitrification of municipal wastewater that has undergone carbon oxidation. Examples of packed bed denitrification treatment plants are listed in Table 14.4. Similar units are also used to reduce nitrate in industrial wastewater systems (67).

5.4. Design Criteria

The design criteria for both coarse and fine media PBRs, as stated in US EPA manuals (63, 67), are given below.

5.4.1. Coarse Media Beds

(a) Optimum pH = 6.5–7.5
(b) Voids = 70–96%
(c) Specific surface = 65−274 ft^2/ft^3 = 213.2−898.7 m^2/m^3
(d) Media size = greater than15 mm
(e) Loading rate in lb NO_3-N/ft^2 packing surface/d is a function of temperature up to 0.5×10^{-4} at 5°C, 0.2–0.8 $\times 10^{-4}$ at 15°C and 0.8–1.3 $\times 10^{-4}$ at 25°C. Here 1 1b/ft^2/d = 4.88 kg/d/m^2
(f) Surface loading rate = 2.5 gal/ft^2/d for a flow of 0.3 MGD and 4.1 gal/ft^2/d for a flow 0.5 MGD. Here 1 gal/ft^2/d = 0.0408 m^3/m^2/d; 1 MGD = 43.8 L/s
(g) Amount of the most common energy source, methanol, required may be estimated at 2.47 mg/L CH_3OH per mg/L of NO_3-N and 1.53 mg/L CH_3OH per mg/L NO_2-N in the inlet to the process

5.4.2. Fine Media Beds

(a) Flow Scheme: Downflow (although upflow systems with different design criteria have been utilized)
(b) Optimum pH = 6.5–7.5
(c) Voids = 40–50%
(d) Specific surface = 85–300 ft^2/ft^3 = 278.8–784 m^2/m^3
(e) Media diameter (d_{50}) = 2–15 mm
(f) Surface loading Rates = 0.5–7.0 gpm/ft^2 = 1.22–17.08 m^3/h/m^2
(g) Column depth = 3–20 ft (function of specific surface and contact time) = 0.91–6.10 m
(h) Backwash rate = 8–25 gpm/ft^2 = 19.52–61 m^3/h/m^2
(i) Backwash cycle frequency = 0.5–4.0 d

Table 14.4
Installation list of Packed Bed Denitrification Systems (26)

Faculty and location	Description of packed-bed denitrification system	Capacity	
		L/s	MGD
Tampa, Florida Hookers Point AWTP	Twelve 97 m^2 (1,050 sq ft) fillers Nineteen 93 m^2 (1,000 sq ft) filters	4,208	96.0
Seminole County, Florida NW Area Regional WW Facility Expansion	Two 46 m^2 (500 sq ft) filters	110	2.5
Port Orange, Florida	Six 52 m^2 (560 sq ft) filters	351	8.0
Hillsborough County, Florida Valrico Wastewater Facility	Three 46 m^2 (500 sq ft) filters	132	3.0
U.S. Home Brandon, Florida	One 19 m^2 (200 sq ft) filters	33	0.75
Purity Farms Clearwater, Florida	One 9.3 m^2 (100 sq ft) filters	10	0.23
Hillsborough County, Florida Dale Mabry AWTP	Five 60 m^2 (650 sq ft) filters	264	6.0
Piney Orchards, Maryland	Four 9.3 m^2 (100 sq ft) filters	53	1.2
Hillsborough County, Florida Falkenburg RD AWTP	Five 46 m^2 (500 sq ft) filters	264	6.0
Altamonte Springs, Florida	Seven 56 m^2 (600 sq ft) filters deep-bed filters for tertiary filtration, denitrification, and virus control of municipal sewage treatment plant effluent	110 548	2.5 (avg.) 12.5 (peak)
Florida Cities Water Co. Fiesta Village Fort Myers, Florida	Four 37 m^2 (400 sq ft) filters for nitrate reduction and SS removal	96 220	2.2 (avg.) 5.0 (peak)
Kanapaha Wastewater Treatment Plant Gainesville, Florida	Six 46 m^2 (500 sq ft) filters	308 770	7.0 (avg.) 17.5 (peak)
Parkland III Expansion Islip, New York	Deep-bed gravity denitrification effluent polishing system including four 5.6 m^2 (60 sq ft)	11	0.26
Fairfield Village, New York	Two 5.6 m^2 (60 sq ft) deep-bed sand filters for effluent polishing and denitrification	4	0.085
Southhampton Hospital Southhampton, New York	Two 4.7 m^2 (60 sq ft) deep-bed sand filters for effluent polishing and denitrification	4	0.1
Blue Ridge Condo. Medford	One deep-bed sand filter system. System includes three deep-bed gravity filler cells 5.6 m^2 (60 sq ft) each	9	0.2

(*Continued*)

Table 14.4
(Continued)

Faculty and location	Description of packed-bed denitrification system	Capacity L/s	Capacity MGD
Brookhaven, New York			
Parkland III	One deep-bed gravity filtration system for effluent polishing and denitrification. System includes four deep-bed filter cells 5.6 m^2 (60 sq ft) each	28	0.65
Islip, New York			
Parr Village	Three 48 m^2 (52 sq ft) deep-bed sand filters	20	0.45
Yaphank, New York			

(j) Amount of the most common energy source, methanol, required may be estimated at 2.47 mg/L CH_3OH per mg/L of NO_3-N and 1.53 mg/L CH_3OH per mg/L NO_2-N in the inlet to the process.

5.5. Performance

As with trickling filters, the efficiency and performance of nitrifying packed bed reactors can be expected to correlate to the effective surface area for biofilm growth, although growth of active nitrifiers in the voids of the media may affect this correlation. Thus, both the surface loading and the volumetric loading are likely to influence nitrification efficiency and performance in packed bed reactors. Other factors, such as the concentration of DO, $CBOD_5$, and ammonium in the reactor, environmental conditions (i.e., temperature and pH), and media characteristics (i.e., surface-to-volume ratio and percent voids), will influence the correlations between loading and nitrification performance. Although surface and volumetric loading information applicable to the design of packed bed reactors for nitrification of municipal wastewaters is available (61–65), onsite piloting is recommended if the technology is being considered for use on a full scale.

Packed bed reactors are capable of converting nearly all nitrates in a nitrified secondary effluent to gaseous nitrogen. Overall nitrogen removals of 70–90% are achievable. In fine media beds Suspended solids removals of up to 93% have been achieved. Under controlled pH, temperature, loading and chemical feed high levels of reliability are achievable. Studies on the effects of environmental factors, modeling and kinetics in full scale submerged denitrification PBRs can be found in refs. (68) and (69).

With controlled supplemental carbon feed rates, little excess sludge is generated. Sludge production varies between 0.6 and 0.8 lb/lb NH_3-N reduced.

5.6. Case Study: Hookers Point WWTP (Tampa, Florida)

Operating data for downflow packed bed systems are shown in Table 14.5. The 4,208-L/s (96 MGD) Hookers Point Wastewater Treatment Plant (WWTP) includes preliminary treatment, primary treatment, biological treatment, postaeration, and effluent disinfection.

Table 14.5
Operating Data for downflow Packed Bed Reactor Systems (26)

Facility	Florida location	Capacity L/s	Capacity MGD	Average rate $m^3/m^2/d$	Average rate gpm/sq ft	Number of denit. filters	Filter size	Media depth m	Media depth in.	Media size, mm
Hookers Point	Tampa	4,208	96.0	123	2.1	20	3 m × 32 m (10 ft × 105 ft)	1.47	54	2.3
Fiesta Village	Ft. Myers	220	5.0	117	2.0	4	3 m × 13.4 m (10 ft × 44 ft)	1.83	72	3.0
Altamonte Springs	Altamonte	548	12.5	123	2.1	7	3 m × 18.3 m (10 ft × 60 ft)	1.83	72	Dual media
Faulkensand Road, Hillsborough Co.	Tampa	264	6.0	29	0.5	5	3 m × 15.2 m (10 ft × 50 ft)	1.22	48	3.0
Dale Mabry	Tampa	264	6.0	123	2.1	5	3 m × 19.8 m (10 ft × 65 ft)	1.83	72	2.3
Port Orange	Port Orange	526	12.0	123	2.1	7	3 m × 17.1 m (10 ft × 56 ft)	1.07	42	1.8

Note: All plants have a 3 mg/L permit limit

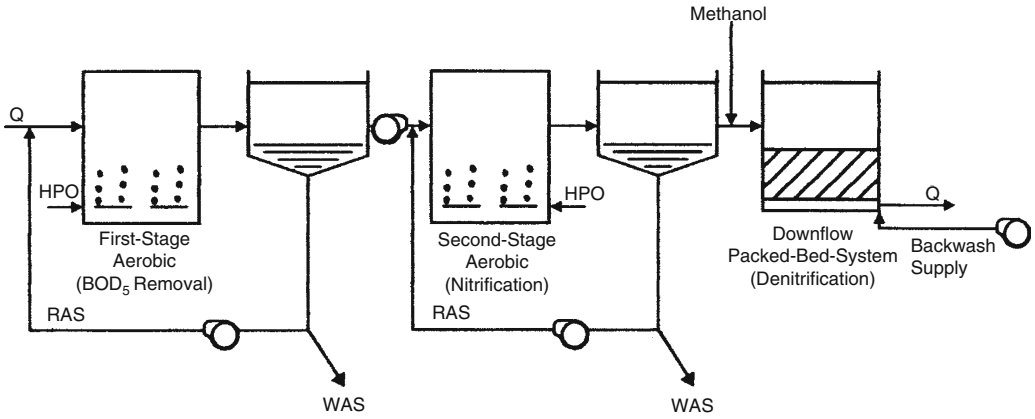

Fig. 14.15. Flow diagram of Hookers point advanced wastewater treatment plant (26).

The biological treatment system includes two-stage carbonaceous oxidation/nitrification using high-purity oxygen and a separate-stage downflow packed bed denitrification system with methanol feed. A flow diagram is shown in Fig. 14.15.

The downflow packed bed denitrification system consists of 20 filters measuring 3 m × 32 m (10 ft × 105 ft). Each filter is filled with 142 cm (56 in.) of coarse sand (2.3 mm), loaded at an average rate of 59–117 $m^3/m^2/d$ (1–2 gpm/ft^2) and having an empty bed contact time of 45 min at average flow.

The Hookers Point WWTP receives domestic wastewater, with a 30% contribution from breweries (70). The influent wastewater has a BOD_5 of 224 mg/L, TSS of 221 mg/L, and TKN of 32 mg/L. The current effluent limits of the plant are 5 mg/L for BOD_5 and TSS, 3 mg/L for TN on an annual average basis, and 7.5 mg/L for total phosphorous (TP). The average month's effluent is below 3 mg/L TN 83% of the time, with an average over 3-year period of 2.33 mg/L. It should be noted that the effluent limit was changed to 3 mg/L TN in 1990. Prior to that time, the limit was 4 mg/L TN in summer and 5 mg/L in winter. The average effluent TSS is 2 mg/L and is relatively stable. Hookers Point has a process loading rate of 1.32 kg NO_x-N/m^2/d (0.27 lb/ft^2/d). The brewery waste may contribute significantly to the background nitrogen removal by synthesis. The plant's overall efficiency in removing nitrogen and SS has been 93 and 99%, respectively.

5.7. Energy Requirement

5.7.1. Coarse Media Beds

Pumping energy can be computed from the following equation (67):

$$\text{kwh/year} = (1,140\,\text{MGD} \times \text{ft of total average head})/\text{wire-to-water efficiency}$$

For a 0.5 MGD (1.89 MLD) plant treating 14 mg/L of NO_3-N, two 10-ft (3.05 m) diameter by 10-ft (3.05 m) deep tanks would be required. Therefore, using 15 ft of total head and a wire-to-water efficiency of 0.60, 14,250 kwh will be required for wastewater pumping.

Backwashing at a rate of 20 gpm/ft^2 (814 Lpm/m^2), once a month for 4 h would require an additional energy consumption of 1,425 kwh/year.

Upflow and downflow operations consume roughly the same amount of energy.

5.7.2. Fine Media Beds

The energy requirement for PBR fine media beds is shown in Fig. 14.16. The assumptions for energy determination are as follows (71):

(a) Influent NO_3-N = 25 mg/L; effluent = 0.5 mg/L
(b) Media size = 2–4 mm
(c) Temperature is 15°C
(d) Methanol feed rate = 3 : 1(CH_3OH : NO_3-N)
(e) Loading rate = 1.7 gpm/ft^2 = 4.15 $m^3/m^2/h$ = 69.2 Lpm/m^2
(f) Depth = 6 ft = 1.83 m
(g) Backwash for 15 min at 25 gpm/ft^2 (1017.5 Lpm/m^2) and 25 ft (7.6 m) TDH once per 2 d for pressure and daily for gravity system.

5.8. Costs

5.8.1. Coarse Media Beds

The construction cost for PBR coarse media beds is determined as follows: for a 0.5 MGD (21.9 L/s) plant treating 14 mg/L NO_3-N, two 10-ft (3.05 m) diameter by 10-ft (3.05 m) deep tanks would be required. Construction costs (1972 Dollars, Utilities Index = 141.94) for such a system was approximately $200,000 (63). To obtain the value in terms of the present 2009

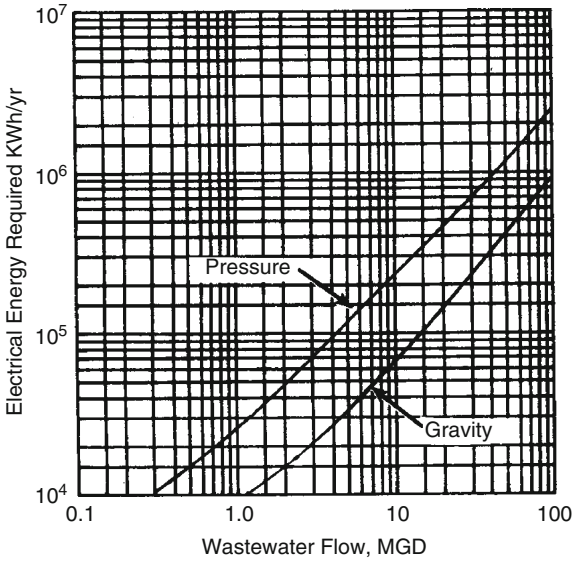

Fig. 14.16. Energy requirements for PBR system (67). (Conversion factor: 1 MGD = 3.785 MLD = 43.8 L/s)

U.S. Dollars, using the Cost Index for Utilities (Appendix), multiply the cost by a factor of 570.38/141.94 = 4.02 (72). Thus, the 2009 construction cost for 0.5 MGD (21.9 L/s) PBR beds would be 200, 000 × 4.02 = U.S.$804, 000.

The cost for chemicals (Methanol) is \$0.03 × 4.02 = \$0.12/1, 000 gal (63, 67, 72). The O & M cost for labor is \$0.03 × 4.02 = \$0.12/1, 000 gal. Thus the total operation and maintenance cost in terms of 2009 U.S. Dollars would be \$0.24 per 1,000 gal treated. Here \$1.00/1,000 gal = \$0.2642/1,000 L = \$0.2642/m^3.

5.8.2. Fine Media Beds

Construction costs (1975 Dollars, Utilities Index = 190.49) for PBR fine media beds are shown in Fig. 14.17 (67). To obtain the values in terms of the present 2009 U.S. Dollars, using the Cost Index for Utilities (Appendix), multiply the costs by a factor of 570.38/190.49 = 2.99 (72).

The operation and maintenance costs for a 0.5 MGD (21.9 L/s) plant treating 14 mg/L NO_3-N is determined as follows: The cost for chemicals (Methanol) is \$0.03 × 4.02 = \$0.12/1, 000 gal (63, 67, 72). The O & M cost for labor (including normal maintenance and daily backwash) is \$0.04 × 4.02 = \$0.16/1, 000 gal. Thus, the total operation and maintenance cost in terms of 2009 U.S. Dollars would be \$0.28 per 1,000 gal treated. Here \$1/1,000 gal = \$0.2642/1,000 L = \$0.2642/m^3.

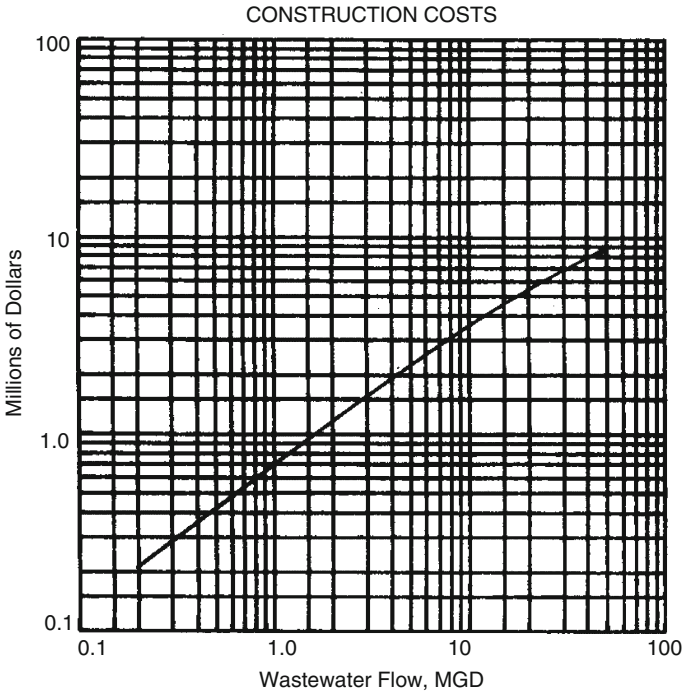

Fig. 14.17. Construction cost for PBR system (67). (Conversion factor: 1 MGD = 3.785 MLD = 43.8 L/s)

6. BIOLOGICAL AERATED FILTER

6.1. BAF Process Description

In the biological aerated filter (BAF), the media are submerged in the reactor, and primary clarified wastewater is introduced at the top of the reactor (26). As noted in an US EPA-sponsored study (73), BAF systems are very similar in both physical appearance and mode of operation to a downflow water filter or tertiary wastewater filter except that:

(a) A coarser, low-density medium is utilized.
(b) Air is diffused upward through the media during operation.

The air is introduced into the media through an air diffusion system located approximately 20–25 cm (8–10 in.) above the filter underdrain system (26, 73). This air is supplied to promote biomass growth in the voids of the packed bed and on the media surface above the air diffusion system. The function of the media below the air diffusion system is to remove SS. As newly grown biomass and influent SS buildup in the reactor, the head loss across the unit increases. The unit is backwashed when a predetermined headloss is reached. The backwashing operation involves a series of air scours and liquid flushes with treated effluent. The intent of this operation is to release SS trapped in the voids of the packed bed and to

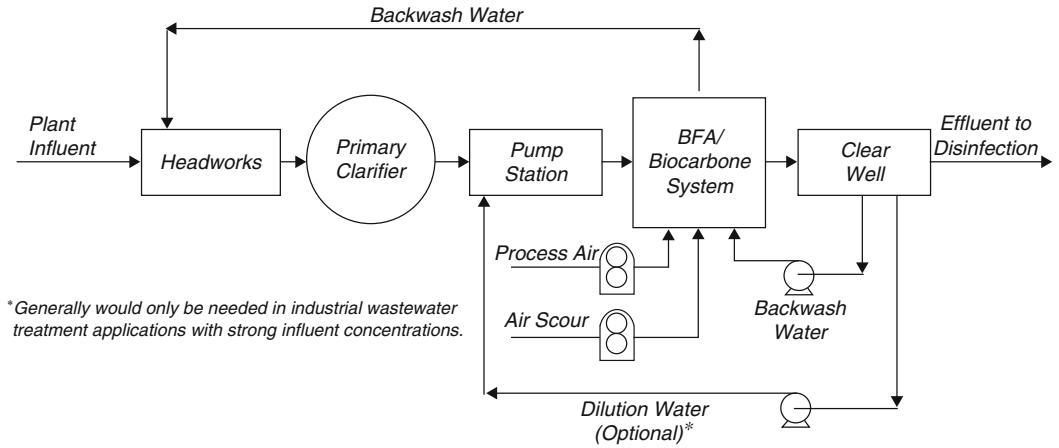

Fig. 14.18. Flow diagram for a biological aerated filter (BAF) system (74).

control the extent of film growth on the media surface (26). The backwash water is either thickened separately or conveyed to primary clarification at the head end of the plant. A common process flow diagram for a complete Biocarbone BAF system is shown in Fig. 14.18 and details of a Bicarbone filter unit is shown in Fig. 14.19. Biocarbone is the trademark name given to Omnium de Traitement et de Valorisation (OTV) commercial embodiment of the process.

When treating primary effluent, the BAF/Biocarbone process can be designed to achieve carbonaceous BOD removal only or carbonaceous BOD removal and nitrification by selecting appropriate loading rates. The process can also be designed to achieve advanced secondary treatment removals of BOD and suspended solids as well as nitrification with either primary or secondary effluent feed (74).

The primary advantage of the BAF is biological treatment and solids separation in the same reactor eliminating the requirement for separate secondary clarification. Consequently, the technology could reduce the space requirements for treatment relative to more conventional technologies such as the activated sludge system (26).

The advantages of the BAF process can be summarized as follows:

(a) Absence of secondary clarifier
(b) Compactness. Good alternative when land availability is low or expensive since the reactor has a compact footprint
(c) Modular design and implementation to suit various flow conditions and effluent quality requirements
(d) Considerable inertia against pollution breakthrough under load variations with peak flows up to three times the average
(e) The rapid startup (relative to activated sludge) allows for adjustment in the number of units in service to match the pollution load arriving at the plant

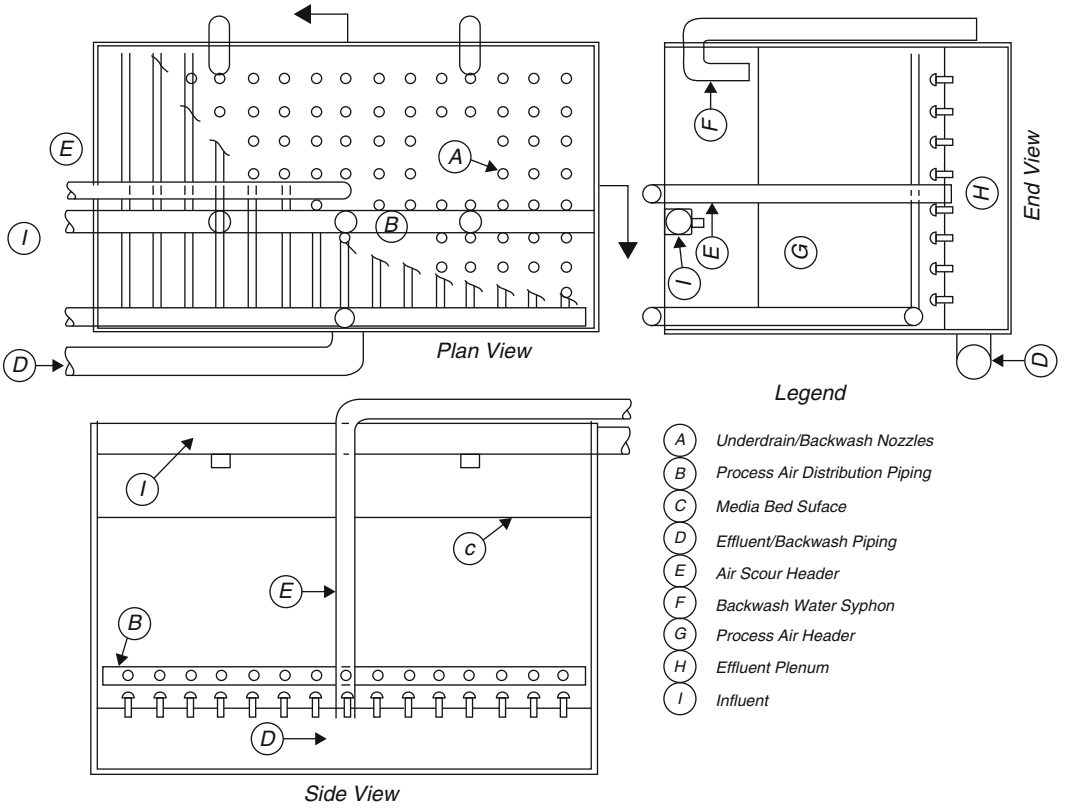

Fig. 14.19. Plan and side views of a BAF/bicarbone unit (74).

6.2. Applications

The first commercial, full-scale BAF system began operation in 1982 in Soissons, France (75). Since that time, a number of systems have been installed in Europe, Japan, and North America (73, 76). As of 1990, there were approximately 30 commercial full-scale Biocarbone BAF systems installed or under construction, designed at wastewater flows of 22 L/s (0.5 MGD) or greater (76). The largest Biocarbone BAF system installed to date is designed to treat approximately 1,056 L/s (24 MGD) (75). Most Biocarbone BAF systems in operation today have been designed for $CBOD_5$ and TSS removal, but the systems can be designed to nitrify primary or secondary effluent.

6.3. BAF Media

The original media employed in the Biocarbone BAF was activated carbon. This material had the desirable characteristics of a porous surface with a high surface-to-volume ratio for enhancing biomass attachment and a low specific gravity to allow for ease of air scouring and backwashing, but it was found too expensive. Subsequently, alternative granular media have

Table 14.6
Recommended BAF media gradation (74)

Media gradation (mm)	Effluent BOD (mg/L)	Effluent TSS (mg/L)
2–4	10	10
3–6	20	20
4–8	30	30

been used for economic reasons. The media in most currently operating BAF systems consist of a kiln-fired clay or shale particle. Biodamine and Biodagene are the names given to two of the media, often used in the Biocarbone BAF (73). Biodamine is an angular-shaped media, whereas Biodagene is more spherical.

The angularity and size range of the media significantly affects the BAF treatment performance and operating requirements. The use of smaller media in the range of 2–4 mm (0.08–0.16 in.), although it offers a superior effluent quality to that of a system with larger sized media, normally requires more frequent backwashing (73). The smaller media have been recommended when nitrification is required (76). Expected effluent quality as a function of media gradation is shown in Table 14.6 (74).

6.4. Process Design and Performance

OTV through years of conducting pilot- and full-scale Biocarbone plant evaluations has developed reliable correlations between applied pollutant and/or hydraulic loading rates and effluent quality or percent pollutant removal (73, 74).

One of these generalized correlations is depicted in Fig. 14.20 for two types of media, activated carbon and biodamjne (vitrified clay particles). Effluent quality from a Biocarbone unit is graphically depicted in Fig. 14.21.

Pilot plant studies by the developer of the Blocarbone BAF system (73) indicate that for a system treating primary effluent wastewater containing a high $CBOD_5$ concentration, nitrification is governed in part by the COD volumetric loading. The volumetric loading is based on the volume occupied by the media (i.e., empty bed volume). The results (Fig. 14.22) indicate that at a COD volumetric loading above 0.2 lb/ft^3/d (3.2 kg/m^3/d), nitrification is substantially reduced because of increased heterotrophic organism growth and associated oxygen consumption. The above loading condition is of concern mainly when primary effluent must be nitrified in conjunction with removing carbonaceous BOD.

Nitrification of secondary effluent, on the other hand, is governed mainly by the TKN loading to a Biocarbone unit. Between nitrogen loadings of 0.010 and 0.037 lb TKN/ft^3/d (0.16 and 0.59 kg/m^3/d), NH_3-N removal decreases at a relatively linear rate, from about 90–84% (Fig. 14.22). Loadings above about 0.037 lb TKN/ft^3/d (0.59 kg/m^3/d) result in substantially reduced NH_3-N removal rates (74).

Based on data from another Biocarbone pilot plant study (76), a COD volumetric load of less than 2.0 kg/m^3/d (0.125 lb/ft^3/d) was required to achieve approximately 90% ammonium oxidation in a single BAF unit. The BAF medium used in the pilot study was metamorphosed shale with a grain size between 3 and 6 mm (0.12–0.24 in.).

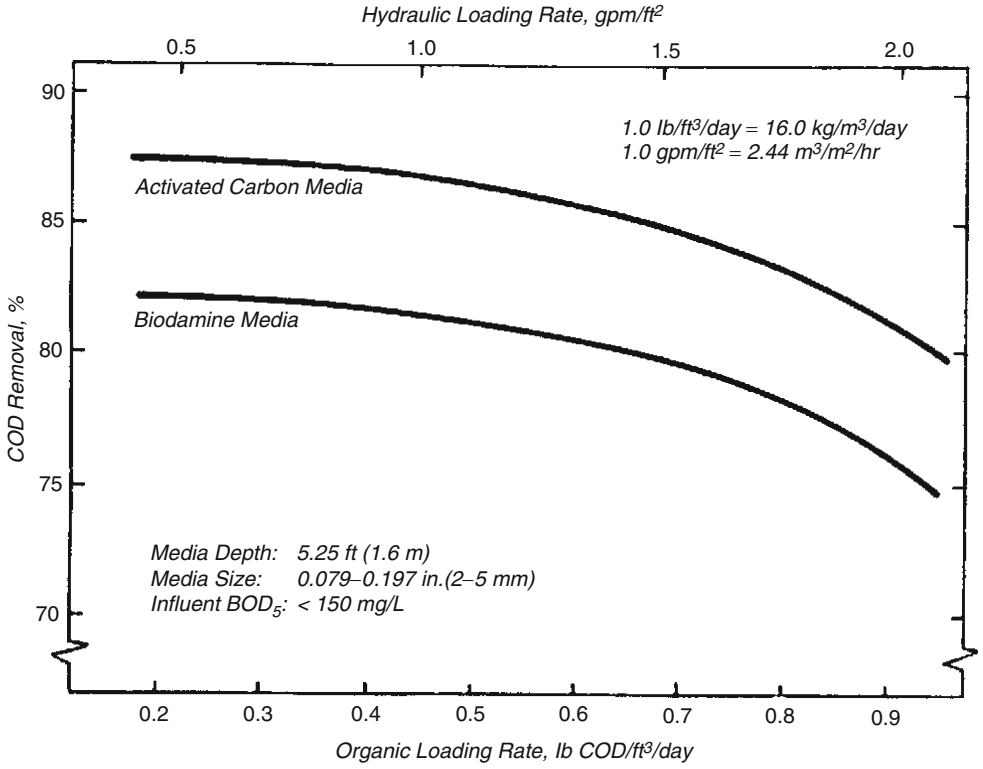

Fig. 14.20. COD removal as a function of BAF influent COD and hydraulic loading rates (74).

Pilot plant studies also provided data on the temperature dependence of NH_3-N oxidation. Based on ammonia-N oxidation in secondary effluent, OTV reported removal rates to approximate the following (74):

(a) At 12°C (54°F) the ammonia-N removal rate is 0.39 kg/m^3/d (0.024 lb/ft^3/d)
(b) At 18°C (64°F) the ammonia-N removal rate is 0.50 kg/m^3/d (0.031 lb/ft^3/d)
(c) At 24°C (75°F) the ammonia-N removal rate is 0.60 kg/m^3/d (0.037 lb/ft^3/d)

According to results from the operation in the United States of a full-scale demonstration Biocarbone BAF plant treating primary municipal wastewater in the mid-1980s (77), the BOD_5 volumetric loading must be limited to approximately 1 kg/m^3/d (0.0624 lb/ft^3/d) to achieve near 90% ammonium oxidation in a single unit. This conclusion is based on operation at temperatures as low as 11°C (52°F) using a vitrified clay medium with an effective size of 3.4 mm (0.13 in.) and a uniformity coefficient between 1.5 and 1.6. Other more recent full-scale Biocarbone BAF plant assessments indicate that to achieve an average effluent ammonia-N concentration of 2.5 mg/L in the treatment of primary effluent, the COD volumetric loading must be limited to approximately 5 kg/m^3/d (0.312 lb/ft^3/d). The volumetric loading rate results indicate that carbonaceous oxidation and nearly complete nitrification of primary

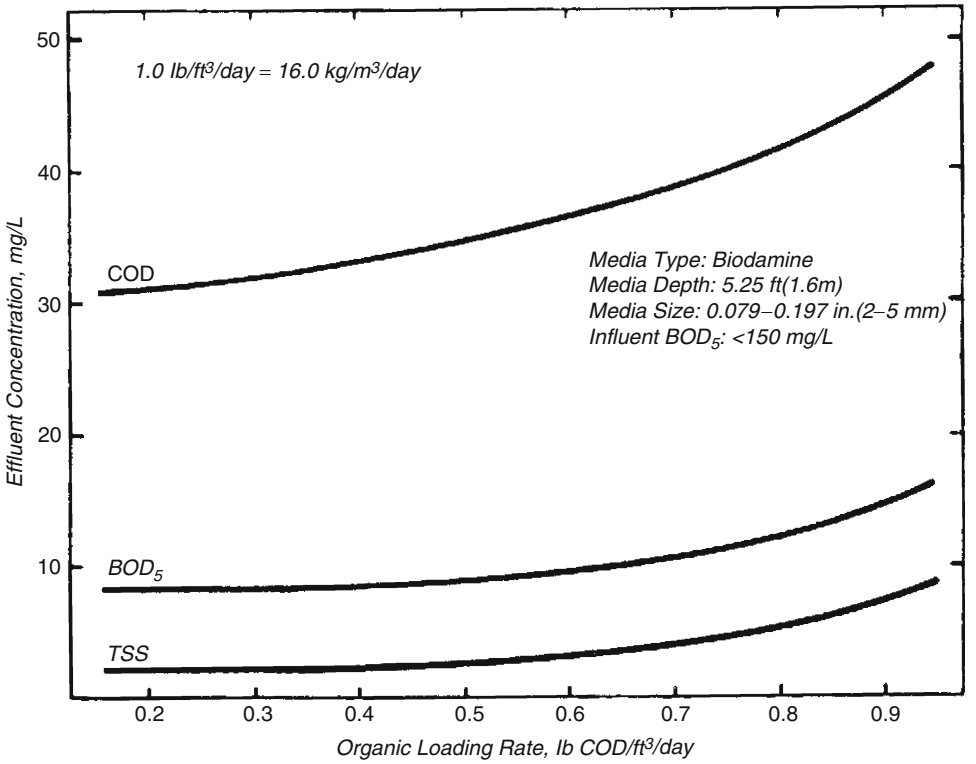

Fig. 14.21. BAF effluent quality as a function of influent COD loading rate (74).

treated wastewater can be achieved in single BAF units at an empty bed hydraulic retention time of approximately 1.5–3.5 h.

BAFs are typically designed to treat municipal wastewaters with low carbonaceous feed concentration, such as that characteristic of secondary effluent. In an US EPA-sponsored, detailed assessment of BAFs (73), information derived from operation of a full-scale BAF unit treating secondary effluent was used to develop a design approach to predict the empty bed hydraulic retention time required to achieve nitrification. At an influent BOD_5 and TSS concentration of approximately 20 mg/L, a hydraulic retention lime of 0.83 h was predicted to be required to reduce the ammonium nitrogen from approximately 21–7 mg/L. These results translate to an ammonium-nitrogen loading of $0.58\,kg/m^3/d$ ($0.036\,lb/ft^3/d$). Other reports indicate that over 90% removal of ammonium nitrogen is achievable at comparable volumetric loading rates at temperatures as low as 13.5°C (56.3°F) (75). Design parameters extracted from various publications (74–87) are listed in Table 14.7.

Although full-scale application of BAFs for municipal wastewater treatment has become widespread in recent years, particularly in Europe (76), the amount of operating and performance information on U.S. installations is limited. The lack at an extensive data base on

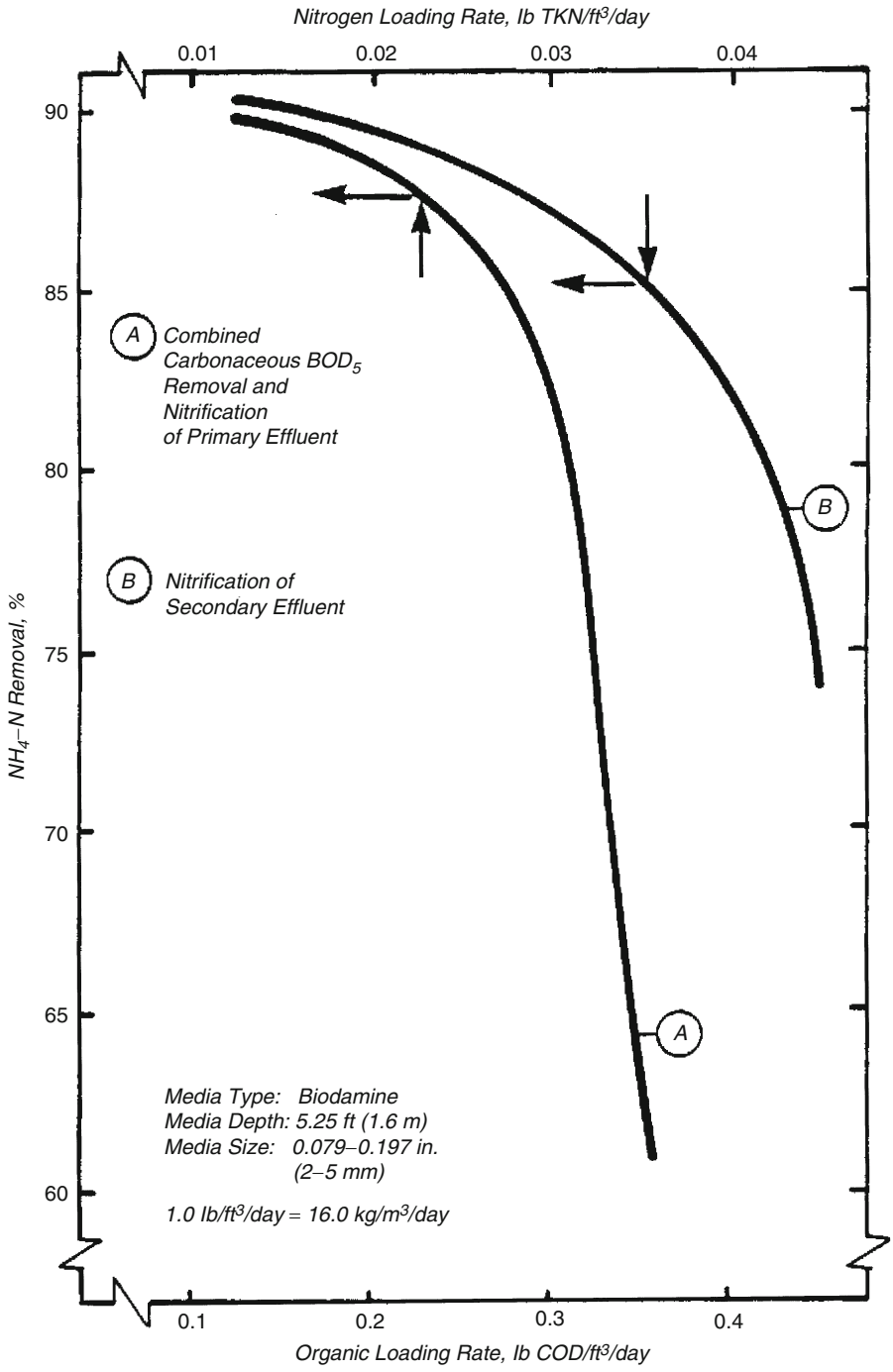

Fig. 14.22. Effect of COD loading on BAF nitrification performance (74).

Table 14.7
Design parameters for biological aerated filter (BAF)

Parameter	Units	Range
Organic loading	kg BOD/m^3/d	3–5
Hydraulic loading	m^3/m^2/d	1–4
Contact time	h	0.5–1
Sludge production	kg SS/kg BOD	0.6–0.9
Bed height	m	2–3
Backwashing	m^3	2.5–3 × filter volume
Backwashing time	min daily	20
Energy consumption	kwh/kg BOD	1.0–1.3

nitrification applications suggests that onsite piloting may be warranted before selecting a technology (26).

6.5. Solids Production

The solids production rate in the BAF/Biocarbone process is a function of, among other factors, the quantities of soluble BOD, nonbiodegradable TSS, NH_3-N, and TKN removed. OTV initially used the historic solids production approximation of 0.7–0.8 lb solids/lb total BOD removed (0.7–0.8 kg solids/kg total BOD removed). A larger data base acquired from both pilot- and full-scale facilities yielded the following two modifications by OTV to their historic solids production value:

Solids production rate (50):

$$= 0.4\,\text{lb/lb soluble } BOD_5 \text{ removed} + 1.0\,\text{lb/lb insoluble } BOD_5 \text{ removed} \quad (2)$$

$$= 0.4\,\text{lb/lb soluble } BOD_5 \text{ removed} + 1.0\,\text{lb/lb TSS removed} \quad (3)$$

$$= 0.4\,\text{kg/kg soluble } BOD_5 \text{ removed} + 1.0\,\text{kg/kg insoluble } BOD_5 \text{ removed} \quad (2a)$$

$$= 0.4\,\text{kg/kg soluble } BOD_5 \text{ removed} + 1.0\,\text{kg/kg TSS removed} \quad (3a)$$

Either of the above predicted models may be used to approximate the net solids production rate.

7. HYBRID BIOLOGICAL-ACTIVATED CARBON SYSTEMS

7.1. General Introduction

While the following processes were developed in laboratory experiments and verified in pilot studies in 1980s, they became popular only recently:

(a) First physicochemical fluidized bed GAC process
(b) First biological fluidized bed GAC process
(c) First physicochemical GAC sequencing batch reactor (SBR)
(d) First biological GAC-SBR
(e) First combined dissolved air flotation (DAF) and GAC process
(f) First DAF-PAC process
(g) First physicochemical PAC-SBR process
(h) First biological PAC-SBR process
(i) First physicochemical PAC-DAF-SBR process
(j) First biological PAC-DAF-SBR process
(k) First ion exchange SBR process
(l) First physicochemical SBR process, and
(m) First regenerable gas phase GAC system.

Because of the importance of the above technologies, many U.S. patents concerning GAC/PAC in combination with SBR, DAF, and precoat filtration were filed by and granted to Wang and his co-workers (88–91)

The biological GAC filtration process was introduced as a competitive process to DAF-GAC process in 1989 (92). Mainstream Bio-Manipulation systems Ltd., adapted both the slow sand filtration and biological GAC filtration processes in 1996 for drinking water production (93). In 2003, the first dual-stage biological GAC filtration plant is the 230-ML/d (230-million liters per day) Ngau Tam Mei Water Works, Hong Kong, China (94). In 2000, the first biological fluidized bed GAC system was built by both Envirogen and US Filter for groundwater decontamination (95).

7.2. Downflow Conventional Biological GAC Systems

7.2.1. Introduction

The granular activated carbon (GAC) adsorption system can remove many adsorbable organics and inorganics, but not nonadsorbable pollutants, such as dimethylnitrosamine, acetone cyanohydrin, butylamine, choline chloride, cyclohexylamine, diethylene glycol, ethylenediamine, triethanolamine, and ethanol. A biological process, on the other hand, can remove biodegradable pollutants and not any nonbiodegradable pollutants. Combination of both processes will solve many traditionally unsolvable environmental pollution control problems.

It has been recognized by researchers and engineers that biological activity plays a major role in the removal of organics by activated carbon. When granular activated carbon is used simultaneously as the filtration and biological growth media in an attached growth biological oxidation–adsorption system, such a combination is called biological GAC adsorption system.

The conventional biological GAC process consists of a fixed bed of granular activated carbon media over which wastewater is applied for aerobic biological and adsorption treatment aiming at the removal of toxic organic substances. Biological slimes form on the GAC media, which assimilate and oxidize substances in the wastewater. The bed is dosed by a distributor system, and the treated wastewater is collected by an underdrain system.

The organic material present in the wastewater is degraded by population of microorganisms attached to the GAC media and partially adsorbed by GAC macropores and micropores. The thickness of the slime layer increases as the microorganisms grow during the bio-oxidation process. The macropores and micropores of GAC are also gradually saturated by the target organic pollutants during adsorption. The microorganisms are also partially responsible for continuous GAC regeneration and prolonged adsorption. Periodically, the GAC bed must be backwashed and regenerated for reuse.

Both downflow pressurized biological GAC system and downflow gravity biological GAC system are technically feasible for water and wastewater treatment as long as oxygen is available for bio-oxidation (96, 97).

7.2.2. Saskatchewan-Canada Biological GAC Filtration Plant for Biological Treatment of Drinking Water

Slow sand filtration (water moves through such filters 10–20 times slower than in rapid sand filters) relies on the formation of a biological layer at the top of the filter. The filter does not become effective until this layer has been formed (92, 93). The American Water Works Association (AWWA) states: "The slow sand filtration process is expected to remove such biological particles as cysts, algae, bacteria, viruses, parasite eggs, nematode eggs, and amorphous organic debris at 100- to 10,000-fold levels when the filter is biologically mature." As effective as sand filtration can be, it is possible to maintain much greater numbers of microorganisms if the support material is GAC instead of sand. It is therefore preferable to use GAC for the removal of dissolved organics (93).

Mainstream Bio-Manipulation Systems Ltd., Canada, has, with the support of the National Research Council, worked on adapting both the slow sand filtration and biological GAC filtration processes. Such treatment systems have been installed at three different sites across Saskatchewan. One site has been in operation since 1996 and removal rates of turbidity, dissolved organic carbon, and color have been good for both the sand filter and the biological GAC filter. Both have provided high-quality household water with no color or odor (removal rates of turbidity, dissolved organic carbon, and color are consistently above 50%). For drinking water purposes, the water is polished by a reverse osmosis unit. All of the household water was hauled before installation of the biological treatment system. Based on successes like this one, it is anticipated that biological treatment will become one of the most common future treatment tools for dealing with surface waters on the Canadian prairie (93).

7.2.3. Ngau Tam Mei Water Works, Hong Kong, China

In 1994, facing projected shortfalls of potable water for the North Western New Territories of Hong Kong, the water supplies department initiated new facilities for treatment,

conveyance, and storage of water from its major supply, the Dongjiang River in Guangdong Province, People's Republic of China, via the Western Aqueduct.

In 2000, the Ngau Tam Mei water treatment works was commissioned, officially opening on December 2. It is the first water treatment plant worldwide to use dual-stage biological filtration with granular activated carbon (GAC) to remove ammonia, replacing break-point chlorination (94). The HK$ 1.8 billion (US$ 227 million) project treats river raw water, which is contaminated by wastewater. The plant was designed with an initial capacity of 230 ML/d, expandable to 450 ML/d. Here 1 ML/d = 1 MLD = 1 million liters per day.

The innovative plant was able to meet or surpass the required water quality goals by employing the following treatment units:

(a) Four preozone contact tanks with a design detention time of 5 min
(b) Twelve triple-deck sedimentation basins with a designed surface loading rate of 1.3 m/h
(c) Intermediate ozone contact tanks with a design retention time of 15 min for achieving 1-log inactivation of Cryptosporidium
(d) Twelve first-stage GAC (1.5-m depth) filters with minimum filters run time of 24 h and filtration rate of 12 m/h, followed by 12 s-stage GAC (1.8-m depth) filters with a filtration rate of 8 m/h, and
(e) Ozone peak dosage of 5 mg/L, ozone production rate of 1,150 kg/d, and ozone concentration of 7.5%.

The plant has been designed such that it is able to reduce O & M cost by:

(a) Generating high-quality oxygen on site, eliminating more costly truck-delivered liquid oxygen
(b) Using dual-stage GAC filters to remove ammonia, eliminating break-point chlorination
(c) Providing flexibility for operating in direct-filtration mode during periods of acceptable raw water quality to reduce coagulant chemical doses and sludge production, and
(d) Reducing labor cost and improving plant management through a supervisory control and data acquisition (SCADA) system (94)

The three special advanced features of this largest biological GAC filtration plant include: (94)

(a) *Dual-stage biological GAC filtration*. A first-of-its-kind application in drinking water treatment. First-stage filters remove turbidity, biodegradable organic carbon, and taste- and odor-causing compounds. Second-stage filters remove ammonia, eliminating break-point chlorination and associated high chlorine doses. Results since commissioning show complete removal of ammonia (effluent concentration < 0.02 mg/L)
(b) *Ozonation for primary disinfection*. This inactivates Giardia and Cryptospordium, and reduces chlorine usage, helping to eliminate formation of chlorinated byproducts (THMs) and enhancing downstream biological filtration by oxygenating water and increasing formation of biodegradable organic carbon.
(c) *Ozonation for manganese removal*. Process uses preozonation for oxidation of reduced manganese to its insoluble form (manganese dioxide) for subsequent removal by coagulation and settling, followed by intermediate ozonation, which oxidizes remaining manganese in the settled water to permanganate for subsequent catalytic removal by first-stage GAC filters.

7.3. Upflow Fluidized Bed Biological GAC System

Upflow fluidized bed biological GAC system (FBB-GAC) has less clogging problem than the two downflow biological GAC systems introduced previously. Accordingly, the downflow

biological GAC filtration process is mainly used for potable water treatment, while the upflow fluidized bed biological GAC system may be used for both water and wastewater treatment (92, 98). Many researchers are studying the upflow fluidized bed biological GAC systems (95, 98–100). The first fluidized bed biological GAC system was designed and built in 2000 by Envirogen and US Filter for groundwater decontamination (95).

The FBB-GAC system (Hydroxyl Systems' Fluidized Bed Bioreactor) shown in Fig. 14.23 can be used in aerobic, anoxic, or anaerobic conditions, and can accommodate a variety of granular media (101). When adsorbent media, such as granular activated carbon (GAC), is used, the FBB combines the benefits of adsorption and bio-oxidation. Contaminants are adsorbed onto the media surface and oxidized by the biofilm, which is formed on the GAC surface. Unlike other biological treatment systems, the requirement for operator attention is minimal and unattended operation is practical. One of the most outstanding features of the FBB-GAC is that treatment detention times are typically minutes rather than hours.

The FBB-GAC system is supplied either as a single skid module of shippable height, incorporating a low profile reactor, or as a two-piece unit with a detachable tall cylindrical reactor. The system is used for aerobic, anoxic, or anaerobic treatment of waterborne biodegradable matter, particularly adsorbable contaminants in low mg/L concentrations. Typical applications include treatment of groundwater contaminated with BTEX and as a complement to advanced oxidation technologies for complete mineralization of biorefractory contaminants. As an anaerobic reactor, the FBB-GAC system can be used to treat high-strength wastewaters. Typical treated contaminants include BTEX, glycol, MTBE, soluble Oil & Grease and organic solvents.

The FBB-GAC system has the following special features (101):

(a) Fast bio-oxidation
(b) Fully automated with PLC control

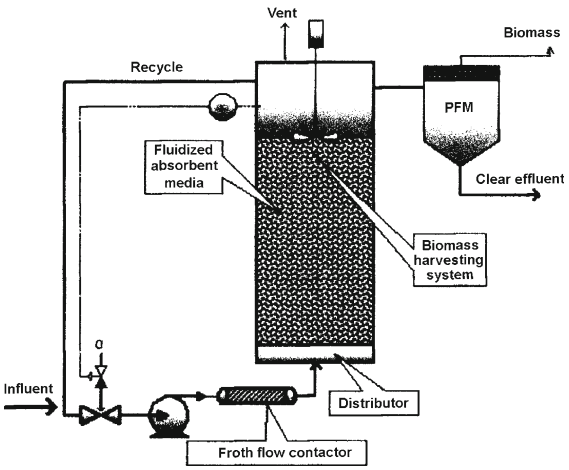

Fig. 14.23. Fluidized bed biological (FBB)-GAC system (101).

(c) Weatherproof container (optional)
(d) No plugging or sludge bulking
(e) No post-clarification required
(f) Very compact and portable
(g) Unattended operation, and
(h) No off-gas

An extremely high concentration of biomass develops in the reactor because of the huge surface area provided by the media, abundant oxygen, and optimized mass transfer conditions. Excess biomass is periodically and automatically removed by extracting media, shearing the biomass and returning the cleaned biomass to the reactor. The effluent from the FBB-GAC is typically very low in suspended solids, allowing effluent discharge without further treatment (101).

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APPENDIX

U.S. Army Corps of Engineers Civil Works Construction Yearly Average Cost Index for Utilities (72)

Year	Index	Year	Index
1967	100	1989	383.14
1968	104.83	1990	386.75
1969	112.17	1991	392.35
1970	119.75	1992	399.07
1971	131.73	1993	410.63
1972	141.94	1994	424.91
1973	149.36	1995	439.72
1974	170.45	1996	445.58
1975	190.49	1997	454.99
1976	202.61	1998	459.40
1977	215.84	1999	460.16
1978	235.78	2000	468.05
1979	257.20	2001	472.18
1980	277.60	2002	484.41
1981	302.25	2003	495.72
1982	320.13	2004	506.13
1983	330.82	2005	516.75
1984	341.06	2006	528.12
1985	346.12	2007	539.74
1986	347.33	2008	552.16
1987	353.35	2009	570.38
1988	369.45		

15
Sequencing Batch Reactor Technology

Lawrence K. Wang and Nazih K. Shammas

CONTENTS

Abstract Such Sequencing Batch Reactor (SBR) processes as Aqua SBR, Omniflo, Fluidyne, CASS, ICEAS and their applicability on a treatment plant are considered in this chapter. Advantages and disadvantages and such design criteria as process parameters, construction of reactor, and process safety altogether with process performance, operation, maintenance, and costs altogether with packaged SBR for onsite systems are described.

Key Words Sequencing batch reactor • SBR • reactor construction • packaged SBR • Aqua SBR • Omniflo • Fluidyne • CASS • ICEAS.

1. BACKGROUND AND PROCESS DESCRIPTION

The sequencing batch reactor (SBR) is a fill-and-draw activated sludge system for wastewater treatment (1). The prototype for the activated sludge concept was developed on a fill-and-draw basis (2). Shortly after that initial study, the emphasis switched to continuous flow "conventional" activated sludge. in an SBR system, wastewater is added to a single

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"batch" reactor, treated to remove undesirable components, and then discharged. Equalization, aeration, and clarification can all be achieved using a single batch reactor. To optimize the performance of the system, two or more batch reactors are used in a predetermined sequence of operations. SBR systems have been successfully used to treat both municipal and industrial wastewater. They are uniquely suited for wastewater treatment applications characterized by low or intermittent flow conditions.

Fill-and-draw batch processes similar to the SBR are not a recent development as commonly thought. Between 1914 and 1920, several full-scale fill-and-draw systems were in operation. Interest in SBRs was revived in the late 1950s and early 1960s, with the development of new equipment and technology. Innovations in aeration devices, control logic, level sensors, solenoids, and hydraulic energy dissipators have surmounted the earlier limitations and revitalized interest in SBR technology (3). The resurgence of interest in SBRs was initially limited to small treatment applications; however, the need for greater treatment efficiencies due to increasingly stringent effluent limits has resulted in the adoption of SBR technology in installations as large as 660 L/s (15 MGD) (4).

The first modern, full-scale plant for SBR treatment of municipal wastewater in the United States was the Culver, Indiana, wastewater treatment facility (5). Retrofitted for the SBR process, operation was initiated in May 1980 (6). Since that time, SBR technology has become widespread in the United States, with more than 150 plants in operation (7). SBRs can be modified to provide carbonaceous oxidation, nitrification, and biological nutrient removal (BNR). Approximately, 25% of all SBR systems were designed to achieve nutrient removal (8).

The unit processes of the SBR and conventional activated sludge systems are the same. A US EPA report summarized this by stating that the SBR is no more than an activated sludge system that operates in time rather than in space (1, 3). The difference between the two technologies is that the SBR performs equalization, biological treatment, and secondary clarification in a single tank using a timed control sequence. This type of reactor does, in some cases, also perform primary clarification. In a conventional activated sludge system, these unit processes would be accomplished by using separate tanks.

The SBR consists of a self-contained treatment system incorporating equalization, aeration, anoxic reaction, and clarification within one basin. Intermittently fed SBRs consist of the following basic steps (1, 3, 9):

1. *Fill* – The fill operation consists of adding the waste and substrate for microbial activity. The fill cycle can be controlled by float switches to a designated volume or by timers for multireactor systems. A simple and commonly applied mode to control the fill cycle is based on reactor volume, resulting in fill times inversely related to influent flow rates. The fill phase can include many phases of operation and is subject to various modes of control, termed static fill, mixed fill, and react fill. Static fill involves the introduction of waste influent with no mixing or aeration. This type of fill method is most common in plants requiring nutrient control. In such applications, the static fill will be accompanied by a mixed fill stage such that the microorganisms are exposed to sufficient substrate, while maintaining anoxic or anaerobic conditions. Both mixing and aeration are provided in the react fill stage. The system may alternate among static fill, mixed fill, and react fill throughout the fill cycle.

2. *React* – The purpose of the react stage is to complete reactions initiated during fill. The react stage may comprise mixing or aeration, or both. As was the case in the fill cycle, desired processes may require alternating cycles of aeration. The length of the react phase may be controlled by timers, by liquid level controls in a multitank system, or when the desired degree of treatment has been attained, verified by monitoring reactor contents. Depending upon the amount and timing of aeration during fill, there may or may not be a dedicated react phase.
3. *Settle* – Liquid–solid separation occurs during the settle phase, analogous to the operation of a conventional final clarifier. Settling in an SBR can demonstrate higher efficiencies than a continuous-flow settler, since total quiescence is achieved in an SBR.
4. *Draw* – Clarified effluent is decanted in the draw phase. Decanting can be achieved by various apparatus, the most common being floating or adjustable weirs. The decanting capability is one of the operational and equipment limitations of SBR technology. Adaptation or development of equipment compatible with a fluctuating liquid level is required.
5. *Idle* – The final phase is termed as the idle phase and is only used in multibasin applications. The time spent in the idle phase will depend on the time required for the preceding basin to complete its fill cycle. Biosolids wastage will typically be performed during the idle phase.

A typical SBR process sequence schematic is shown in Fig. 15.1.

Denitrification can occur during the fill or react stages by cycling the aerators and during the settle and draw period. An obvious advantage of an SBR system with low flows is that the reactor contents can be retained until the desired level of treatment is achieved, providing that sufficient tankage exists to equalize or accommodate the additional influent.

2. PROPRIETARY SBR PROCESSES

SBR manufacturers have adapted the sequence of batch treatment cycles in various ways. One classification of SBR systems distinguishes those which operate with continuous feed and intermittent discharge (CFID) from those which operate with intermittent feed and intermittent discharge (IFID). IFID reactors are characteristic of the conventional fill-and-draw SBR reactors in that the influent flow to the reactor is discontinued for some portion of each cycle. The CFID reactors receive wastewater during all phases of the treatment cycle. A key design consideration with such systems is minimization of short-circuiting between influent and effluent. This is accomplished by locating the feed and withdrawal points at opposite ends of the tank, using rectangular reactors with length-to-width ratios of at least 2–1 and providing baffling.

The steps and associated conditions and purpose of a complete, typical cycle for a single tank operated as part of an IFID SBR system designed to achieve nitrification are described in Table 15.1. Nitrification takes place during the react phase and during the portions of the fill period when aeration is practiced.

Several proprietary process and equipment innovations have been developed to enhance treatment, simplify operation, or control biosolids characteristics (9–15). All proprietary SBR manufacturers will guarantee TN effluent concentrations $< 5\,\text{mg/L}$. To illustrate the variety of options available, the proprietary aspects of five SBR manufacturers are discussed in the following section.

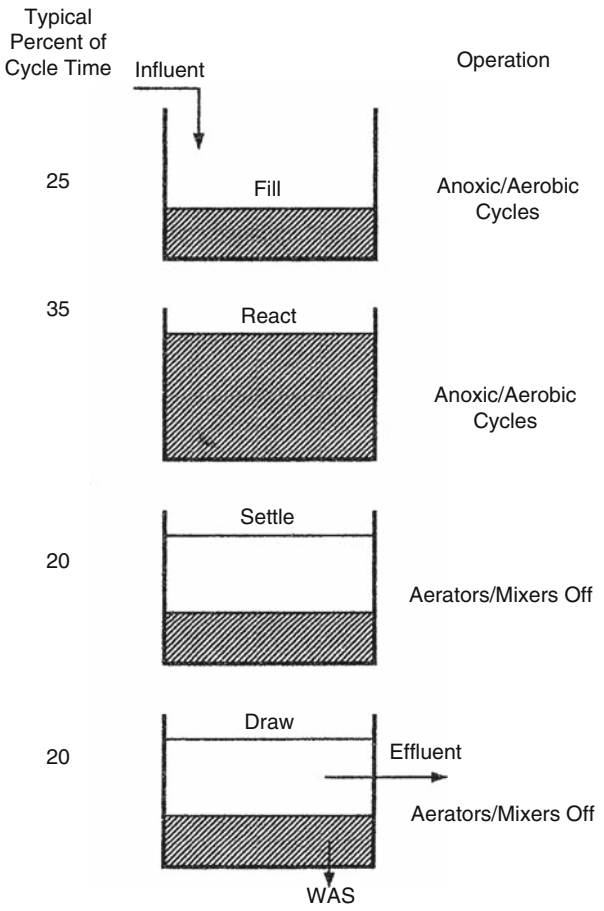

Fig. 15.1. Sequencing batch reactor (SBR) (Source: US EPA).

2.1. Aqua SBR

The Aqua SBR system provided by Aqua-Aerobic Systems, Inc. (11) is not a patented process, but the process does include a proprietary floating direct drive mixer, an effluent decanter, and a microprocessor control system. The floating decanter is designed to prohibit MLSS from entering the decanter during mixed or react phases, and it also withdraws supernate 30 cm (0.5 ft) below the water surface to mitigate scum losses to the effluent. If long settling times are provided, clear effluent can be obtained at high SVIs (Sludge Volume Index).

2.2. Omniflo

Jet Tech, Inc. (12) has developed SBR equipment and also has a patented logic control for their aeration system. The proprietary equipment includes dry pit pumps, headers, manifolds,

Table 15.1
Typical cycle for a single tank in a dual tank SBR system designed for nitrification (Source: US EPA)

Step	Conditions	Purpose
Fill	Influent flow into SBR Aeration occurs continually or intermittently Time = half of cycle time	Addition of raw wastewater to the SBR; COD removal and nitrification
React	No influent flow to SBR Aeration Time typically = 1–2 h (varies widely depending on nitrification kinetics, waste strength, and amount of aeration during fill)	Carbonaceous oxidation and nitrification
Settle	No influent flow to SBR No aeration Time = approximately1 h (depends on settling characteristics)	Allow SS to settle, yielding a clear supernatant
Draw	No influent flow to SBR No aeration Effluent is decanted Time = 1 h (variable)	Decant–remove clarified effluent from reactor; 15–25% of the reactor volume is typically decanted, depending on hydraulic considerations and SBR manufacturers design
Idle	No influent flow to SBR No aeration Sludge is wasted Time = variable (determined by flow rate)	Multi-tank system, which allows time for one reactor to complete the fill step before another starts a new cycle; waste sludge – remove excess solids from reactors

Note: A typical total cycle time is 4–6 h.

influent distribution hardware, jet aerators, and decanter apparatus. A proprietary aspect of the SBR process provided by Jet Tech is the Batch Proportional Aeration System. The function of this aeration system is to relate the volumetric change rate during the fill phase to the aeration capacity requirements by sensing the DO level in the reactor, optimizing nitrification and denitrification cycles.

2.3. Fluidyne

The Fluidyne Corp. (13, 14) offers a system with effluent decanters fixed in position to the reactor wall. The device excludes MLSS (missed liquor suspended solids) entry during aeration. These systems also commonly employ jet aeration with a combination of aeration and static conditions during fill.

2.4. CASS

The Cyclic Activated Sludge System (CASS) was developed and is marketed by Transenviro, Inc. CASS uses a similar sequence of operation as other batch technologies, but is configured with a proprietary captive selector reactor. The selector can also receive continuous flow. The selector is a baffled compartment that receives raw wastewater or primary effluent

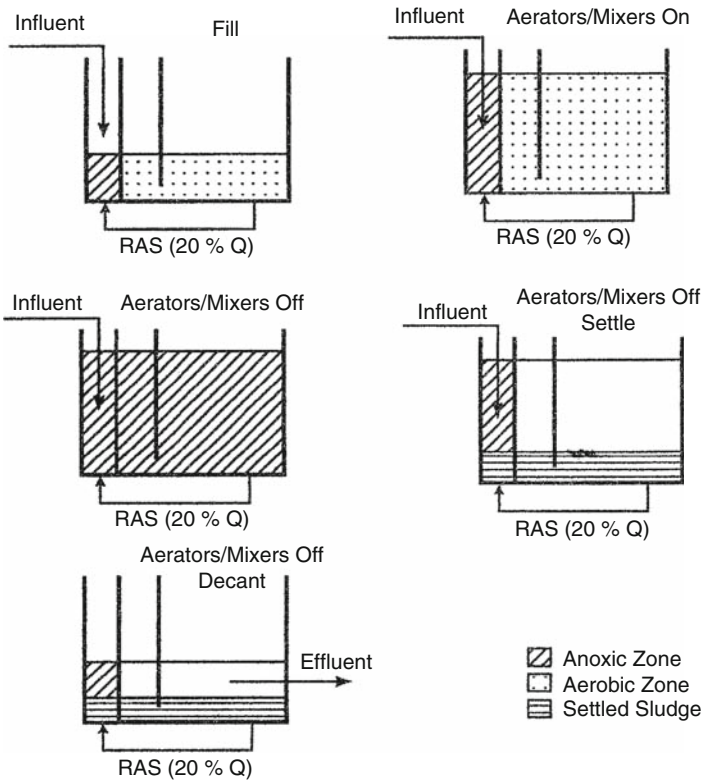

Fig. 15.2. Cyclical activated sludge system (CASS) (Source: US EPA).

where it is mixed with RAS or internally recycled MLSS. The selector then conveys flow to the reactor basin. By limiting or eliminating aeration to the selector, oxygen deficient conditions can be attained, while concurrent high substrate levels are maintained. This mode of operation is claimed to favor the propagation of floc formers and to inhibit growth of filamentous strains (15). A process schematic is presented in Fig. 15.2.

2.5. ICEAS

A modified batch system is available from Austgen-Biojet (ABJ). The ABJ system is termed as Intermittent Cycle Extended Aeration System (ICEAS) and is depicted schematically in Fig. 15.3. The distinguishing feature of ICEAS is that continuous inflow is incorporated in all phases, compared to other variable volume processes that do not receive continuous inflow. Noncontinuous inflow operation can be provided, if requested. Austgen-Biojet maintains that the continuous inflow mode is preferable to noncontinuous flow operation, as the distribution box used by ABJ will ensure that variations in load and flow are distributed evenly between the reactors and prevent diurnal variations or shock loads from continually overloading one reactor. The manufacturer asserts an additional advantage of the ICEAS flow regime is that continuous flow via the distribution box reduces the valving and

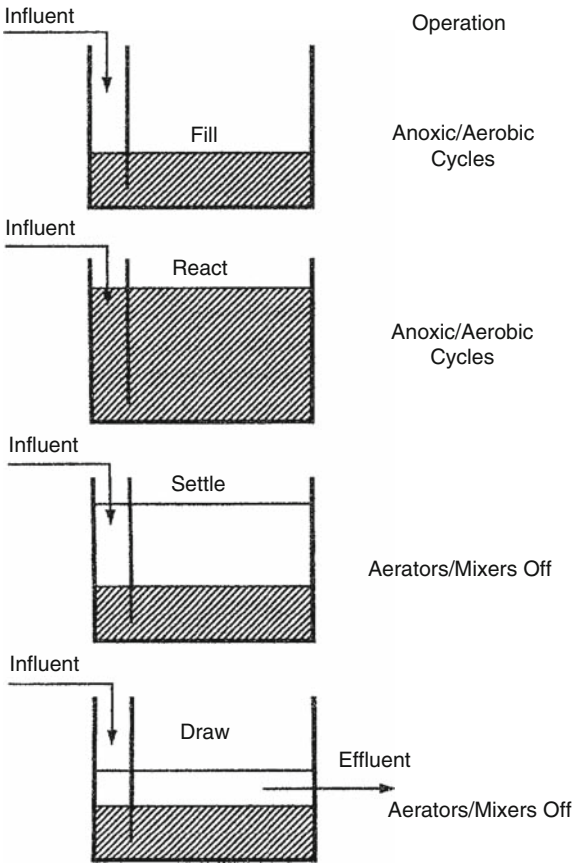

Fig. 15.3. Intermittent cycle extended aeration system (Source: US EPA).

head-works engineering compared to requirements for a noncontinuous flow SBR. A complete ICEAS treatment cycle consists of three phases: Aeration, Settle, and Draw. Since influent is received during all phases, ICEAS does not offer total quiescence during the settle phase, a characteristic of an intermittently fed SBR. Although ICEAS is proprietary, no royalty or license fees are imposed. ICEAS uses a patented anoxic selector to provide denitrification and to promote growth of zoogleal microorganism, and to inhibit filamentous strains. The ABJ selector has characteristics similar to the patented CASS selector, but ABJ claims to be the developer of the original selector concept.

3. DESCRIPTION OF A TREATMENT PLANT USING SBR

A typical process flow schematic for a municipal wastewater treatment plant using an SBR is shown in Fig. 15.4 (1, 3). Influent wastewater generally passes through screens and grit removal prior to the SBR. The wastewater then enters a partially filled reactor, containing biomass, which is acclimated to the wastewater constituents during preceding cycles. Once the

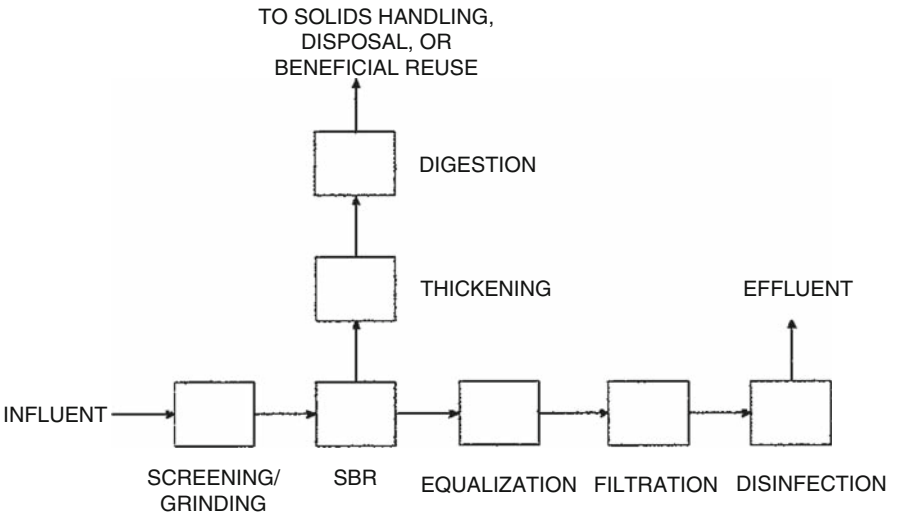

Fig. 15.4. SBR process flow diagram (Source: US EPA).

reactor is full, it behaves like a conventional activated sludge system, but without a continuous influent or effluent flow. The aeration and mixing is discontinued after the biological reactions are complete, the biomass settles, and the treated supernatant is removed. Excess biomass is wasted at any time during the cycle. Frequent wasting results in holding the mass ratio of influent substrate to biomass nearly constant from cycle to cycle. Continuous flow systems hold the mass ratio of influent substrate to biomass constant by adjusting return activated sludge (RAS) flowrates continually as influent flowrates, characteristics, and settling tank underflow concentrations vary. After the SBR, the "batch" of wastewater may flow to an equalization basin where the wastewater flow to an additional processing unit can be controlled at a determined rate. In some cases, the wastewater is filtered to remove additional solids and then disinfected.

As illustrated in Fig. 15.4, the solids handling system may consist of a thickener and an aerobic digester. With SBRs there is no need for RAS pumps and primary sludge (PS) pumps like those associated with conventional activated sludge systems. With the SBR, there is only one sludge biomass (biosolids) to handle. The need for gravity thickeners prior to digestion is determined on a case by case basis depending on the characteristics of the biosolids.

An SBR serves as an equalization basin when the vessel is filling with wastewater, enabling the system to tolerate peak flows or peak loads in the influent and to equalize them in the batch reactor. In many conventional activated sludge systems, separate equalization is needed to protect the biological system from peak flows, which may wash out the biomass, or peak loads, which may upset the treatment process.

It should also be noted that primary clarifiers are typically not required for municipal wastewater applications prior to an SBR. In most conventional activated sludge wastewater treatment plants, primary clarifiers are used prior to the biological system. However, primary clarifiers may be recommended by the SBR manufacturer if the total suspended solids (TSS)

or biochemical oxygen demand (BOD) are greater than 400–500 mg/L. Historic data should be evaluated and the SBR manufacturer consulted to determine whether primary clarifiers or equalization are recommended prior to an SBR for municipal and industrial applications.

Equalization may be required after the SBR, depending on the downstream process. If equalization is not used prior to filtration, the filters need to be sized in order to receive the batch of wastewater from the SBR, resulting in a large surface area required for filtration, Sizing filters to accept these "batch" flows is usually not feasible, which is why equalization is used between an SBR and downstream filtration. Separate equalization following the biological system is generally not required for most conventional activated sludge systems, because the flow is on a continuous and more constant basis.

4. APPLICABILITY

SBRs are typically used at flowrates of 219 L/s (5 MGD) or less (1, 3). The more sophisticated operation required at larger SBR plants tends to discourage the use of these plants for large flowrates. The SBR technology is particularly attractive for treating smaller wastewater flows. The majority of plants were designed at wastewater flow rates of less than 22 L/s (0.5 MGD) (7). The cost-effectiveness of SBRs may limit their utilization to flows less than 440 L/s (10 MGD) (6). Depending on the number of SBR reactors in a plant and the duration of the discharge cycle, the downstream units often must be sized for two or more times the influent flow rate. Plants with four or more separate reactors may have the reactor process cycles offset such that the discharge is nearly continuous.

As these systems have a relatively small footprint, they are useful for areas where the available land is limited. In addition, cycles within the system can be easily modified for nutrient removal in the future, if it becomes necessary. This makes SBRs extremely flexible to adapt to regulatory changes for effluent parameters like nutrient removal. SBRs are also very cost effective if treatment beyond biological treatment is required, such as filtration.

5. ADVANTAGES AND DISADVANTAGES

Some advantages and disadvantages of SBRs are listed in the following section (1, 3, 8).

5.1. Advantages

1. Equalization and the ability to tolerate peak flows and shock loads of BOD_5
2. Primary clarification (in most cases), biological treatment, and secondary clarification can be achieved in a single reactor vessel
3. Operating flexibility and control of effluent discharge
4. Minimal footprint
5. Potential capital cost savings by eliminating clarifiers and other equipment

5.2. Disadvantages

1. A higher level of sophistication is required (compared to conventional systems), especially for larger systems of timing units and controls
2. Higher level of maintenance (compared to conventional systems) associated with more sophisticated controls, automated switches, and automated valves

Table 15.2
SBR design parameters for conventional load (Source: US EPA)

	Municipal	Industrial
Food to mass (F:M)	0.15–0.4/day	0.15–0.6/day
Treatment cycle duration	4.0 h	4.0–24 h
Typically low water level mixed liquor suspended solids	2,000–2,500 mg/L	2,000–4,000 mg/L
Hydraulic retention time	6–14 h	Varies

3. Potential of discharging floating or settled biosolids during the draw or decant phase with some SBR configurations
4. Potential plugging of aeration devices during selected operating cycles, depending on the aeration system used by the manufacturer
5. Potential requirement for equalization after the SBR, depending on the downstream processes

6. DESIGN CRITERIA

For any wastewater treatment plant design, the first step is to determine the anticipated influent characteristics of the wastewater and the effluent requirements for the proposed system. These influent parameters typically include design flow, maximum daily flow BOD_5, TSS, pH, alkalinity, wastewater temperature, total Kjeldahl nitrogen (TKN), ammonia-nitrogen (NH_3-N), and total phosphorus (TP). For industrial and domestic wastewater, other site specific parameters may also be required.

The state regulatory agency should be contacted to determine the effluent requirements of the proposed plant. These effluent discharge parameters will be dictated by the state in the National Pollutant Discharge Elimination System (NPDES) permit. The parameters typically permitted for municipal systems are flowrate, BOD_5, TSS, and Fecal Coliform (FC). In addition, many states are moving toward requiring nutrient removal. Therefore, total nitrogen (TN), TKN, NH_3-N, or TP may also be required. It is imperative to establish effluent requirements because they will impact the operating sequence of the SBR. For example, if there is a nutrient requirement and NH_3-N or TKN is required, then nitrification will be necessary. If there is a TN limit, then nitrification and denitrification will be necessary.

6.1. Design Parameters

Once the influent and effluent characteristics of the system are determined, the engineer will typically consult SBR manufacturers for a recommended design. Based on these parameters, and other site specific parameters such as temperature, key design parameters are selected for the system. An example of these parameters for a wastewater system loading is listed in Table 15.2.

A unified approach to SBR technology has yet to be developed (16); however, the principles used to design nitrification–denitrification facilities in single anoxic or dual anoxic zone systems, such as flow and loadings, may be applied with some modifications. One factor to consider specifically for the design of an SBR is the flow volume which will determine

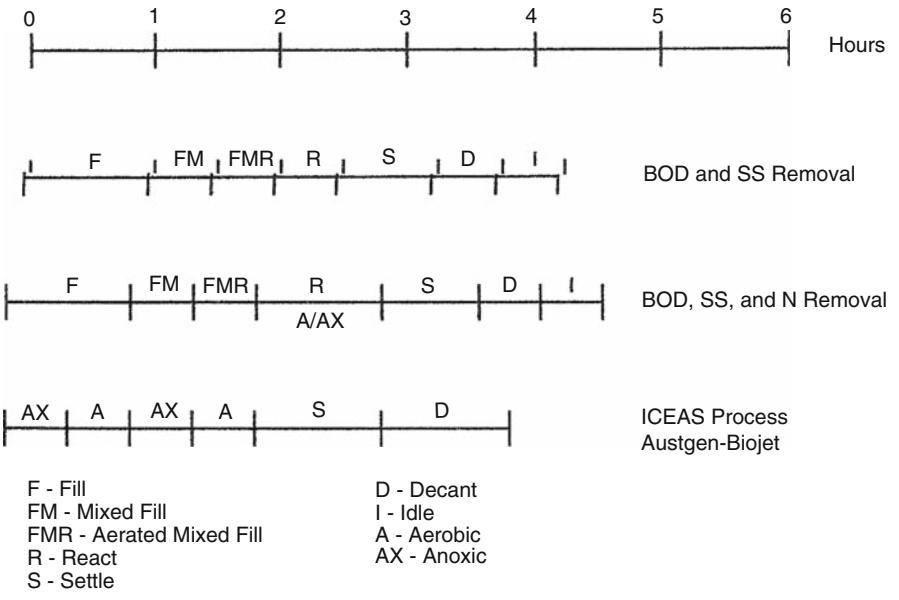

Fig. 15.5. Operating strategies for SBR systems (Source: US EPA).

whether one reactor will suffice (generally for flows $< 2\,L/s$ or 0.05 MGD) or whether a two-vessel system is required. Additional vessels should be considered for sites that experience a wide transient variation in either organic or hydraulic loading. Conditions, including wet weather with ingress of surface or ground waters, may be accommodated by effecting more frequent decant cycles, without causing washout of the reactor biomass. The SBR process can accommodate peak hourly flows 3–10 times as large as the design flow without adverse effects, if excess capacity is available. The F/M ratio must be determined by the desired effluent quality which in turn dictates reactor sizing.

The critical operational feature is the cycle time for fill, react, settle, and draw, and the amount of oxygen that is supplied. A typical cycle for an intermittent-feed, intermittent-discharge SBR based on average flow conditions is 4-h duration; 2 h allocated to fill/aeration/anoxic react, 1 h to settling, and 1 h to decant and idle. The total time for a batch cycle consists of the time allowed for each component phase. Design cycle times in full-scale plants have varied from 2 to 24 h (17). A suggested strategy is presented in Fig. 15.5. Some typical design criteria are presented in Table 15.3.

SBR systems are typically designed and operated at long solids residence times (>15 day) and low F/M (less than 0.1 kg BOD_5/kg MLSS/day). Consequently, partial or complete nitrification is nearly always observed (7, 8). In an evaluation of 19 SBR treatment plants (8) (all originally designed for nitrification), influent and effluent ammonia-nitrogen data were reported for eight of the plants (Table 15.4). The average effluent ammonium-nitrogen concentration for the eight plants was less than 2.0 mg/L, implying that a high degree of nitrification

Table 15.3
Typical design criteria for SBRs (Source: US EPA)

Parameter	SBR	ICEAS
BOD load, g/day/m^3	80–240	
Cycle time, h		
Fill (aeration)	1–3	
Settle	0.7–1	
Draw	0.5–1.5	
MLSS, mg/L	2,300–5,000	
MLVSS, mg/L	1,500–3,500	
HRT, h	15–40	36–50
θ_c, day	20–40	—
F/M, g BOD/g MLVSS/day	0.05–0.20	0.04–0.06

Table 15.4
Nitrification performance information for SBR operating plants[a] (Source: US EPA)

Plant location	Period of evaluation	Wastewater flow		Percent of design flow	BOD_5, mg/L		Ammonia-N, mg/L	
		m^3/day	MGD		Influent	Effluent	Influent	Effluent
Buckingham, PA	04/89–04/91	439	0.116	49	324	8	25.3	1.1
Clarkston, MI (Chateau estates)	11/89–04/91	208	0.055	50	192	12	39.1	1.7
Grundy center, IA	12/89–11/90	2,176	0.575	72	195	4	15.8	1.2
Marlette, MI	07/90–06/91	1,578	0.417	60	103	4	10.1	0.5
Mifflinburg, PA	10/88–03/91	2,763	0.73	81	105	12	7.8	0.4
Monticello, IN (white oaks resort)	10/89–05/91	15	0.004	8	131	5	3.1	0.3
Muskegon heights, MI (clover estates)	01/88–10/90	132	0.035	78	185	9	21.2	0.7
Windgap, PA	02/90–10/90	2,116	0.559	56	160	7	12.9	0.6

[a] Average monthly values based on all data available.

was achieved in all cases. These efficiencies reflect the long design solids residence times that are employed and operations that are generally well below the design flow.

The design mixed liquor volume can be calculated from the selected MLSS concentration, which decreases throughout the fill cycle. The MLSS concentration at the end of the draw phase is that of settled mixed liquor and is similar to that in a conventional clarifier underflow (18). Once the tank volumes have been calculated, the cycle times can be determined. If the

cycle times are unsatisfactory, the tank volumes can be adjusted accordingly. The number of cycles per day, number of basins, decants volume, reactor size, and detention times can then be calculated.

Other site specific information is needed to size the aeration equipment, such as site elevation above mean sea level, wastewater temperature, and total dissolved solids concentration. The sizing of aeration equipment is done according to criteria for complete nitrification and BOD removal, except that the required oxygen transfer must be accomplished in a shorter period. The actual amount of aeration time per cycle must be considered when sizing the aeration equipment.

The operation of an SBR is based on the fill-and-draw principle, which, as discussed in a previous section, consists of five basic steps: Idle, Fill, React, Settle, and Draw. More than one operating strategy is possible during most of these steps. For industrial wastewater applications, treatability studies are typically required to determine the optimum operating sequence. For most municipal wastewater treatment plants, treatability studies are not required to determine the operating sequence because municipal wastewater flowrates and characteristic variations are usually predictable and most municipal designers will follow conservative design approaches.

The Idle step occurs between the Draw and the Fill steps, during which treated effluent is removed and influent wastewater is added. The length of the Idle step varies depending on the influent flowrate and the operating strategy. Equalization is achieved during this step if variable idle times are used. Mixing to condition the biomass and biosolids wasting can also be performed during the Idle step, depending on the operating strategy.

Influent wastewater is added to the reactor during the Fill step. The following three variations are used for the Fill step and any or all of them may be used depending on the operating strategy: static fill, mixed fill, and aerated fill. During static fill, influent wastewater is added to the biomass already present in the SBR. Static fill is characterized by no mixing or aeration, meaning that there will be a high substrate (food) concentration when mixing begins. A high food to microorganisms (F:M) ratio creates an environment favorable to floc forming organisms versus filamentous organisms, which provides good settling characteristics for the biosolids. Additionally, static fill conditions favor organisms that produce internal storage products during high substrate conditions, a requirement for biological phosphorus removal. Static fill may be compared to using "selector" compartments in a conventional activated sludge system to control the F:M ratio.

Mixed fill is classified by mixing influent organics with the biomass, which initiates biological reactions. During mixed fill, bacteria biologically degrade the organics and use residual oxygen or alternative electron acceptors, such as nitrate-nitrogen. In this environment, denitrification may occur under these anoxic conditions. Denitrification is the biological conversion of nitrate-nitrogen to nitrogen gas. An anoxic condition is defined as an environment in which oxygen is not present and nitrate-nitrogen is used by the microorganisms as the electron acceptor. In a conventional BNR activated sludge system, mixed fill is comparable to the anoxic zone which is used for denitrification. Anaerobic conditions can also be achieved during the mixed fill phase. After the microorganisms use the nitrate-nitrogen, sulfate becomes

the electron acceptor. Anaerobic conditions are characterized by the lack of oxygen and sulfate as the electron acceptor.

Aerated Fill is classified by aerating the contents of the reactor to begin the aerobic reactions completed in the React step. Aerated Fill can reduce the aeration time required in the React step.

The biological reactions are completed in the React step, in which mixed react and aerated react modes are available. During aerated react, the aerobic reactions initialized during aerated fill are completed and nitrification can be achieved. Nitrification is the conversion of ammonia-nitrogen to nitrite-nitrogen and ultimately to nitrate-nitrogen. If the mixed react mode is selected, anoxic conditions can be attained to achieve denitrification. Anaerobic conditions can also be achieved in the mixed react mode for phosphorus removal.

Settle is typically provided under quiescent conditions in the SBR. In some cases, gentle mixing during the initial stages of settling may result in a clearer effluent and a more concentrated settled biosolids. In an SBR, there are no influent or effluent currents to interfere with the settling process as in a conventional activated sludge system.

The Draw step uses a decanter to remove the treated effluent, which is the primary distinguishing factor between different SBR manufacturers. In general, there are floating decanters and fixed decanters. Floating decanters offer several advantages over fixed decanters as described in the Tank and Equipment Description Section.

SBR technology requires unique and innovative strategies to accomplish each phase of the process cycle. Large facilities that require dual vessels can accommodate continuous flow by alternating fill cycles between reactors; single-vessel facilities except for ICEAS systems will require flow equalization or a selector. Compartments or baffles may be included within a selector to control the hydraulic regime and biosolids characteristics. Several criteria have been proposed that can be used to design an appropriate selector (19, 20). The CASS process by Transenviro is a proprietary SBR that includes an integral selector as a part of the process. For more details on SBR design, the readers are referred to Wilderer et al (21) and Toby (22).

6.2. Construction

Construction of SBR systems can typically require a smaller footprint than conventional activated sludge systems because the SBR often eliminates the need for primary clarifiers. The SBR never requires secondary clarifiers. The size of the SBR tanks themselves will be site specific; however, the SBR system is advantageous if space is limited at the proposed site. A few case studies are presented in Table 15.5 to provide general sizing estimates at different flowrates. Sizing of these systems is site specific and these case studies do not reflect every system at that size.

SBR reactors have been constructed with a variety of shapes including rectangular, oval, circular, and with sloped sidewalls. Design bottom water levels after decant are typically 3–4 m (10–13 ft) and design top water levels are typically 4.3–5.5 m (14–18 ft). A freeboard of 1 m (3 ft) is common.

The actual construction of the SBR tank and equipment may be comparable or simpler than a conventional activated sludge system. For BNR plants, an SBR eliminates the need for RAS pumps and pipes. It may also eliminate the need for internal Mixed Liquor Suspended Solid

Table 15.5
Case studies for several SBRs facilities (Source: US EPA)

Flow	Reactors			Blowers	
(MGD)	No.	Size (feet)	Volume (MG)	No.	Size (HP)
0.012	1	18 × 12	0.021	1	15
0.10	2	24 × 24	0.069	3	7.5
1.2	2	80 × 80	0.908	3	125
1.0	2	58 × 58	0.479	3	40
1.4	2	69 × 69	0.678	3	60
1.46	2	78 × 78	0.910	4	40
2.0	2	82 × 82	0.958	3	75
4.25	4	104 × 80	1.556	5	200
5.2	4	87 × 87	1.359	5	125

Conversion factors: 1 MGD = 43.8L/s; 1 feet = 0.3048 m; 1 MG = 3.785 ML; 1 HP = 0.7457 kW

(MLSS) recirculation, if this is being used in a conventional BNR system to return nitrate-nitrogen.

The control system of an SBR operation is more complex than a conventional activated sludge system and includes automatic switches, automatic valves, and instrumentation. These controls are very sophisticated in larger systems. The SBR manufacturers indicate that most SBR installations in the United States are used for smaller wastewater systems of less than 2 MGD (87.6 L/s) and some references recommend SBRs only for small communities where land is limited. This is not always the case, however, as the largest SBR in the world is currently a 10 MGD (438 L/s) system in the United Arab Emirates (23).

6.3. Tank and Equipment Description

The SBR system consists of a tank, aeration and mixing equipment, a decanter, and a control system. The central features of the SBR system include the control unit and the automatic switches and valves that sequence and time the different operations. SBR manufacturers should be consulted for recommendations on tanks and equipment. It is typical to use a complete SBR system recommended and supplied by a single SBR manufacturer. It is possible, however, for an engineer to design an SBR system, as all required tanks, equipment, and controls are available through different manufacturers. This is not typical of SBR installation because of the level of sophistication of the instrumentation and controls associated with these systems.

The SBR tank is typically constructed with steel or concrete. For industrial applications, steel tanks coated for corrosion control are most common while concrete tanks are the most common for municipal treatment of domestic wastewater. For mixing and aeration, jet aeration systems are typical as they allow mixing either with or without aeration, but other aeration and mixing systems are also used. Positive displacement blowers are typically used for SBR design to handle wastewater level variations in the reactor. The varying liquid volume restricts

the feasibility of fixed mechanical surface aerators. The most common aeration system in SBRs are diffused bubblers; but both the floating aerator as manufactured by Aqua SBR and diffused bubble aeration systems will benefit from submerged mixers used to ensure proper agitation of the reactor contents under anoxic conditions.

As previously mentioned, the decanter is the primary piece of equipment that distinguishes different SBR manufacturers. Types of decanters include floating and fixed. Floating decanters offer the advantage of maintaining the inlet orifice slightly below the water surface to minimize the removal of solids in the effluent removed during the DRAW step. Floating decanters also offer the operating flexibility to vary fill-and-draw volumes. Fixed decanters are built into the side of the basin and can be used if the Settle step is extended. Extending the Settle step minimizes the chance that solids in the wastewater will float over the fixed decanter. In some cases, fixed decanters are less expensive and can be designed to allow the operator to lower or raise the level of the decanter. Fixed decanters do not offer the operating flexibility of the floating decanters.

6.4. Health and Safety

Safety should be the primary concern in every design and system operation. A properly designed and operated system will minimize potential health and safety concerns. Manuals such as the Manual of Practice (MOP) No. 8, Design of Municipal Wastewater Treatment Plants (24), and MOP No. 11, Operation of Municipal Wastewater Treatment Plants (25) should be consulted to minimize these risks. Other appropriate industrial wastewater treatment manuals, federal regulations, and state regulations should also be consulted for the design and operation of wastewater treatment systems.

7. PROCESS PERFORMANCE

The performance of SBRs is typically comparable to conventional activated sludge systems and depends on system design and site specific criteria. Depending on their mode of operation, SBRs can achieve good BOD and nutrient removal. For SBRs, the BOD removal efficiency is generally 85–95% and nitrogen removal can be considerably higher than in conventional activated sludge systems (26–32). Performance results from full-scale facilities are provided in Table 15.6.

SBR manufacturers will typically provide a process guarantee to produce an effluent of less than (1, 3):

1. 10 mg/L BOD
2. 10 mg/L TSS
3. 5–8 mg/L TN
4. 1–2 mg/L TP

One of the primary features of SBR technology is the flexibility to exercise control as a function of time rather than space (as in conventional flow-through systems). Several key aspects include (1, 3):

Table 15.6
Summary of SBR plant operating data (Source: US EPA)

Plant	Flow m^3/day, (MGD)	Influent BOD_5, mg/L	Influent TKN (Total N), mg/L	Effluent TKN (Total N), mg/L	Influent NH_4^+-N, mg/L	Effluent NH_4^+-N, mg/L	Effluent NO_x-N, mg/L	Effluent Total N, mg/L	% N Removal
Nonproprietary Culver, IN	N/A	170	N/A	N/A	20.0	1.0	N/A	1.0[a]	88
Cass Deep River, CT	189 (0.05)	100	54.5	3.6	40.4	1.3	1.0	4.6	92
Cass Dundee, Ml	N/A	123	28.9	2.2	16.9	0.5	4.9	2.7	75
Nonproprietary Grundy Center, IA	1,249 (0.33)	210	N/A	N/A	17.3	0.8	2.8	3.6[a]	90
Aqua SBR Grundy Center, IA	3,028 (0.8)	140	28.0	4.4	19.0	1.6	0.5	4.9	83
Aqua SBR Rock Falls, IN	530 (0.14)	109	39.8	1.8	35.9	0.6	1.0	2.8	93
Aqua SBR Oak Hill, MI	416 (0.11)	220	N/A	N/A	25.0	0.6	3.5	4.1[a]	84
Jet Tech Oak Pt., Ml	227 (0.06)	142	N/A	N/A	19.0	0.6	2.8	3.4[a]	82
Jet Tech Cow Creek, OK	9,841 (2.6)	119	24.0	2.7	17.0	1.8	1.9	4.6	81
Jet Tech Del City, OK	13,248 (3.5)	115	(28.3)	(5.4)	17.6	0.9	3.5	5.4	81
ICEAS Buckingham, PA	492 (0.13)	349	N/A	N/A	29.2	0.6	0.9	1.5[a]	95
ICEAS Burkeville, PA	530 (01.4)	296	35.7	3.6	19.3	0.3	1.0	4.6	87
ICEAS Shiga Kogen	757 (0.2)	484	(36.9)	(5.4)	N/A	N/A	N/A	5.4	85

N/A – Data not available.
[a] Based on effluent NH_4^+-N + NO_x-N.

1. The SBR system can tolerate shock loads and peak flows because of the equalizing basin characteristics of the fill phase.
2. Periodic effluent discharge may permit retention of reactor contents until desired clarity or treatment quality is achieved.
3. A fraction of the total volume may be used during low flow periods, resulting in lower aeration requirements. If aerators or blowers have turn-down capability, O&M costs may be reduced.
4. No RAS or internal recycles are required; however, some systems (e.g., CASS) include recycle to an antecedent basin or selector chamber.

5. With intermittently fed SBRs, clarification occurs under total quiescence, thereby eliminating short-circuiting. Consequently, small flocs will settle in an SBR that would be washed out in a continuous-flow regime.
6. Filamentous growth can be controlled by operational strategies along with adjustments during the fill phase.

Readers interested in the performance of SBR systems in industrial wastewater treatment are referred to Refs. (33–35).

8. OPERATION AND MAINTENANCE

The SBR typically eliminates the need for separate primary and secondary clarifiers in most municipal systems, which reduces operations and maintenance (O&M) requirements. In addition, RAS pumps are not required. In conventional BNR systems, anoxic basins, anoxic zone mixers, toxic basins, toxic basin aeration equipment, and internal MLSS nitrate-nitrogen recirculation pumps may be necessary. With the SBR, this can be accomplished in one reactor using aeration/mixing equipment, which will minimize operation and maintenance requirements otherwise be needed for clarifiers and pumps.

Since the heart of the SBR system is the controls, automatic valves, and automatic switches, these systems may require more maintenance than a conventional activated sludge system. An increased level of sophistication usually equates to more items that can fail or require maintenance. The level of sophistication may be very advanced in larger SBR wastewater treatment plants requiring a higher level of maintenance on the automatic valves and switches (1, 3). The recent advances and cost reductions of microprocessors have been some of the causes of the revival of interest in SBR technology, permitting automated control of the timing and sequence of process phases and operation. The use of timers and DO monitors can be used to reduce costs attributable to over aeration, thereby reducing the lag period of DO depletion and allowing the maximum time for denitrification to occur.

Significant operating flexibility is associated with SBR systems. An SBR can be set up to simulate any conventional activated sludge process, including BNR systems. For example, holding times in the aerated react mode of an SBR can be varied to achieve simulation of a contact stabilization system with a typical hydraulic retention time (HRT) of 3.5–7 h or, on the other end of the spectrum, an extended aeration treatment system with a typical HRT of 18–36 h. For a BNR plant, the aerated react mode (oxic conditions) and the mixed react modes (anoxic conditions) can be alternated to achieve nitrification and denitrification. The mixed fill mode and mixed react mode can be used to achieve denitrification using anoxic conditions. In addition, these modes can ultimately be used to achieve an anaerobic condition at which phosphorus removal can occur. Conventional activated sludge systems typically require additional tank volume to achieve such flexibility. SBRs operate in time rather than in space and the number of cycles per day can be varied to control desired effluent limits, offering additional flexibility with an SBR.

9. COST

This section includes some general guidelines as well as some general cost estimates for planning purposes. It should be remembered that capital and construction cost estimates are site-specific.

Budget level cost estimates presented in Table 15.7 are based on projects that occurred from 1995 to 1998 (1). Budget level costs include such as the blowers, diffusers, electrically operated valves, mixers, biosolids pumps, decanters, and the control panel. All costs in this chapter have been updated to year 2009 costs, using the Cost Index for Utilities shown in Appendix (36). The 1998 costs were multiplied by a factor = 570.38/459.40 = 1.24 i.e., costs were increased by 24% to obtain their values in terms of 2005 US Dollars.

In Table 15.8, a range of equipment costs for different design flowrates is provided (1).

Again the equipment cost items provided do not include the cost for the tanks, sitework, excavation/backfill, installation, contractor's overhead and profit, or legal, administrative, contingency, and engineering services. These items must be included to calculate the overall construction costs of an SBR system. Costs for other treatment processes, such as screening, equalization, filtration, disinfection, or aerobic digestion, may be included if required.

The ranges of construction costs for a complete, installed SBR wastewater treatment system are presented in Table 15.9 (1). The variances in the estimates are due to the type of biosolids handling facilities and the differences in newly constructed plants versus systems that use

Table 15.7
SBR equipment costs based on different existing facilities (Source: US EPA)

Design flowrate (MGD)	Budget level equipment costs ($)
0.012	117,000
0.015	169,000
1.0	419,000
1.4	502,000
1.46	502,000
2.0	699,000
4.25	1,448,000

Costs are adjusted to current 2009 US dollars. 1 MGD = 43.8 L/s.

Table 15.8
Equipment costs based on flowrates (Source: US EPA)

Design flowrate (MGD)	Budget level equipment costs($)
1	187,000–433,000
5	568,000–903,000
10	1,348,000–1,695,000
15	2,722,000
20	2,600,000–3,712,000

Costs are adjusted to current 200918 US dollars. 1 MGD = 43.8 L/s.

Table 15.9
Installed costs per gallon treated (Source: US EPA)

Design flowrate (MGD)	Budget level equipment costs ($/gal)
0.5–1.0	2.40–6.19
1.1–1.5	2.27–3.33
1.5–2.0	2.05–4.07

Costs are adjusted to current 2009 US dollars. 1 MGD = 43.8 L/s; 1 gal = 3.785 L.

existing plant facilities. As such, in some cases these estimates include other processes required in an SBR wastewater treatment plant.

There is typically an economy of scale associated with construction costs for wastewater treatment, meaning that larger treatment plants can usually be constructed at a lower cost per gallon than smaller systems. The use of common wall construction for larger treatment systems, which can be used for square or rectangular SBR reactors, results in this economy of scale.

Operations and Maintenance costs associated with an SBR system may be similar to a conventional activated sludge system. Typical cost items associated with wastewater treatment systems include labor, overhead, supplies, maintenance, operating administration, utilities, chemicals, safety and training, laboratory testing, and solids handling. Labor and maintenance requirements may be reduced in SBRs because clarifiers, clarification equipment, and RAS pumps may not be necessary. On the other hand, the maintenance requirements for the automatic valves and switches that control the sequencing may be more intensive than for a conventional activated sludge system. Operations and Maintenance costs are site specific and may range, in terms of 2009 US Dollars, from $1,000 to $2,500/MG (1).

10. PACKAGED SBR FOR ONSITE SYSTEMS

As discussed earlier, SBRs can be designed and operated to enhance removal of nitrogen, phosphorus, and ammonia, in addition to removing TSS and BOD. The intermittent flow (IF) SBR accepts influent only at specified intervals and, in general, follows the five-step sequence (Fig. 15.6). There are usually two IF units in parallel. Because this system is closed to influent flow during the treatment cycle, two units may be operated in parallel, with one unit open for intake while the other runs through the remainder of the cycles. In the continuous inflow SBR, influent flows continuously during all phases of the treatment cycle. To reduce short-circuiting, a partition is normally added to the tank to separate the turbulent aeration zone from the quiescent area (37).

The SBR system is typically found in packaged configurations for onsite and small community or cluster applications. The major components of the package include the batch tank, aerator, mixer, decanter device, process control system (including timers), pumps, piping, and appurtenances (37). Aeration may be provided by diffused air or mechanical devices. SBRs are often sized to provide mixing as well and are operated by the process control timers. Mechanical aerators have the added value of potential operation as mixers or aerators. The

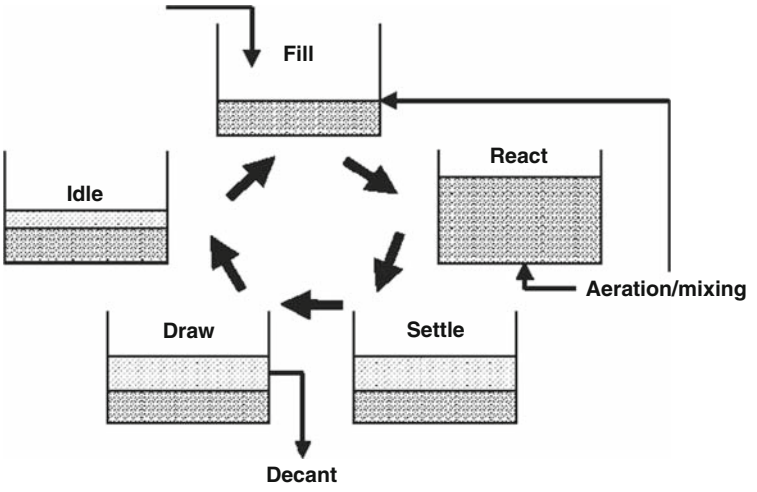

Fig. 15.6. SBR design principle for onsite systems (Source: US EPA).

decanter is a critical element in the process. Several decanter configurations are available, including fixed and floating units. At least one commercial package employs a thermal processing step for the excess biosolids produced and wasted during the "idle" step. The key to the SBR process is the control system, which consists of a combination of level sensors, timers, and microprocessors. Programmable logic controllers can be configured to suit the owner's needs. This provides a precise and versatile means of control.

10.1. Typical Applications

SBR package plants have found application as onsite systems in some states and counties where they are allowed by code. They are normally used to achieve a higher degree of treatment than a continuous-flow, suspended-growth aerobic system (CFSGAS) unit by eliminating impacts caused by influent flow fluctuations. For discharge to surface waters, they must meet effluent permit limits on BOD, TSS, and possibly ammonia. Additional disinfection is required to meet effluent FC requirements. For subsurface discharge, they can be used in situations where infiltrative surface organic loadings must be reduced. There are data showing that a higher quality effluent may reduce soil absorption field area requirements. The process may be used to achieve nitrification as well as nitrogen and phosphorus removal prior to surface and subsurface discharge (37).

10.2. Design Assumptions

Typical IF system design information is provided in Table 15.10 (37). With CF-type (continuous flow) SBRs, a typical cycle time is 3–4 h, with 50% of that cycle devoted to aeration (step 2), 25% to settling (step 3), and 25% to decant (step 4). With both types, downstream or subsequent unit processes (e.g., disinfection) must be designed for greater capacity (because the effluent flow is several times the influent flow during the decant period) or an equalization tank must be used to permit a consistent flow to those processes.

Table 15.10
Design parameters for IF-Type SBR systems (Source: US EPA)

Parameter[b]	SBR systems
Pretreatment	Septic tank or equivalent
MLSS (mg/L)	2,000–6,500
F/M load (lb BOD/day/lb MLVSS)	0.04–0.20
Hydraulic retention time (h)	9–30
Total cycle times (h)[a]	4–12
Solids retention time (day)	20–40
Decanter overflow rate (gpm/ft^2)	$<$100
Biosolids wasting	As needed to maintain performance

[a]Cycle times should be tuned to effluent quality requirements, wastewater flow, and other site constraints.
[b]Conversion factors: 1 gpm/ft^2 = 40.7 Lpm/m^2; 1 1b BOD/day/1b MLVSS = 1 kg BOD/day/kg MLVSS

Onsite package units should be constructed of non corrosive materials, such as coated concrete, plastic, fiberglass, or coated steel. Some units are installed aboveground on a concrete slab with proper housing to protect against local climatic concerns. The units can also be buried underground as long as easy access is provided to all mechanical parts, electrical control systems, and water surfaces. All electric components should meet NEC code and should be waterproofed and/or sheltered from the elements. If airlift pumps are used, large-diameter pipes should be provided to avoid clogging. Blowers, pumps, and other mechanical devices should be designed for continuous heavy-duty use. Easy access to all moving parts must be provided for routine maintenance. An effective alarm system should be installed to alert home owners or management entities of malfunctions (38).

10.3. Performance

With appropriate design and operation, SBR plants have been reported to produce high quality BOD and TSS effluents. Typical ranges of $CBOD_5$ (carbonaceous 5-day BOD) are from 5 to 15 mg/L. TSS ranges from 10 to 30 mg/L in well-operated systems. Fecal Coliform removal of 1–2 logs can be expected. Normally, nitrification can be attained most of the time unless cold temperatures persist. The SBR systems produce a more reliable effluent quality than CFSGAS owing to the random nature of the wastewater generated from an individual home. The CF/SBR is also capable of meeting secondary effluent standards (30 mg/L of CBOD and TSS), but more subject to upset by randomly generated wastewaters than the IF/SBR (39) if short-circuiting cannot be minimized.

10.4. Management Needs

Long-term management (including operation and maintenance) of SBRs through homeowner service contracts or local management programs is an important component of the operation and maintenance program. Homeowners do not typically possess the skills needed or the desire to learn to perform proper operation and maintenance. In addition, homeowner neglect, ignorance, or interference (e.g., disabling alarm systems) has contributed to

operational malfunctions. No wasting of biomass should be practiced until a satisfactory concentration has developed. Intensive surveillance by qualified personnel is desirable during the first months of startup.

Most operating parameters in SBR package systems can be controlled by the operator. Time clock controls may be used to regulate cycle times for each cycle, adjusted for and depending on observed performance. Alarm systems that warn of aerator system failure and/or pump failure are essential.

Inspections are recommended three to four times per year; septage pumping (biosolids wasting) is dependent upon inspection results. Operation and maintenance requires semi-skilled personnel. Based on field experience, 5–12 person-hours per year, plus analytical services, are required. The process produces 0.6–0.9 lb TSS/lb BOD (0.6–0.9 kg TSS/kg BOD) removed and requires between 3.0 and 10 kwh/day for operation (37). Operating personnel prefer these systems to CFSGAS for their simplicity of O/M tasks. The key operational components are the programmer and the decanter, and these must be maintained in proper working order.

10.5. Risk Management Issues

With proper management, a package SBR system is reliable and should pose no unacceptable risks to the homeowner or the environment (37). If neglected, however, the process can result in environmental damage through production of poor-quality effluent that may pose public health risks and can result in the premature failure of subsurface systems. Odor and noise may also create some level of nuisance. SBRs are less susceptible to flow and quality loading changes than other aerobic biological systems, but they are still not suitable for seasonal applications. They are similarly susceptible to extreme cold and should be buried and/or insulated in areas subjected to these extremes. Local authorities can provide guidance on climatic effects on equipment and how to prevent them. The controller should be located in a heated environment. Long power outages can result in odors and effluent degradation, as is the case with other aerobic biological systems.

10.6. Costs

For residential applications, typical system equipment costs, in term of 2009 US Dollars, are \$9,000–\$11,000. Installation costs vary depending on site conditions; installation costs between \$1,770 and \$3,740 are typical for uncomplicated sites with good access (37). It should be noted that additional system components (e.g., subsurface infiltration system) will result in additional costs.

Annual operation and maintenance costs include electricity use (<\$370/year), sludge removal (>\$120/year), and equipment servicing. Some companies are providing annual service contracts for these units for \$300–\$500 (37). Actual costs will vary depending on the location of the unit and local conditions.

Various biological and physicochemical SBRs were developed by Dr. Lawrence K. Wang, Dr. Lubomyr Kurylko, and Dr. Mu-Hao S. Wang (40–42). Today the biological SBR becomes a main stream biological process, but the physicochemical SBR remains to be an innovative

process which should be further researched promoted. The case history of a major physico-chemical SBR plant in Europe can be found from the literature (42).

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APPENDIX

US Army Corps of Engineers Civil Works Construction Yearly Average Cost Index for Utilities (36)

Year	Index	Year	Index
1967	100	1989	383.14
1968	104.83	1990	386.75
1969	112.17	1991	392.35
1970	119.75	1992	399.07
1971	131.73	1993	410.63
1972	141.94	1994	424.91
1973	149.36	1995	439.72
1974	170.45	1996	445.58
1975	190.49	1997	454.99
1976	202.61	1998	459.40
1977	215.84	1999	460.16
1978	235.78	2000	468.05
1979	257.20	2001	472.18
1980	277.60	2002	484.41
1981	302.25	2003	495.72
1982	320.13	2004	506.13
1983	330.82	2005	516.75
1984	341.06	2006	528.12
1985	346.12	2007	539.74
1986	347.33	2008	552.16
1987	353.35	2009	570.38
1988	369.45		

16
Flotation Biological Systems

Lawrence K. Wang, Nazih K. Shammas, and Daniel B. Guss

CONTENTS

Abstract Dissolved air flotation, air dissolving tube, friction valve, flotation chamber, spiral scoops, flotation system configurations, and flotation biological systems (FBS) for conventional activated sludge process with sludge recycle are considered in the chapter. The case studies of FBS treatment systems in petrochemical industry effluent treatment, municipal effluent treatment, and paper manufacturing effluent treatment, altogether with operational difficulties and their remedy are described in the chapter.

Key Words Dissolved air flotation • flotation biological system • flotation chamber • flotation system configurations • flotation of activated sludge • petrochemical industry effluent • municipal effluent treatment • paper manufacturing effluent treatment.

1. INTRODUCTION

Activated sludge consists of biological flocs that are matrices of microorganisms, nonliving organics, and inorganic substances. The microorganisms include bacteria, fungi, protozoa (Sarcodina, Mastigophora, Sporozoa, Ciliata, and Suctoria), rotifers, viruses, and higher forms of animals such as insect larvae, worms, and crustaceans. The activated sludge process is one of the most common biological wastewater treatment processes. It can be defined as a

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suspended growth system in which biological flocs are continuously circulated to come into contact and to oxidize the organic waste substances in the presence of oxygen and nutrients (1, 2). The waste organic matter is aerobically converted to gaseous carbon dioxide, cell tissue of microorganisms ($C_5H_7NO_2$), and other simple soluble end products. Part of the microorganisms (i.e. activated sludge) is returned to the aeration basin in order to maintain a constant microbial population (i.e. constant mixed liquor suspended solids). The wastewater is considered to be adequately treated when the excess microorganisms (i.e. excess waste sludge) and residual suspended solids are separated from the aqueous phase by clarification, and when the clarified effluent meets the Federal and State Effluent Standards. The most common clarification method used today is sedimentation (3–5).

The recent and accelerating emphasis on water pollution control has necessitated the rapid development of improved biological waste treatment systems to aid in cost and energy savings. The use of secondary flotation clarification in place of, or in assistance to, secondary sedimentation clarification in the activated sludge process system is one such recent advancement. The potential of this development, in terms of higher suspended solids and BOD (Biochemical Oxygen Demand) removals compared to existing plants and the expansion of hydraulic capacity at significantly reduced cost, is expected to result in extremely rapid acceptance of the process by municipalities and industries (6, 7).

The primary distinguishing feature of the improved activated sludge treatment system is that high rate dissolved air flotation (DAF) unit is the secondary clarifier for separation of suspended solids from the aeration basin effluent, as opposed to secondary sedimentation alone in conventional activated sludge systems. The concept of using flotation for water-solid separation is not new; many engineers have applied the flotation technology in sludge separation since the early 1920s. The major deterrent to flotation use in the municipal and industrial processes envisaged by these early practicing engineers was economics, with objections centering mainly on the cost of gas bubble generation and retention. Table 16.1 indicates the evolution of dissolved air flotation clarifiers during the last 50 years (8–10). The following progress has been made:

(a) Specific clarification load increased from 1.5 gpm/ft^2 (61 L/min/m^2) to 3.5 gpm/ft^2 (140 L/min/m^2) and for triple stacked unit to 10 gpm/ft^2 (400 L/min/m^2)
(b) The retention time of water in the flotation clarifier decreased from 30 to 3 min
(c) The largest unit size increased from 260 gpm (1,000 L/min) to 7,900 gpm (30,000 L/min) and for triple stacked units to 23,700 gpm (90,000 L/min)
(d) The size of modern DAF units is much smaller. It allows construction predominately in stainless steel prefabricated for easy erection
(e) The smaller size and weight 120 lb/ft^2 (60 kg/m^2) allows installation on posts leaving free passage under the unit; therefore, it is easier to find available space for indoor installation and to construct inexpensive housing
(f) Air dissolving is improved and now requires only 10 s retention time in the air dissolving tube instead of the previous 60 s; accordingly, this reduction in retention time results in smaller air dissolving tubes, which are predominantly built from stainless steel
(g) Availability of excellent flocculating chemicals gives a high stability of operation and high clarification degree

Table 16.1
Dissolved air flotation clarifiers: brief history of development (Conversion factors: 1 MGD = 3.785 MLD; 1 gpm/ft^2 = 40.7 Lpm/m^2)

Year	Type	Maximum capacity gpm (MGD)	Rate (gpm/ft^2)	Retention time (min)	Dissolved air type	Air dissolving tank retention time (s)
1920	SVEEN PETERSON	790 (1.1)	2.0	25	Full	60
1930–1935	ADKA SAVALLA	600 (0.85)	2.0	20	Vacuum	–
1948	KROFTA Unifloat ADKA Simplex KOMLINE SANDERSON	2,500 (3.8)	2.0	20	Full Partial	60
1955	KROFTA Flotator	2,800 (4.0)	4.0	20	Full	60
1965	KROFTA Sedifloat ADKA Standard INFILCO Carborundum	4,700 (6.6)	2.0	40	Partial	60
1970	PERMUTIT Erpac	4,000 (5.8)	3.0	12	Full Partial	60
1975	KROFTA SUPRACELL	8,000 (11.5)	3.5	3	Partial Recycle	10
1993	KROFTA Sandfloat BP	20,000 (28.8)	5.0	5	Partial Recycle	10

The comparison between a DAF clarifier and a conventional sedimentation clarifier shows that (6)

(a) DAF floor space requirement is only 15% of that required for sedimentation
(b) DAF volume requirement is only 5% of that required for sedimentation
(c) The degree of clarification of both clarifiers is the same with the same flocculating chemical addition
(d) The operational cost of the DAF clarifier is slightly higher than that for sedimentation, but this is offset by the considerably lower cost of installation financing
(e) DAF clarifiers are mainly prefabricated in stainless steel for erection cost reduction, corrosion control, better construction flexibility, and possible future changes, contrary to in situ constructed heavy large concrete sedimentation tanks. Ideally for design and construction of a new activated sludge wastewater treatment plant, it will be more cost-effective if secondary flotation is used instead of conventional secondary sedimentation

The primary objective of this chapter, however, is to introduce the secondary flotation concept which can be applied for improving treatment efficiency of an existing overloaded activated sludge plant, or for expansion of the existing plants hydraulic capacity to handle additional wastewater flows. A high rate dissolved air flotation clarifier can be applied in series between the aeration basin and secondary sedimentation in a conventional activated sludge process to separate the living microorganisms before settling in the existing secondary sedimentation

basins. This results in a flotation activated sludge (FAS) system that provides the following improvements in the existing plant:

(a) Solids and hydraulic loading rates on overloaded secondary sedimentation units are reduced resulting in increasing clarification efficiency and saving construction cost on expansion of secondary sedimentation facilities
(b) A reduction in recycle sludge volume due to higher solids content in the recycled sludge reduces the hydraulic loading on an aeration basin thus increases retention time without increasing aeration basin size
(c) Higher solids content in the waste sludge represents cost saving and improved operation of biosolids thickening, dewatering, and disposal
(d) The living microorganisms, separated by DAF, are returned to the aeration basin quickly (in less than 15 min) in a better aerobic condition and are more active than comparable settled microorganisms, and the oxygen requirement for the mixed liquor suspended solids is also significantly reduced
(e) The problems of sludge rising and sludge bulking can be completely eliminated when using secondary flotation

In general, the application of a DAF clarifier following a biological treatment unit results in a flotation biological system (FBS). Upgrading of such systems is not limited to the improvement of the activated sludge process (FAS system), but rather can be used for upgrading almost any biological treatment scheme as follows:

1. Flotation activated sludge (FAS)
2. Flotation trickling filter (FTF)
3. Flotation rotating biological contactors (FRBC)
4. Flotation contact stabilization (FCS)
5. Flotation sequencing batch reactor (FSBR)
6. Flotation oxygen activated sludge (FOAS)
7. Flotation stabilization ponds (FSP)
8. Flotation fluidized bed (FFB)
9. Flotation vertical shaft (FVS)
10. Flotation nitrification-denitrification (FND)
11. Flotation anoxic/oxic (FA/O)
12. Flotation anaerobic/anoxic/oxic (FA2/O)

2. FLOTATION PRINCIPLES AND PROCESS DESCRIPTION

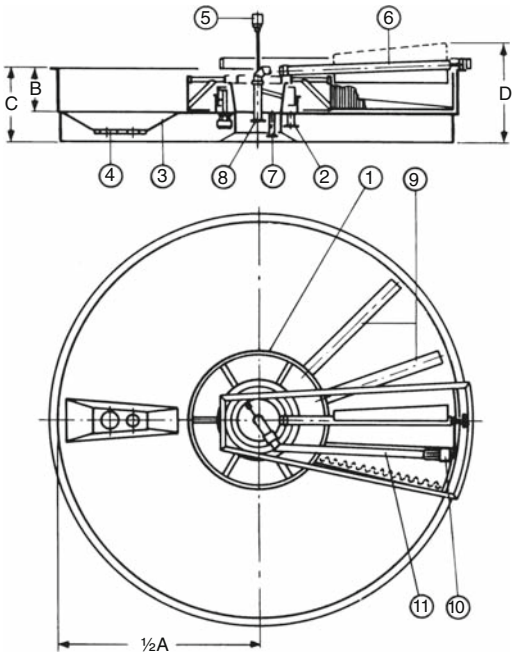
2.1. Dissolved Air Flotation

Dissolved air flotation is mainly used to float suspended and colloidal solids by decreasing their apparent density. The influent feed liquid can be raw water, wastewater, liquid sludge or industrial process water (11–36).

The flotation system consists of eight major components: Influent feed pump, air supply, pressurizing pump, air dissolving tube (retention tank), friction valve, flotation chamber, spiral scoop, and effluent extraction pipe. Figures 16.1 and 16.2 show a single cell and a double cell, respectively, of high rate dissolved air flotation clarifiers.

DESCRIPTION

1 ROTATING CENTER SECTION
2 CLARIFIED WATER OUTLET
3 SETTLED SLUDGE SUMP
4 SETTLED SLUDGE OUTLET
5 (KROFTA) ROTARY CONTACT
6 (KROFTA) SPIRAL SCOOP
7 FLOATED SLUDGE OUTLET
8 UNCLARIFIED WATER INLET
9 CLARIFIED WATER EXTRACTION PIPES
10 GEAR MOTOR
11 DISTRIBUTION DUCT



A DIAMETER of SUPRACELL
B DEPTH of SUPRACELL TANK
C DEPTH of SUPRACELL TANK with BOTTOM SUPPORT
D MINIMUM OVERALL HEIGHT of SUPRACELL

TYPE		DIMENSIONS						FLOW		
A ft	A mm	B in	B mm	C in	C mm	D in	D mm	m^3/mm	US GPM	m^3/hr
8	2400	23.5	600	33	850	45	1150	0,56	148	34
10	3200	23.5	600	33	850	49	1250	1,00	263	60
12	3900	25.5	650	35	900	51	1300	1,50	394	90
15	4500	25.5	650	37	950	57	1450	2,00	525	120
18	5500	25.5	650	37	950	58	1480	3,00	789	180
20	6100	25.5	650	37	950	61	1560	3,65	961	219
22	6700	25.5	650	37	950	62	1580	4,40	1160	264
24	7200	25.5	650	37	950	63	1600	5,08	1340	305
27	8100	25.5	650	37	950	67	1700	6,44	1695	386
30	9000	25.5	650	37	950	71	1820	7,95	2090	477
33	10000	25.5	650	37	950	72	1840	9,80	2580	588
36	11000	25.5	650	37	950	73	1860	11,87	3125	712
40	12200	26	660	38	960	76	1920	14,60	3840	876
44	13400	27	685	39	985	78	1980	17,60	4630	1056
49	14800	27	685	39	985	82	2070	21,50	5650	1290
55	16800	27	685	39	985	87	2200	27,70	7290	1662
62	19900	29.5	750	37.5	1050	87	2200	35,20	9265	2112
70	21300	30,7	780	42.7	1080	90,5	2300	44,90	11800	2692

Fig. 16.1. Single cell high rate DAF system.

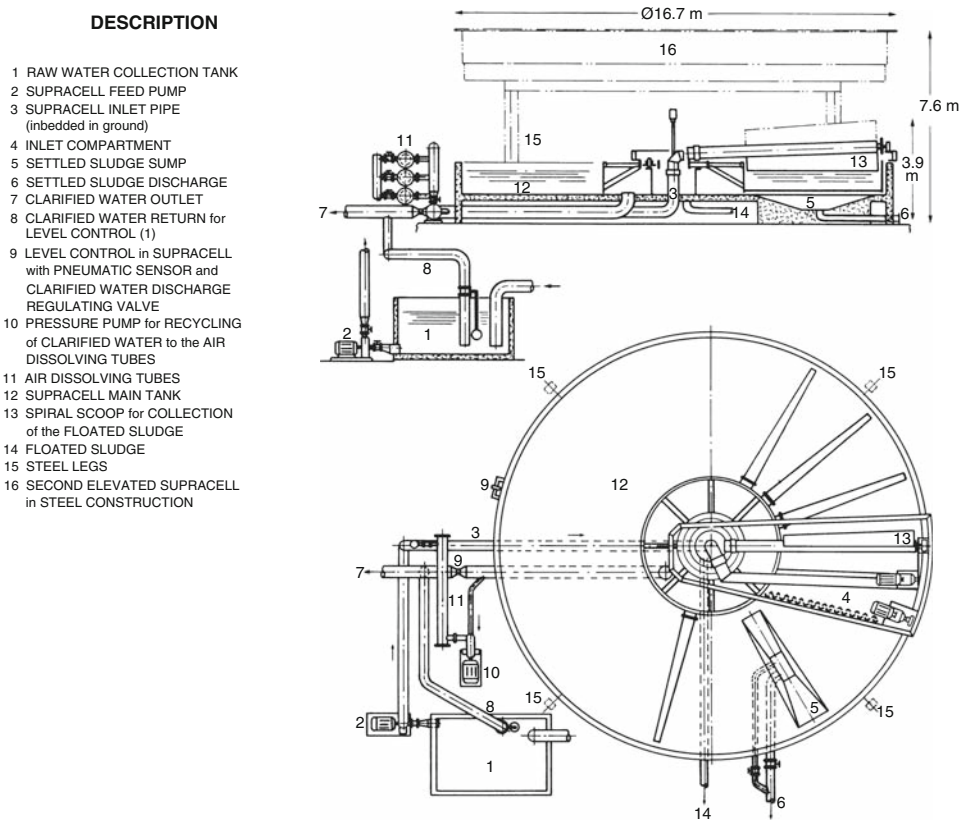


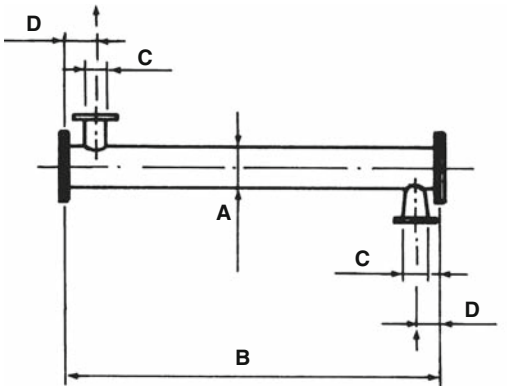
Fig. 16.2. Double cell high rate DAF system.

It is seen from Fig. 16.1 that the single unit can be as large as 55 ft (16.76 m) in diameter handling a maximum flow of 7,290 gpm (10.5 MGD or 39.74 MLD). However, for doubling the capacity vertically in order to save some land space, a second flotation unit can be installed on four legs over the bottom one, as shown in Fig. 16.2. This second unit is built in steel with steel supports. Three units installed one over the other have also been built, and are all incorporated in light-weight housing.

The flotation unit is delivered fully prefabricated. Larger units are delivered in parts which are flanged together. Generally, no heavy foundation or support structure is needed for a single cell unit as the total load factor when filled with water weighs less than 150 lb/ft^2 (732 kg/m^2), which is less than the load for a parking lot. A flat concrete ground pad is usually sufficient.

The inlet, outlet, and sludge removal mechanisms are contained in the central rotating section. This section and the spiral scoop rotate around the tank at a speed synchronized with the flow.

Unclarified water, first passing through an air dissolving tube and a friction valve, is released through a rotary joint in the center of the tank. It then passes into the distribution



DIMENSIONS OF AIR DISSOLVING TUBES

TYPE	500	1000	1500	2000	2500	3000
FLOW USGPM	132	263	395	526	658	789
A diam. inches	12"	12"	18"	18"	18"	18"
B feet inches	3' 10"	6' 8"	10' 2"	6' 8"	8' 5"	10' 2"
C diam. inches	2½"	6"	6"	6"	6"	6"
D inches	4"	6"	6"	6"	6"	6"

Fig. 16.3. Dimensions of air dissolving tube. (Conversion factors: $1' = 1$ ft $= 0.3048$; $1'' = 1$ in $= 2.54$ cm)

duct that moves backward with the same velocity as the forward incoming water. The settling and the flotation processes take place in the quiescent state in the flotation chamber.

The spiral scoop takes up the floated sludge, pouring it into the stationary center section where it is discharged by gravity for either recycling or disposal.

Clarified water is removed by effluent extraction pipes, which are attached to the moving center section. The clarified water, which normally contains less than 30 mg/L of suspended solids, can be recycled in the process and/or sewered.

Wiper blades attached to the moving distribution duct scrape the bottom and the sides of the tank and discharge settled sludge into the built in sump for periodic purging.

The variable speed gear motor drives the rotating elements and scoop. Electrical current for the gear motor feeds from a rotary contact mounted on the central shaft.

2.2. Air Dissolving Tube and Friction Valve

According to Henry's law, the solubility of gas (such as air) in aqueous solution increases with increasing pressure. The influent feed stream can be saturated several times at atmospheric pressure (45–85 psig or 312–590 kPa) by a pressurizing pump. The pressurized feed stream is held at this high pressure for about 10 s in an air dissolving tube designed to provide efficient dissolution of air into the water or wastewater stream to be treated. The pressurized stream usually enters the air dissolving tube tangentially at one end and is discharged at the opposite end. During the short passage, the water cycles inside the tube and passes repeatedly by an insert, fed by compressed air. Very thorough mixing under pressure then dissolves the air in the water. The small dimensions of the tube allow its economical construction using stainless steel. Figure 16.3 indicates the dimensions of air dissolving tubes, and Fig. 16.4 illustrates two typical models.

The pressurized water is decompressed in a friction valve (see Fig. 16.5), where the liquid is forced through a narrow slot in a coil spring. High shear is produced and dissolved air is forced out of the solution.

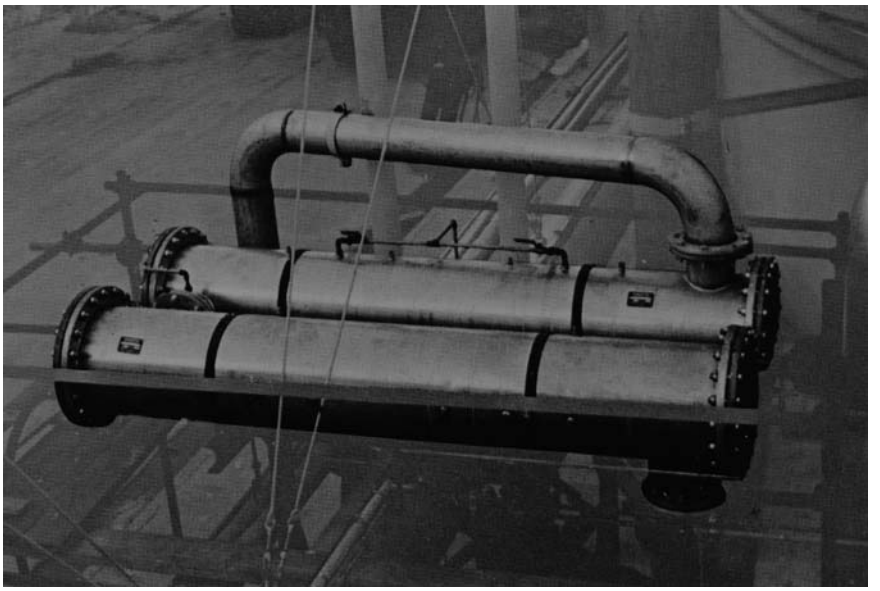


Fig. 16.4. Air dissolving tubes.

Fig. 16.5. Friction valve for pressure reduction.

2.3. Flotation Chamber

From the friction valve, the stream is released back to atmospheric pressure in the flotation chamber. Most of the pressure drop occurs after the friction valve and in the transfer line between the air dissolving tube and the flotation chamber, so that the turbulent effects of the depressurization can be minimized. The sudden reduction in pressure in the flotation chamber results in the release of microscopic air bubbles (average diameter 80 μm or smaller), which attach themselves to suspended or colloidal particles in the process water. This results in agglomeration, which, due to the entrained air, gives a net combined specific gravity less than

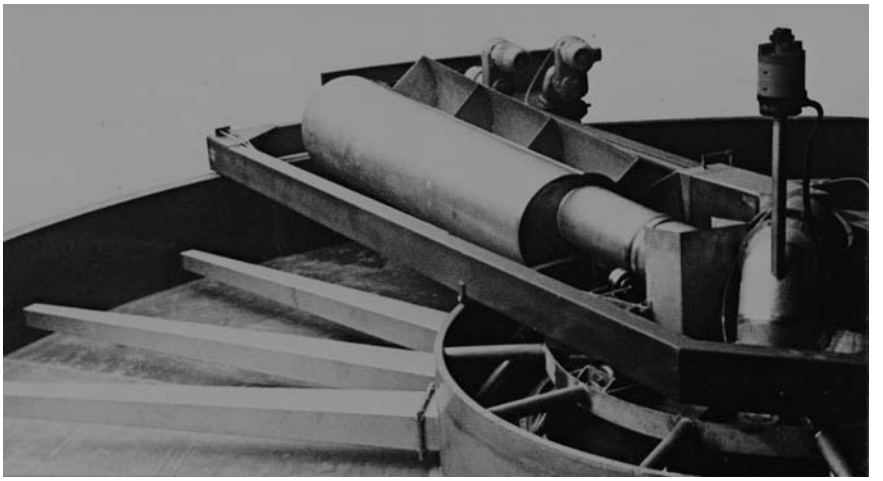


Fig. 16.6. Spiral scoop and effluent extraction pipes.

that of water and causes the flotation phenomenon. The vertical rising rate of air bubbles ranges between 0.5 and 2.0 ft/min (0.15–0.6 m/min). The floats rise to the surface of the flotation chamber to form a floated layer, which is carried away by a spiral scoop shown in Fig. 16.6. Clarified water effluent is usually drawn off from the bottom of the flotation chamber through effluent extraction pipes (see Fig. 16.6) and either recovered for reuse or discharged.

The unique compact and efficient design of the flotation cell is made possible by the use of the principle of "zero velocity." As mentioned earlier, the influent distribution duct moves backward with the same velocity as the forward incoming water. The "zero velocity" quiescent state in the flotation chamber is thus created to produce an ideal condition for flotation.

The retention time in flotation chambers is usually about 2.5–4 min depending on the characteristics of process water and the performance of the flotation unit. The process effectiveness depends upon the attachment of air bubbles to the particles to be removed from the process water. The attraction between the air bubbles and particles is primarily a result of the particle surface charges and bubble-size distribution. The more uniform the distribution of water and the microbubbles, the shallower the flotation unit can be. Generally, the depth of effective modern flotation units is only between 16 and 24 in (40.6 and 60.9 cm).

2.4. Spiral Scoops

Specially designed spiral scoops (see Figs. 16.6 and 16.7) continuously remove the floats, and subsequently pour them into the stationary center section of the flotation chamber from which they are discharged by gravity.

The surface layer of accumulated sludge thickens with time and can attain a thickness of several inches. Although the floats are relatively stable for some time, undue delays in removal will cause some release of particulates back to the liquid.

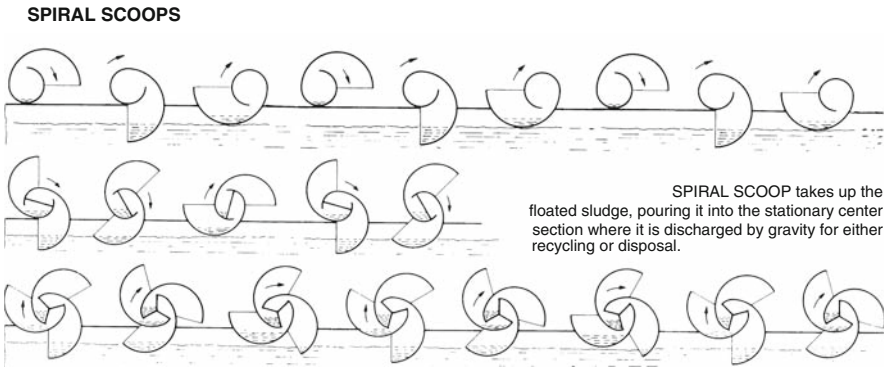


Fig. 16.7. Spiral scoop operation.

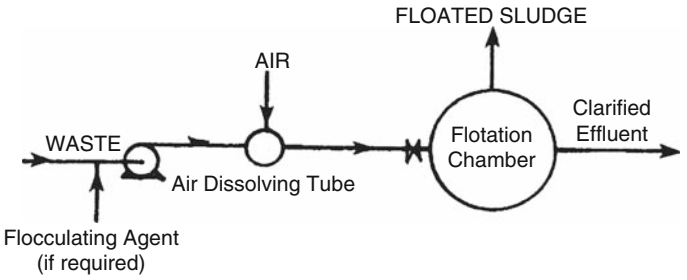


Fig. 16.8. Full flow pressurization system.

2.5. Flotation System Configurations

There are three common flotation system configurations, which are graphically illustrated in Figs. 16.8–16.10 respectively:

(a) Full flow pressurization
(b) Partial flow pressurization without effluent recycle
(c) Recycle flow pressurization

In the full flow pressurization system (Fig. 16.8), the entire influent feed stream is pressurized by a pressurizing pump and held in the air dissolving tube. The system is usually applicable to a feed stream with suspended solids exceeding 800 mg/L in concentration and not susceptible to the shearing effects caused by the pressurizing pump and the high pressure drop at the friction valve. It is occasionally used for separating some discrete fibers and particles, which require high volume of air bubbles. It is particularly applicable for solid-water separation where the suspended solids flocculate rapidly upon the addition of chemical coagulants in the inlet compartment in the presence of the released air. The air bubbles may become entrapped within the floc particles resulting in a strong air to solids bond, thus in a highly efficient separation process.

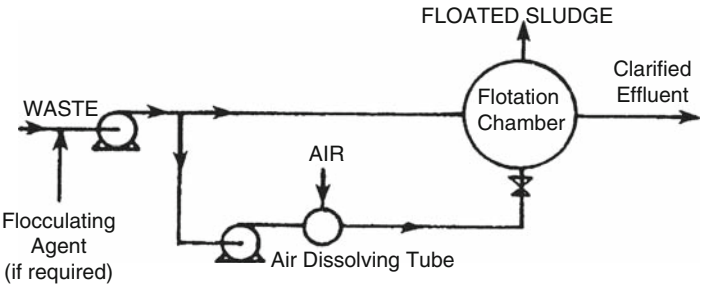


Fig. 16.9. Partial flow pressurization without effluent recycle system.

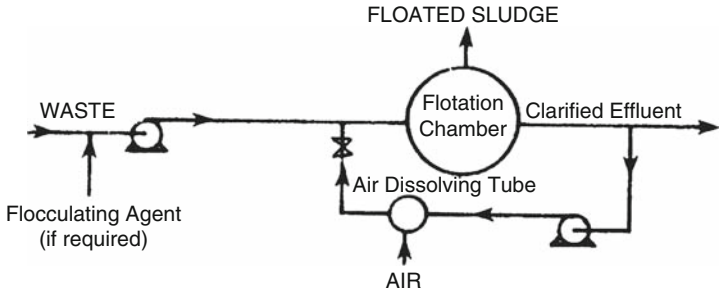


Fig. 16.10. Recycle flow pressurization system.

In the partial flow pressurization without effluent recycle system (Fig. 16.9), only about 30–50% of the influent feed stream is pressurized by a high pressure pump and held in the air dissolving tube. The remaining portion of influent stream is fed by gravity or low pressure pump to the inlet compartment of the flotation chamber where it mixes with the pressurized portion of the influent stream. Materials with low specific gravity can be removed using the partial flow pressurization system. This system is again not recommended to be used when the suspended solids are susceptible to the shearing effects of pressurizing pump and the high pressure drop at the friction valve. It is generally employed in applications where the suspended solids concentrations are low, resulting in lower air requirement, in turn, lower operation and maintenance costs.

In the recycle flow pressurization system (Fig. 16.10), a portion (15–50%) of the clarified effluent from the flotation chamber is recycled, pressurized, and semisaturated with air in the air dissolving tube. The recycled flow is mixed with the unpressurized main influent stream just before admission to the flotation chamber, with the result that the air bubbles come out of the aqueous phase in contact with the suspended particulate matter at the inlet compartment of the flotation chamber. This system is usually employed in applications where preliminary chemical addition and flocculation are necessary ahead of flotation. It eliminates the problems with shearing the floc particles since only clarified effluent passes through the pressurizing pump and the friction valve. It should be noted, however, that the increased hydraulic flow

in the flotation chamber due to flow recirculation must be taken into account in the flotation chamber design.

While all the aforementioned three system configurations can be used for sludge separation, only the recycle flow pressurization system is recommended for water purification and wastewater treatment.

3. FLOTATION BIOLOGICAL SYSTEMS

3.1. General Principles and Process Description

Activated sludge is a continuous flow biological treatment process characterized by the suspension of aerobic microorganisms, maintained in a relatively homogeneous state by the mixing and turbulence induced by aeration. The microorganisms oxidize soluble and colloidal organics to CO_2 and H_2O in the presence of molecular oxygen. The process is generally but not always, preceded by a primary sedimentation clarifier. The mixture of microorganisms and wastewater formed in the aeration basins, called mixed liquor, is transferred to gravity clarifiers for liquid solid separation. The major portion of the microorganisms settling out in the clarifiers can be recycled to the aeration basins to be mixed with incoming wastewater, while the excess, which constitutes the waste sludge, is sent to the sludge handling facilities (37). The rate and concentration of activated sludge returned to the aeration basins determines the mixed liquor suspended solids (MLSS) level developed and maintained in the basins. During the oxidation process, a certain amount of the organic material is synthesized into new cells, some of which then undergoes auto-oxidation (self-oxidation, or endogenous respiration) in the aeration basins, the remainder forming net growth or excess sludge. Oxygen is required in the process to support the oxidation and synthesis reactions. Volatile compounds are driven off to a certain extent in the aeration process. Metals will also be partially removed and accumulated in the sludge. Activated sludge systems are classified as high rate, conventional, or extended aeration (low rate) based on the organic loading. In the conventional activated sludge plant, the wastewater is commonly aerated for a period of 4–8 h (based on average daily flow) in a plug flow hydraulic mode. Either surface or submerged aeration systems can he employed to transfer oxygen from air to wastewater.

A partial listing of design criteria for the conventional activated sludge process is summarized as follows (1, 2, 38, 39):

Volumetric loading (lb BOD_5/day/1, 000 ft^3): 25–50
Aeration detention time (based on avg. daily flow) (h): 4–8
MLSS (mg/L): 1,500–3,000
F/M (lb BOD_5/day/lb MLVSS): 0.25–0.5
Air requirement (std. ft^3/lb BOD_5 removed): 800–1,500
Mean cell residence time (days) 5–10

Here

1 lb BOD_5/day/1,000 ft^3 = 16.018 g BOD_5/day/m^3
1 lb BOD_5/day/lb MLVSS = 1 kg BOD_5/day/kg MLVSS

$1\ \text{ft}^3/\text{lb} = 0.0624\ \text{m}^3/\text{kg}$

The success of an activated sludge process in producing a high quality effluent depends on a continuous growth of biological flocs having a good separating characteristic. The growth of biological flocs is accompanied by the organic substrate removal. The rate of microbial growth and the rate of substrate utilization are interrelated. If one assumes that the Michaelis–Menten enzymatic kinetics can be applied to the substrate utilization by microorganisms in the process, then (40)

$$\begin{aligned} U &= (\mathrm{d}S/\mathrm{d}t)/X \\ &= k_\mathrm{m} S/(K_\mathrm{s} + S) \\ &= Q(S_\mathrm{o} - S)/(VX) \\ &= (F/M)E/100 \end{aligned} \tag{1}$$

in which,

U = specific substrate utilization rate, or soluble organics utilization rate = change of soluble substrate concentration per unit time per unit microbial concentration
S = substrate concentration in solutions, mass per unit volume (mg/L)
X = microbial concentration (VSS) in reactor, mass per unit volume (mg/L)
k_m = maximum rate of specific substrate utilization, time^{-1}
K_s = Michaelis–Menten constant, or half velocity coefficient = to the substrate concentration when $U = k_\mathrm{m}/2$, mass per unit volume (mg/L)
S_o = initial substrate concentration, mass per unit volume (mg/L)
Q = volumetric wastewater flow rate, volume per unit time
V = reactor volume
F/M = the food to microorganism ratio = $S_\mathrm{o}/(tX)$
t = hydraulic detention time of reactor = V/Q
E = process efficiency = $100(S_\mathrm{o} - S)/S_\mathrm{o}$

Biological growth is the result of the coupled synthesis-endogenous respiration reactions. The net result can be expressed as:

$$\mu = (\mathrm{d}X/\mathrm{d}t)/X = YU - b \tag{2}$$

in which,

μ = net specific growth rate = change of microbial concentration per unit time per unit microbial concentration, time^{-1}
Y = growth yield coefficient, mass microbial growth per unit mass substrate utilized
b = endogenous or decay coefficient, time^{-1}

3.2. Kinetics of Conventional Activated Sludge Process with Sludge Recycle

There are four conventional activated sludge process schemes: (a) complete-mix reactor with sludge recycle (b) complete-mix reactor without sludge recycle (c) plug-flow reactor

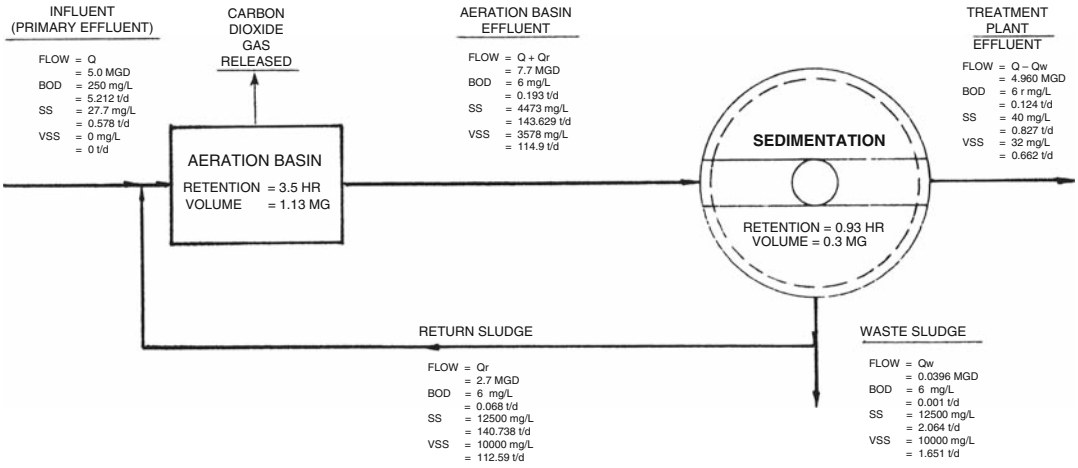


Fig. 16.11. Conventional activated sludge process. (Conversion factors: 1 MGD = 3.785 MLD; 1 MG = 3.785 ML; 1 t = 1 ton = 2000 lb = 907.2 kg = 0.9072 metric ton)

with sludge recycle and (d) plug-flow reactor without sludge recycle. These process schemes are described elsewhere in detail (1, 2, 38, 41). This chapter introduces only the conventional system using complete-mix reactor with sludge recycle for the purpose of comparison between a conventional system and an improved system, flotation activated sludge (FAS) process using secondary flotation.

In the conventional activated sludge process with biological sludge recycle, shown in Fig. 16.11, the mean cell residence time or sludge retention time is longer than the hydraulic retention time. When sludge wasting is accomplished from the recycle line, the sludge retention time is calculated as θ_c in the following:

$$\theta_c = VX/[Q_w X_r + (Q - Q_w)X_e] \tag{3}$$

in which,

Q_w = wasted sludge flow rate, volume per unit time
X_r = return sludge concentration, mass per unit volume
X_e = sludge concentration in the treated effluent from the final sedimentation clarifier

Assuming that X_e is very small, Eq. (3) can be rewritten as:

$$Q_w = VX/(\theta_c X_r) \tag{4}$$

By writing the mass balance equation for sludge in the entire system, as shown in Fig. 16.11, and assuming X_o is in negligible amounts (X_o = sludge concentration in the primary effluent), one can get the following:

$$V(dX/dt) = (YUX - bX)V - [Q_w X_r + (Q - Q_w)X_e] \tag{5}$$

where

$V(\mathrm{d}X/\mathrm{d}t)$ is the rate of change of microorganism concentration in reactor; $(YUX - bX)V$ is the net rate of microorganism growth in reactor and $Q_w X_r + (Q - Q_w)X_e$ is the rate of microorganism outflow from reactor. Making use of Eq. (3) and considering steady state conditions, Eq. (5) can be simplified and rearranged to yield

$$1/\theta_c = \mu = YU - b \tag{6}$$

in which,

both $1/\theta_c$ and μ are called the net specific growth rate. The following are the working equations of substrate (S), mixed liquor suspended solids concentration (X) and aeration volume (V) for the sludge recycle model:

$$S = K_s(1 + b\theta_c)/\left[\theta_c(Yk_m - b) - l\right] \tag{7}$$

$$X = \theta_c Y(S_o - S)/t(1 + b\theta_c) \tag{8}$$

$$V = YQ\theta_c(S_o - S)/X(1 + b\theta_c) \tag{9}$$

It is important to know from Eq. (7) that the performance of a complete-mix with recycle system does not depend on hydraulic retention time. For a specific wastewater, a biological culture, and a particular set of environmental conditions, all coefficients K_s, b, Y, and k_m become constant. It is apparent from Eq. (7) that the system performance is a function of mean cell residence time.

A typical overloaded complete-mix activated sludge treatment plant is illustrated in Fig. 16.11. The treatment plant treats 5.0 MGD (18.93 MLD) of settled wastewater having a 5-day BOD of 250 mg/L. The plant effluent consistently contains over 40 mg/L of suspended solids (SS) and about 6 mg/L of soluble 5-day BOD. The effluent SS violate the effluent standard because of the overloaded existing sedimentation clarifier. Assume that the following field conditions are applicable:

(a) Wastewater temperature 20°C
(b) Return sludge concentration = 12, 500 mg/L SS
(c) Volatile suspended solids (VSS) = 0.8 SS
(d) Mean cell residence time $\theta_c = 10$ days
(e) Growth yield coefficient $Y = 0.65$ lb cells per lb of 5-day BOD utilized = 0.65 kg cell/kg 5-day BOD
(f) Endogenous or decay coefficient $b = 0.1\ \mathrm{day}^{-1}$
(g) Waste contains adequate nitrogen, phosphorus, and other necessary trace nutrients for biological growth
(h) Aeration basin volume $V = 1, 130, 000$ gal = 4,277,050 L
(i) Sedimentation clarifier volume = 300, 000 gal = 1, 135, 500 L

The process conditions of the existing system will be:

(a) Mixed liquor suspended solids $X = 4, 375$ mg/L
(b) Hydraulic detention time of aeration basin, $t = 3.5$ h
(c) Hydraulic detention time of sedimentation clarifier, $t = 0.935$ h
(d) Return sludge flow $Q_r = 2.7$ MGD = 10.22 MLD

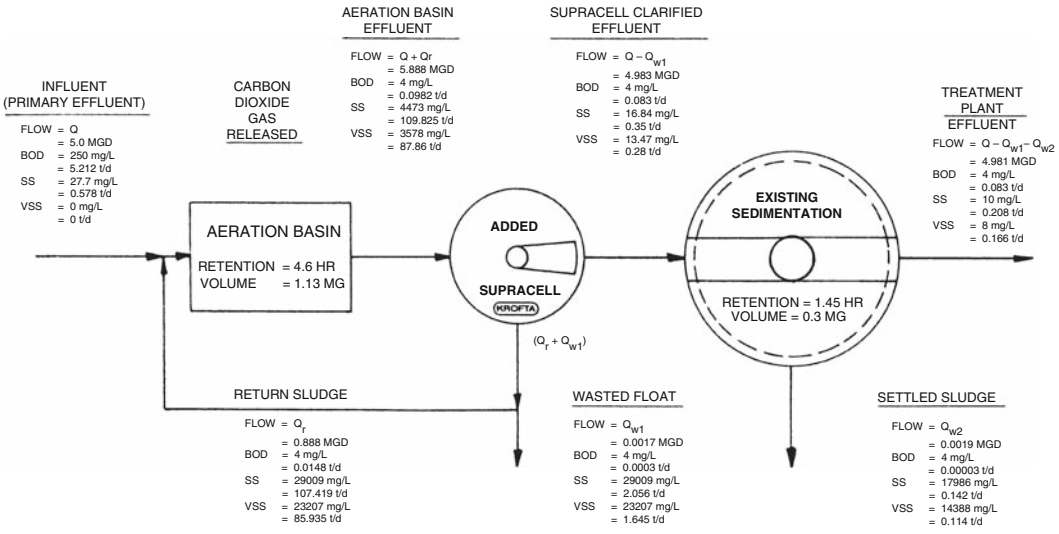


Fig. 16.12. Upgraded flotation activated sludge (FAS) process. (Conversion factors: 1 MGD = 3.785 MLD; 1 MG = 3.785 ML; 1 t = 2000 lbs = 1 ton = 907.2 kg = 0.9072 metric ton)

(e) Sludge production rate $(dX/dt) = 3{,}300\,\text{lb VSS/day} = 4{,}125\,\text{lb SS/day} = 1497\,\text{kg VSS/day} = 1871\,\text{kg SS/day}$
(f) Waste sludge flow $Q = 0.04\,\text{MGD} = 0.15\,\text{MLD}$
(g) Specific substrate (soluble 5-day BOD) utilization rate $U = 0.31\,\text{day}^{-1}$

3.3. Kinetics of Flotation Activated Sludge Process Using Secondary Flotation

Figure 16.12 shows the improved activated sludge process (FAS) in which a new secondary flotation unit is applied in series between the aeration basin and the final sedimentation clarifier for increasing the overall treatment performance and hydraulic capacity of an originally overloaded existing plant.

A microbial mass balance equation can be established for the FAS system shown in Fig. 16.12:

$$V(dX/dt) = (YUX - bX)V - [Q_{w1}X_r + Q_{w2}X_{w2} + (Q - Q_{w1} - Q_{w2})X_e] \qquad (10)$$

where

$V(dX/dt)$ = the rate of change of microorganism concentration in reactor
$(YUX - bX)V$ = the net rate of microorganism growth in reactor
$[Q_{w1}X_r + Q_{w2}X_{w2} + (Q - Q_{w1} - Q_{w2})X_e]$ is the rate of microorganism outflow from the reactor
Q_{w1} = flow rate of waste sludge from secondary flotation, volume per unit time
X_{w1} = concentration of waste sludge (float) from secondary flotation, mass per unit volume

Q_{w2} = flow rate of waste sludge from the existing final sedimentation clarifier, volume per unit time

X_{w2} = concentration of waste sludge from the existing final sedimentation clarifier, mass per unit volume

The sludge retention time (θ_c) can he calculated from the following equation:

$$\theta_c = VX/[Q_{w1}X_r + Q_{w2}X_{w2} + (Q - Q_{w1} - Q_{w2})X_e] \tag{11}$$

Assuming that the sludge concentration in the treated plant effluent (X_e) is very low, Eq. (11) can be rewritten as

$$\theta_c = VX/(Q_{w1}X_r + Q_{w2}X_{w2}) \tag{12}$$

Again making use of Eqs. (1), (2), and (11), and considering steady state conditions, Eq. (11) can also be simplified and rearranged to yield Eq. (6). It is, therefore, concluded that the design equation of net specific growth rate (μ or $1/\theta_c$) for the conventional activated sludge system is identical to that of the improved FAS system. The numerical values of the two net specific growth rates, however, are different. Figure 16.13 shows the specific substrate utilization rate vs. the limiting substrate concentration for the two activated sludge systems considered. Both systems use identical biological flocs, and naturally, the maximum specific substrate utilization rates (k_m) of the two systems are the same. The living biological flocs, separated by secondary flotation, are returned to the aeration basin quickly (in less than 15 min), and thus stay in aerobic conditions at all times. Accordingly, the returned sludge (i.e. biological flocs) from secondary flotation (Fig. 16.12) are more active (in terms of lower K_s value in Fig. 16.13) than comparable settled sludge from conventional secondary sedimentation (Fig. 16.11). According to Eq. (1), the improved FAS system (Fig. 16.12) having a relatively lower K_s value (Fig. 16.13) will definitely have a higher specific substrate utilization rate (U), signifying a higher biological treatment efficiency.

Microscopic examinations of floated sludge from secondary flotation and settled sludge from secondary sedimentation have been made to further demonstrate the aforementioned facts. Unstained samples of floated and settled sludges showed a marked difference in the number and viability of free-swimming and stalked ciliates (protozoa). Settled sludge contained only a few stationary cells (noted in 100 microscopic fields); floated sludge contained about 200 times more motile protozoan cells. Since protozoa are an integral and very important segment of the biological community, flotation is a desirable follow-up to the provision of dissolved oxygen (DO) within an aeration basin.

The net specific growth rate Eq. (6) holds true for both conventional and improved FAS systems. The latter having comparatively higher specific substrate utilization rate (U) has higher net specific growth rate (μ), and requires less mean cell residence time (θ_c) provided that the growth yield coefficient (Y) and the decay coefficient (b) of the floated sludge and the settled sludge are assumed to be the same.

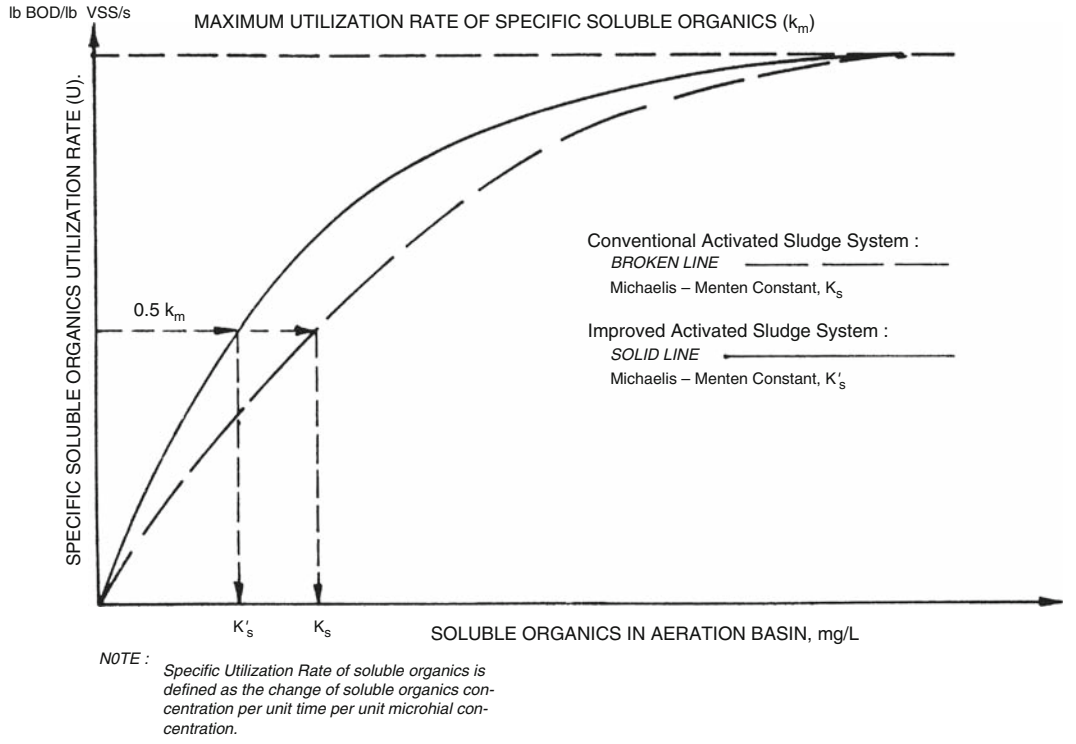


Fig. 16.13. Specific substrate utilization rate vs. substrate concentration. (Conversion factor: 1 lb BOD/lb VSS/s = 1 kg BOD/kg VSS/s)

The mean hydraulic retention time (t) can be determined by Eq. (13), regardless of the types of treatment system used:

$$t = \sum_{i=1}^{n} \frac{V_i}{Q} \tag{13}$$

For example, the mean hydraulic retention time for the entire FAS system is

$$t = (V_p + V + V_f + V_s)/Q \tag{14}$$

Where

V_P = volume of primary clarifier
V = volume of aeration basin
V_f = volume of secondary flotation
V_s = volume of final sedimentation
Q = total wastewater flow to the system

For the secondary sedimentation alone is

$$t = V_s/(Q - Q_{w1}) \tag{15}$$

For the aeration basin alone is

$$t = V/(Q + Q_r) \tag{16}$$

A typical overloaded conventional complete-mix activated sludge treatment plant (shown in Fig. 16.11) has been described earlier. The same conventional plant can be improved by the addition of a secondary flotation unit (shown in Fig. 16.12). Some advantages of the improved FAS system are presented mathematically below.

The hydraulic detention time of the secondary sedimentation clarifier of original overloaded conventional system is

$$t = V_s/Q = 0.935\,\text{h} \quad \text{(see Fig. 16.11)} \tag{17}$$

In comparison with Eq. (15) for the improved FAS system, it is seen that the hydraulic loading on the originally overloaded secondary sedimentation can be reduced significantly by a parameter of Q_{w1}, thus saving the construction cost required for the expansion of the secondary sedimentation facilities. With the addition of a small flotation cell (detention time = 3 min), the detention time of the sedimentation clarifier can be increased by 55% (i.e. from 0.935 to 1.45 h), as shown in Figs. 16.11 and 16.12.

The hydraulic detention time of the conventional system's aeration basin is also expressed by Eq. (16). However, the return sludge flow (Q_r) of the improved FAS system is only 33% ($0.888/2.7 = 0.33$) of the conventional system, assuming the suspended solid concentrations (i.e. consistencies) of floated sludge and settled sludge are 2.8 and 1.25%, respectively. Accordingly, the hydraulic loading on an aeration basin can be reduced significantly by the addition of the flotation cell. The addition increases the hydraulic retention time (from 3.5 to 4.6 h or a 31% increase) without actually increasing the size of the aeration tank.

The higher solids content (X_{w1}) of the waste sludge produced from the improved FAS system, shown in Fig. 16.12, represents another cost saving because of the improved operation of sludge thickening, dewatering, and disposal. The waste sludges produced from the FAS system are 0.0170 MGD at 29,009 mg/L and 0.0019 MGD at 17,986 mg/L, i.e. a combined sludge of 0.0189 MGD at 27,900 mg/L. Here 1 MGD = 3.785 MLD.

The comparable conventional system (Fig. 16.11), on the other hand, generates 0.0396 MGD of waste sludge at a concentration of 12,500 mg/L. The sludge treatment cost of an improved FAS system will, therefore, be reduced to one-half because of a reduction in sludge flow and an increase in sludge consistency.

The most important fact is that both the effluent suspended solids (X_e) and effluent soluble 5-day BOD (S) of the improved FAS system will be able to meet the governmental effluent standards.

4. CASE STUDIES OF FBS TREATMENT SYSTEMS

Many pilot-scale and full-scale trials involving the use of a flotation cell as a secondary flotation unit in biological treatment plants were conducted by the Lenox Institute of Water Technology (LIWT) in Lenox, MA. Only partial operational data are selected for the presentation in the following three case studies.

4.1. Petrochemical Industry Effluent Treatment

The first example is for a petrochemical industrial facility located in Texas with an existing conventional activated sludge treatment plant. The waste loading was projected to increase when plant production increases in the following years. Consequently, the plant needs to meet present state discharge limits and possible future more stringent limits. Mechanical breakdown of the secondary sedimentation clarifier or extremely high hydraulic loads due to sudden rainstorms have in the past caused severe problems with high loadings, rising sludge, bulking sludge, and poor effluent water quality. Figure 16.11 shows the conventional treatment plant before upgrading by the addition of an intermediate secondary flotation unit.

Full-scale trials with a flotation cell clarifier were conducted with excellent pilot results in the removal of solids, sludge consistency, and water clarity. The following are the conclusions drawn from the investigation (see Table 16.2):

(a) *Suspended solids (SS) loading:* SS Loadings of 111–175 lb/day/ft^2 were maintained while still obtaining an acceptable quality of clarified water (125–475 mg/L of SS, or 94–98% SS removal). Improvement in water quality could be obtained with lower SS loadings (60–77 lb/day/ft^2). At a loading of 60 lb/day/ft^2 and with chemical addition very clean water (less than 20 mg/L of SS) was obtained. Here 1 lb/day/ft^2 = 4.8824 kg/day/m^2.

(b) *Chemical treatment:* Acceptable operation was obtained without chemical aids. It is recommended, however, that the aids be available for full scale operation. A small chemical addition dosage of cationic polymer (Pearl River Chemical 560 or equivalent) in the 10–20 mg/L range may be desirable for good sludge compaction and improvement in overall clarification. Larger doses in the range up to 100 mg/L of cationic polymer gave exceptionally clear water. This chemical dose would be used in those cases when the cleanest possible water must be obtained (i.e. during breakdown of the existing settling unit).

(c) *Aeration system:* The demonstration plant was operated in the "full flow pressurization" mode in which all of the incoming water, including dilution water is pumped through the air dissolving tube. For power savings in large installations, only the recycled water would be aerated (i.e. the recycle flow pressurization system would be used; see Fig. 16.10). This will not change the amount of air available for flotation, as the clarified water is a more effective absorption medium than the incoming water. If the raw incoming water is pumped into the unit with a low shear type pump, or by gravity flow, less shearing and breakup of the flocs would be expected in the larger unit, than was experienced in the demonstration plant.

(d) *Settled material:* The great majority of the incoming solids floated, however, some settled material was observed in the bottom purge. For the full-scale unit, an automatic timed purge valve is to be installed opening a few seconds each 1/2 to 1 h. The bottom purge goes directly to the existing sedimentation clarifier. This purge keeps the flotation cell continuously clean, avoiding any build-up on the bottom which could occur during operation over an extended period of time.

Fig. 16.14. 49-ft Diameter flotation cell clarifier.

(e) *Sizing for full-scale unit:* Normal flotation cell influent flow from the aeration basin is estimated at 2,000 gpm of discharged water plus the recycled sludge flow. At normal solids loading of 4,000 mg/L from the aeration basin and minimum floated sludge consistency of 2.2%, the total hydraulic loading on the flotation unit is estimated at 2,400 gpm (9,084 Lpm). A 49-ft (15-m) diameter flotation unit would have a SS loading under normal conditions of 60 lb/day/ft^2 (293 kg/day/m^2). During a rainstorm, both SS and hydraulic loadings would be significantly increased. If the solids concentration is increased to 6,000 mg/L, the load would increase by approximately 65%. If both conditions occur together, the loading may go as high as 160 lb/day/ft^2, (781 kg/day/m^2), which is near the highest rate run in the demonstration plant. The 49-ft (15-m) unit would be the best choice, giving the ability for excellent clarification under normal conditions and acceptable operation under the worst expected loadings.

Based on the test results, improvements in the hydraulic characteristics of the plant alone were very attractive for the industrial complex to install a 49-ft (15-m) diameter flotation cell clarifier for secondary clarification (see Fig. 16.14). In addition to the hydraulic improvements, improved microbial activity will increase the performance of the treatment plant. The trials have demonstrated that under normal operating conditions, the flotation cell can produce the same clarity as the existing settling unit, thus giving the unit 100% backup. Under overload conditions, which can be caused by rainstorms or mechanical breakdown in sludge wasting, etc., the flotation cell will cushion the shock of heavy SS loading by removing 90% of the solids. The total project cost was far less than the comparable expansion of the aeration basin and the secondary sedimentation clarifier not withstanding the fact that the flotation cell will use less power (42).

4.2. Municipal Effluent Treatment

The Municipal Effluent Treatment Plant in Haltern, West Germany, was designed for a wastewater capacity generated from 37,000 population units. The influent flow ranges from 310 m^3/h in dry weather to 626 m^3/h in rainy weather. The activated sludge plant treats about

Table 16.2
Operational data: petrochemical industry wastewater treatment plant (Conversion factors: 1 gpm = 3.785 Lpm = 3.785 L/min)

Test runs	Raw SS (mg/L)	Raw SS (lb/day)	Influent SS (mg/L)	Clarified SS (mg/L)	SS removal (%)	FLOAT SS (%)	Raw flow (gpm)	Clarified flow (gpm)	Sludge flow (gpm)	Recycle flow (gpm)	Total flow (gpm)	Polymer addition (mg/L)
1	8,305	121	3,620	143	98.3	2.8	12.2	10.5	2.2	16.4	28.6	None
2	8,480	175	5,105	125	98.5	2.4	17.2	12.6	2.4	10.6	28.7	None
3	8,410	130	5,015	160	98.1	2.5	12.9	7.6	5.3	9.0	21.9	None
4	8,130	113	4,280	235	97.1	2.3	11.6	7.4	4.2	11.0	22.6	None
5	8,070	112	4,240	153	98.1	2.2	11.6	7.4	4.2	10.8	22.4	None
6	8,680	122	4,070	190	97.8	2.5	11.8	7.6	4.2	14.0	25.8	None
7	8,890	129	4,320	475	94.6	2.6	12.1	8.0	4.1	14.3	26.4	None
8	9,080	132	4,970	460	94.9	2.6	12.1	8.0	4.1	11.0	23.1	None
9	9,030	137	5,180	375	95.8	2.5	12.7	8.5	4.2	10.1	22.8	None
10	8,740	114	4,650	180	97.9	2.3	10.9	8.4	2.5	9.9	20.8	49
11	8,760	111	5,060	280	96.8	2.1	10.6	8.1	2.5	8.2	19.0	49
12	8,550	77	3,060	115	98.6	2.2	7.5	5.4	2.1	13.9	21.4	49
13	8,530	73	3,330	120	98.6	1.9	7.4	5.5	1.9	12.0	19.4	70
14	8,630	73	3,190	145	98.3	3.1	7.1	6.0	1.1	12.7	19.8	70
15	8,530	60	1,800	0	(100)	3.7	5.9	4.3	1.6	22.0	27.9	100

Notes: (a) Polymer used was Pearl River Chemical 560 cationic, Cost at residual SS of 10 mg/L = \$0.06/1, 000 gal = \$0.016/1,000 L.

(b) All suspended solids testing was done in the field, using procedures as outlined in Standard Methods (30). Particle size retention of the glass fiber filter pad used was 0.3 μm.

(c) SUPRACELL operation parameters: air dissolving tube pressure 60 PSI = 416 kPa, compressed air 70 PS1 = 486 kPa, scoop speed 6 rpm, rotometer 10 SCFH = 0.283 m^3/h, water temperature 81°F = 27.2°C, pH of water 7.1. Diameter of SUPRACELL: 4 ft = 1.22 m.

(d) SUPRACELL influent is the mixture of raw wastewater and recycle flow.

(e) Suspended solids (SS) percent removal is calculated based on the equation: 100(raw SS – clarified SS)/(raw SS).

1,000 kg BOD_5/day. The total volume of aeration basins is 1, 180 m^3. Both solid loading and hydraulic loading of the plant are high; the plant engineers were actively seeking feasible solutions from both technical and economical viewpoints. Many pilot plant operations were conducted under the direct supervision of the plant's manager. The use of a flotation clarifier for secondary clarification in the activated sludge treatment plant proved to be a feasible and good option.

The pilot plant (Fig. 16.10) had a diameter of 6 ft (1.8 m) designed for a hydraulic capacity of 12 m^3/h and operated as a recycle flow pressurization system. The maximum pump pressure was 4.0 bars. The pilot flotation cell was installed between the aeration tank and the secondary sedimentation tank. A centrifugal pump was used to feed the aeration tank effluent to the pilot cell. The flotation cell effluent was discharged into the existing sedimentation tank; the floated sludge was partially returned to the aeration tank, and partially wasted, as shown in Fig. 16.12.

Table 16.3 highlights some of the operational data. It can be seen that the flotation cell effluent was fully aerated with an oxygen content ranging from 6.2 to 7.4 mg/L. The dissolved oxygen in the floated sludge was above 3 mg/L except for the first two runs when the pilot flotation cell was just started. The settleable solids in the clarified flotation cell effluent, in

Table 16.3
Operational data: FAS municipal effluent treatment plant

Test runs	Flows (m^3/h)	Oxygen (mg/L)			Settleable (mL/L)			SVI (mL/g)		SS volatility (%)			Total solids (mg/L)		
	INF	INF	EFF	FLOAT	INF	EFF	FLOAT	INF	FLOAT	INF	EFF	FLOAT	INF	EFF	FLOAT
1	1,440	2.7	7.2	1.5	225	2.0	980	107	48	9.9	75	69	2,100	100	20,400
2	684	2.9	7.0	1.8	225	1.5	970	109	47	–	67	62	2,070	150	20,800
3	342	3.8	6.8	–	270	0.3	998	114	39	62	50	74	2,370	50	25,700
4	288	4.5	–	–	260	0.5	998	96	28	67	–	–	2,700	25	35,400
5	306	7.8	–	–	260	0	–	–	–	–	–	–	–	–	–
6	396	2.7	–	–	200	0	999	95	30	61	–	77	1,900	–	33,200
7	396	6.5	–	–	–	0	–	–	–	–	80	–	–	–	–
8	216	1.0	6.2	–	210	0.1	990	102	32	–	–	–	2,050	40	31,400
9	234	7.5	7.4	3.8	225	–	990	132	39	73	68	65	1,700	19	25,200
10	331	3.5	7.0	3.2	240	–	999	126	35	66	59	79	1,900	8	28,800
11	324	2.8	7.2	3.1	270	0.1	1,000	150	36	60	64	68	1,800	8	28,000

Notes: (a) Temperature of wastewater and floated sludge = 16–19°C.
(b) pH of SUPRACELL influent = 7.3–8.1; pH of SUPRACELL effluent = 7.2–7.6.
(c) Recycle flow pressurization system.
(d) Scoop operation for float collection = 4.1–5.2 rpm.
(e) Depth of float collected by the scoop = 7.5–9 in (19–23 cm) from surface.
(f) Carrier operation = 5–7.3 min /rev.
(g) Air supple = 0.5–0.8 m^3/h.
(h) Pressure of compressed air to the air dissolving tube = 5.4–5.8 bars = 540–580 kPa.

most cases, were below 1 mg/L. It is interesting to see that the sludge volume index (SVI) of the flotation cell influent (i.e. the aeration tank effluent) originally was in the range of 95–150. After DAF treatment, the SVI of the floated sludge was in the range of 28–48. The overall flotation cell treatment efficiency can be judged by the total solids in the influent, clarified effluent, and the float. Table 16.3 indicates that in all cases, the treatment efficiency was between 92 and 99% in terms of total solids removal. The float consistency was between 2 and 3.5%, which could be further concentrated if it is so desired.

Additional investigations were conducted for the comparison of original conventional system using only secondary sedimentation and the improved FAS system using an intermediate secondary flotation cell. It was observed that the effluent of the flotation clarifier contained 6–7 mg/L of dissolved oxygen, while the effluent of the sedimentation clarifier (without DAF) contained only about 2 mg/L. The average characteristics of the settled sludge from the existing sedimentation clarifier and the floated sludge from the flotation clarifier are shown below:

It can be seen that comparatively the floated sludge will have higher dissolved oxygen content, higher total solids consistency, larger solids volume, and a much lower sludge volume index. In conclusion, the quality of the floated sludge is better than that of settled sludge.

It should be noted, however, that the flotation pilot cell installed between the existing aeration basin and sedimentation clarifier was fed by a centrifugal pump. Although the total solids and suspended solids of the flotation cell were extremely low, the turbidity was high.

	Settled sludge	Floated sludge
Dissolved oxygen (mg/L)	0.56	2.5
Total solids (mg/L)	4,780	26,000
Solids volume (mL/L)	492	954
SVI (mL/g)	103	37

The problem of high effluent turbidity can be solved in the full-scale installation by feeding the flotation cell with a non-shearing screw pump.

4.3. Paper Manufacturing Effluent Treatment

A conventional activated sludge treatment plant for a paper manufacturing industry in Houston, Texas, was heavily overloaded. A 4-ft flotation cell was used to evaluate the flotation characteristics of the mixed liquor suspended solids in the effluent from the plant's aeration tank. The pilot cell had a flotation area of 10 ft^2 (0.929 m^2) and a maximum hydraulic loading of 30 gpm (113.55 Lpm). The unit was set up adjacent to the aeration basin at the waste treatment plant. A small sump pump was used to pump mixed liquor to the flotation unit.

The pilot trial was composed of three different test runs:

Run 1: Full flow pressurization without chemical treatment
Run 2: Full flow pressurization with chemical treatment
Run 3: Recycle flow pressurization with chemical treatment

Table 16.4 summarizes the experimental conditions of the three test runs, while Table 16.5 documents the operational data generated from this investigation. The data showed that 93% solids capture was obtained at a hydraulic loading of 2 gpm/ft^2 (4.89 $m^3/m^2/h$). The percent capture was remarkably uniform for all flow rates and chemical treatments. Sludge consistency of 0.8–1.2% was obtained without chemical addition, and up to 1.5% with cost effective chemical addition. It is expected that in the full scale unit with proper flocculation, the goal of 2% or more could be met.

5. OPERATIONAL DIFFICULTIES AND REMEDY

The most common operational difficulties encountered in the conventional activated sludge treatment plant are rising sludge and bulking sludge, resulting in high suspended solids and 5-day BOD in the plant effluent.

The common cause of rising sludge is biological denitrification, in which nitrites and nitrates in the wastewater are converted to nitrogen gas (1, 2, 41, 43, 44). When enough nitrogen gas is formed and trapped in the sludge mass, the sludge in the conventional secondary sedimentation clarifier becomes buoyant and floats to the surface. This phenomenon is called biological flotation (27).

Table 16.4
Operational conditions: paper manufacturing effluent treatment plant

Test 1 – Full flow pressurization without chemical treatment
Operational time = September 16, 17, 18, 1981 (7 h)
Average total flow rates = 22 gpm = 83.27 Lpm
Average sludge flow rates = 4.2 gpm = 15.89 Lpm
Average raw wastewater suspended solids = 1, 930 mg/L
Average clarified water = 129 mg/L
Average sludge consistency = 0.8790%
Solids loading = 58 lb/day/ft^2 = 283.18 kg/day/m^2
Test 2 – Full flow pressurization with chemical treatment
Average chemical dosage = 58 mg/L (Pearl River 560)
Operational time = September 18, 1981 (4 h)
Average total flow rate = 22.3 gpm = 84.41 Lpm
Average sludge flow rate = 2.5 gpm = 9.46 Lpm
Average raw wastewater suspended solids = 1, 781 mg/L
Average clarified water suspended solids 131 mg/L
Average solids consistency = 1.2%
Solids loading = 48 lb/day/ft^2 = 234.36 kg/day/m^2
Test 3 – Recycle flow pressurization with chemical treatment
Average chemical dosage = 1, 547 mg/L (Pearl River 560)
Operational time period = September 18, 21, 22, 1981
Average total flow rates = 21 gpm = 79.49 Lpm
Average sludge flow rate = 3.0 gpm = 11.36 Lpm
Percent recycle flow = 34%
Average raw wastewater suspended solids = 1, 778 mg/L
Average clarified water suspended solids = 121 mg/L
Average sludge consistency = 1.4%
Solids loading = 29 lb/day/ft^2 = 141.59 kg/day/m^2

Rising sludge can also be caused by internal solids overloading and hydraulic overloading to secondary sedimentation. Poor sedimentation clarifier design and operation in terms of flow-through velocity, weir design, etc. are also possible causes (3, 45).

Sludge bulking is another phenomenon that often occurs in activated sludge plants whereby the sludge occupies excessive volumes and will not settle rapidly. There are two principal types of sludge bulking problems (46–48):

(a) The growth of filamentous organisms
(b) The formation of swelling biological flocs through the addition of bound water to the cells to the extent that their density is reduced

Possible causes of sludge bulking include the following (38):

Table 16.5
Operational data: paper manufacturing effluent treatment plant (Conversion factor: 1 gpm = 3.785 Lpm = 3.785 L/min)

Test no. (see Table 16.4)	Time	Total flow (gpm)	FLOAT flow (gpm)	Clarified flow (gpm)	Recycle flow (%)	Influent SS (mg/L)	FLOAT SS (%)	Effluent SS (mg/L)	SS removal (%)	Chemical dosage (mg/L)
1	11:30	24.5	3.5	21.0	0	2,140	0.82	100	95	None
1	12:00	23.0	2.0	19.0	0	1,760	0.74	150	92	None
1	12:30	10.0	2.4	6.9	0	1,840	0.86	120	94	None
1	1:00	9.5	3.0	6.5	0	2,160	0.74	132	94	None
1	2:30	4.2	3.7	17.5	0	1,920	0.88	126	93	None
1	3:00	17.5	3.2	14.2	0	2,140	1.13	164	92	None
1	3:30	23.0	3.5	19.2	0	1,946	1.23	140	93	None
1	4:15	32.3	9.0	23.3	0	1,960	0.76	112	94	None
1	4:45	30.0	9.0	21.0	0	1,810	0.83	140	92	None
1	4:30	Not recorded			0	1,980	0.75	132	93	None
1	7:10	25.4	2.4	23.0	0	1,830	0.83	128	93	None
1	7:30	Not recorded			0	1,760	0.92	108	94	None
1	7:45	Not recorded			0	1,840	0.75	126	93	None
2	8:05	25.0	3.6	21.4	0	1,870	0.96	154	92	51
2	8:30	25.0	3.6	21.4	0	1,710	1.10	122	93	51
2	8:50	25.0	3.6	21.4	0	1,920	1.30	142	93	51
2	9:06	19.0	1.9	17.0	0	1,830	1.50	128	93	67
2	9:27	21.0	2.0	19.0	0	1,760	0.96	132	92	60
2	10:02	21.6	2.3	19.3	0	1,615	1.10	112	93	58
2	10:27	21.6	2.3	19.3	0	1,810	1.30	126	93	65
3	11:47	34.0	2.7	21.3	29	1,740	1.50	132	92	37
3	12:05	34.0	2.7	21.3	29	1,710	1.40	106	94	37
3	12:21	34.0	2.7	21.3	29	1,780	1.40	132	93	37
3	3:35	20.8	6.75	8.0	29	1,810	1.30	112	94	4,227
3	4:00	20.8	6.75	8.0	29	1,690	1.40	108	94	1,920
3	4:30	17.7	2.30	9.4	34	1,840	1.70	94	95	4,500
3	5:00	20.4	4.00	11.4	30	1,820	1.50	116	94	738
3	5:30	19.4	2.60	10.8	31	1,740	1.30	128	93	738
3	9:45	20.5	2.50	12.0	41	1,860	0.98	106	94	None
3	11:00	20.5	1.60	12.7	41	1,740	1.50	114	93	1,100
3	11:15	22.0	1.60	14.4	38	1,820	1.50	98	95	960
3	11:30	22.0	1.60	14.4	38	1,760	1.30	116	93	960
3	11:45	Not recorded				1,840	1.30	126	93	None
3	12:35	18.7	1.70	11.4	43	1,810	1.30	104	94	1,210
3	2:30	22.2	2.30	13.0	45	1,760	1.40	112	94	649
3	3:05	23.0	2.40	14.4	37	1,780	1.50	124	93	95

Note: Polymer used = Pearl River Chemical 560, cationic.

(a) Absence of certain necessary trace elements in wastewater
(b) Wide fluctuations in wastewater pH
(c) Limited dissolved oxygen in the aeration tank
(d) Inadequate food-to-microorganism ratio (F/M)
(e) Inadequate mean cell residence time (θ_c)
(f) Inadequate return sludge pumping rate
(g) Internal plant overloading
(h) Poor sedimentation clarifier operation

The problems of sludge rising and sludge bulking, when serious, cannot be overcome easily. If rising and bulking conditions continue to persist after all the aforementioned factors have been checked, a critical investigation of the behavior of aeration basin and secondary sedimentation clarifier should be made. It is very possible that the design is at fault, and either changes or expansions must be made in the facilities.

Expansions in the existing aeration basins and secondary sedimentation clarifiers are costly and sometimes unaffordable (42). The easiest facility change will be the addition of a secondary flotation unit, shown in Fig. 16.12. The secondary sludge in the proposed secondary flotation clarifier is floating, thus sludge rising is no longer a problem, in fact, becomes a big plus.

Rapid changes (within hours) in many operational parameters, such as nutrients, food-to-microorganism ratio, pH, dissolved oxygen, etc. are detrimental to the performance of conventional activated sludge treatment systems. Under these conditions, production of filamentous fungi, which are the major cause of sludge bulking, is almost unavoidable. Fungi can tolerate an environment with a relatively low pH. They also have a low nitrogen requirement and only need about one-half as much as bacteria (49, 50). The ability of the filamentous fungi to survive and function under low pH and nutrient-limiting conditions makes them very important in the biological treatment of certain industrial wastewaters and combined wastewaters with fluctuating characteristics. Sludge bulking is almost an expected phenomenon. Any filamentous living or non-living substances will have poor settleability but excellent flotability. Selection of secondary flotation instead of conventional sedimentation for sludge separation appears to be an ideal solution. Therefore, for a new activated sludge treatment plant treating industrial or combined wastewaters, flotation cells should be considered as an option to replace secondary sedimentation tanks. For an existing conventional activated sludge treatment plant having a fungi problem, installing a flotation cell between the existing aeration basin and final sedimentation tank will be a good remedy.

The state-of-the-art method for controlling sludge bulking in an emergency situation is chemical oxidation by chlorine or hydrogen peroxide. Although chemical oxidation is effective in controlling sludge bulking caused by the growth of filamentous fungi, it is ineffective when sludge bulking is due to lightweight biological flocs containing bound water. Therefore, chemical oxidation is only a temporary solution to the fungi problem. Secondary flotation cells, on the other hand, are a permanent solution to sludge bulking problem caused by both filamentous substances and bound water.

The aeration/suffocation sequence resulting from the exclusive use of secondary sedimentation clarifier by practically all facility designers since 1914 is counter productive and harmful; it constitutes a shock: several inches below the surface of a settling unit, there is

practically no dissolved oxygen (DO). In practical operation of an efficient activated sludge treatment plant, the change in DO should not exceed 0.5 mg/L. Effluent from the aeration basin, maintained at about 2.0 mg/L DO is depleted of dissolved oxygen within minutes after the mixed liquor enters the secondary sedimentation clarifier; the oxygen-requiring microorganisms cannot recover instantly from the shock resulting from such a tremendous change, and the recovery is bound to take up valuable retention time in the aeration basin. Aeration basins with spare retention time and capacity are a rare exception. The addition of secondary flotation can return highly aerobic biological solids to the aeration basin and allow the more settleable solids to collect in the existing secondary settling units. An efficient flotation cell can remove the biological solids within 3 min and concentrates them to over 2.0% in consistency, coincidentally reducing the return flow into the aeration basin since the consistency of sludges from settling is often only about 1.0%; and this could provide additional – and frequently the much needed – retention time in the aeration basin.

The previously discussed kinetics and material balance equations showed how the retention times of an existing aeration basin and a secondary sedimentation clarifier can be increased significantly, and how the excess solids loading to the existing secondary sedimentation clarifier can be reduced by installing a flotation cell (small in dimensions) in series between the aeration basin and the sedimentation clarifier.

Another alternative involves the addition of a secondary flotation unit, which parallels the existing secondary sedimentation unit in a conventional existing activated sludge treatment plant.

The use of secondary flotation as the sole secondary clarification unit in a new activated sludge treatment plant should also be considered as an option. This type of application has been practiced in Italy (34) and in West Germany (51).

6. SUMMARY AND CONCLUSIONS

The flotation cell is a high rate DAF clarifier. The unit has minimum volume (less than 16 in. water depth), low cost and flexibility in application due to its small size. It has been very successfully applied for in-process and secondary flotation in industrial and municipal applications. The flotation cell has the following advantages when used to separate biological solids from activated sludge mixed liquor:

(a) Sludge consistency for the floated biological sludge is about three times higher than comparable settled sludge. This fact has been recognized for years in the use of flotation in thickening wastewater sludges (52).
(b) The floated sludge is aerobic. There is far less kill of the biological community due to anaerobic shock. This has been demonstrated in field comparisons using both floated and settled sludges.
(c) The aerobic floated sludge is returned to the aeration basin in 8–15 min instead of several hours for the conventional activated sludge process using secondary sedimentation clarifier.
(d) Clarified water from a properly operated and sized flotation unit is comparable in quality to settled effluent. This has been demonstrated in field pilot trials. Where a settling unit already exists in the treatment process, the practice is to undersize the flotation cell and install it in series with the existing settling unit. The flotation cell is then the workhorse or "harvester" and the settling unit is the final "polisher" for the effluent. The problems of high solids loading, high

hydraulic loading, sludge rising, and/or sludge bulking in existing conventional activated sludge treatment plant can then be eliminated.

(e) The small size of the flotation cell greatly simplifies the issues involved in the upgrading of existing wastewater treatment plants.

(f) Capital cost of flotation cells is lower than the cost for conventional sedimentation clarifiers or comparable basin expansion.

(g) Stainless steel and prefabricated construction increase the economic feasibility of flotation cells.

In summary, although the present conventional activated sludge process has been in use for many decades, there is still a lot of room for improvement. One big area of weakness of the conventional process is the secondary sedimentation clarifier, which gives low consistency sludge, shocks the living biota by holding them for long periods in anaerobic conditions, and has problems of sludge rising and sludge bulking. The use of a high rate dissolved air flotation unit in series before the final sedimentation clarifier eliminates these deficiencies. The net results are lower solids loading to the existing sedimentation clarifier, higher hydraulic capacity and retention time of aeration tank, easier concentration of waste sludge, more active recycled sludge, better effluent quality and lower wastewater treatment costs. Besides, there will be no sludge rising or sludge bulking problems (53).

ABBREVIATIONS

BOD	Biochemical oxygen demand
DAF	Dissolved air flotation
DO	Dissolved oxygen
EFF	Effluent
FA/O	Flotation anoxic/oxic
FA2/O	Flotation anaerobic/anoxic/oxic
FAS	Flotation activated sludge
FCS	Flotation contact stabilization
FFB	Flotation fluidized bed
FLOAT	Floated sludge or scum
FND	Flotation nitrification-denitrification
FOAS	Flotation oxygen activated sludge
FRBC	Flotation rotating biological contactors
FSBR	Flotation sequencing batch reactor
FSP	Flotation stabilization ponds
FTF	Flotation trickling filter
FVS	Flotation vertical shaft
gpm	Gallons per minute
INF	Influent
Lpm	Liters per minute
MG	Million gallons
MGD	Million gallons per day
ML	Million liters

MLD	Million liters per day
MLSS	Mixed liquor suspended solids
MLVSS	Mixed liquor volatile suspended solids
SS	Suspended solids
SVI	Sludge volume index
VSS	Volatile suspended solids

NOMENCLATURE

b = Endogenous or decay coefficient, time^{-1}, s^{-1}
E = Process efficiency in terms of soluble organics removal = $100(S_o - S)/S_o$, %
F/M = Food to microorganism ratio, s^{-1}
k_m = Maximum rate of specific soluble organics utilization, time^{-1}, s^{-1}
K_s = Michaelis–Menten constant, or half velocity coefficient, mg/L
C = Volumetric wastewater flow rate, volume per unit time, ft^3/s or m^3/s
Q_w = Wasted sludge flow rate, volume per unit time, ft^3/s or m^3/s
Q_{w1} = Flow rate of waste sludge from secondary flotation, volume per unit time, ft^3/s or m^3/s
Q_{w2} = Flow rate of waste sludge from existing secondary sedimentation clarifier, volume per unit time, ft^3/s or m^3/s
S = Soluble substrate concentration, mass per unit volume, mg/L
S_o = Initial substrate concentration, mass per unit volume, mg/L
t = Hydraulic residence time = V/Q, h, min or s
μ = Net specific growth rate, change of microbial concentration per unit time per unit microbial concentration, s^{-1}
U = Specific substrate utilization rate, change of substrate concentration per unit time per unit microbial conc., s^{-1}
V = Biological reactor volume, ft^3 or m^3
V_f = Volume of secondary flotation clarifier, ft^3 or m^3
V_p = Volume of primary clarifier, ft^3 or m^3
V_s = Volume of secondary sedimentation clarifier, ft^3 or m^3
X = Microbial concentration or VSS in biological reactor, mass per unit volume, mg/L
X_e = Sludge concentration in the treated effluent from the final clarifier, mass per unit volume, mg/L
X_o = Sludge concentration in the primary effluent, mass per unit volume, mg/L
X_r = Return sludge concentration, mass per unit volume, mg/L
X_{w1} = Concentration of waste sludge (float) from secondary flotation, mass per unit volume, mg/L
X_{w2} = Concentration of waste sludge from existing clarifier, mass per unit volume, mg/L
Y = Growth yield coefficient, mass microbial growth per unit mass substrate utilized (dimensionless)
θ_c = Mean cell residence time or sludge retention time, days, h, min or s

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17
A/O Phosphorus Removal Biotechnology

Nazih K. Shammas and Lawrence K. Wang

CONTENTS

Abstract Biological treatment systems accomplish phosphorus removal by using phosphorus for biomass synthesis during biochemical oxygen demand (BOD) removal. Phosphorus is an important element in microorganisms for energy transfer and for such cell components as phospholipids, nucleotides, and nucleic acids. The theory for biological phosphorus removal is that anaerobic–aerobic contacting results in a competitive substrate utilization and selection of phosphorus-storing microorganisms. This chapter deals with the biological phosphorus removal, which includes process description, retrofitting existing activated sludge plant, A/O process design, dual phosphorus removal and nitrogen removal A^2/O process, sludge derived from biological phosphorus removal processes and costs.

Key Words Phosphorus removal • nitrogen removal • A/O • A^2/O • P-biosolids • biological phosphorus removal • costs.

1. BACKGROUND AND THEORY

Biological treatment systems accomplish phosphorus removal by using phosphorus for biomass synthesis during biochemical oxygen demand (BOD) removal. Phosphorus is an

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important element in microorganisms for energy transfer and for such cell components as phospholipids, nucleotides, and nucleic acids. Attachment of a phosphate radical bond to adenosine triphosphate (ATP) results in the storage of energy (7.4 kcal/mole P), which is available upon conversion of ATP back to adenosine diphosphate (ADP). Phosphorus is also contained in nucleotides such as nicotinamide adenine dinucleotide (NAD) and flavin adenine dinucleotide (FAD), which are used for hydrogen transfer during substrate oxidation–reduction reactions. Ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) are composed of a deoxyribose sugar structure with attached amino acids of adenine, cytosine, guanine, and thymine or uracil. The deoxyribose molecules are attached by phosphorus bonds. Phosphorus may account for 10–12% of the RNA or DNA mass.

A typical phosphorus content of microbial solids is 1.5–2% based on dry weight. Wasting of excess biological solids with this phosphorus content may result in a total phosphorus removal of 10–30%, depending on the BOD-to-phosphorus ratio, the system sludge age, sludge handling techniques, and sidestream return flows (1).

In 1955, Greenburg et al. (2) proposed that activated sludge could take up phosphorus at a level beyond its normal microbial growth requirements. In 1959, Srinath (3) reported on batch experiments to conclude that vigorous aeration of activated sludge could cause the concentration of soluble phosphorus in mixed liquor to decrease rapidly to below 1 mg/L. In 1965, Levin and Shapiro (4) reported on enhanced biological phosphorus removal using activated sludge from the District of Columbia activated sludge plant. Over 80% phosphorus removal was observed by vigorous aeration of the sludge and without the addition of chemicals. They termed the high phosphorus removal "luxury uptake" by the microorganisms. They observed volutin granules in the bacterial cells, which are reported in the microbial literature to contain polyphosphates. Acidification of the sludge resulted in the release of phosphorus, which led to a proposed treatment flow scheme of exposing return sludge to acidic conditions and stripping of phosphorus. Shapiro et al. (5) later observed high phosphorus uptake at the Baltimore wastewater treatment plant and release in the bottom of the secondary clarifiers under the conditions of zero or low dissolved oxygen (DO). They proposed that the return sludge could be intentionally exposed to such conditions prior to return to the aeration basin to strip out phosphorus. This work led to the development of the PhoStrip process (6, 7).

High levels of phosphorus removal were observed at various full-scale activated sludge plants in the United States, including the Rulings Road plant in San Antonio, Texas (8); the Hyperion plant in Los Angeles, California (9); and the Back River plant in Baltimore, Maryland (10). The three plants reported total phosphorus removals of 85–95% and the phosphorus content of the waste sludge was 2–7.3% on a dry weight basis. All of the plants were of the plug flow configuration using diffused aeration, and the following operating characteristics were judged important in all or some of the plants to maximize phosphorus removal (1):

1. Require a DO concentration of 2.0 mg/L or greater from the middle to end of the plug flow aeration basins
2. Prevent the recycle of phosphorus back to the activated sludge system via sludge handling streams
3. Maintain aerobic conditions in the secondary clarifiers to prevent the release of phosphorus into the effluent

In addition to the development of the PhoStrip biological phosphorus removal process in the early 1970s in the United States, biological phosphorus removal was observed during the development of the Bardenpho four-stage biological nitrification–denitrification system by Barnard (11). The system consists of sequential anoxic–aerobic–anoxic–aerobic stages with an internal mixed liquor recycle from the first aerobic stage to the first anoxic stage. During a period of high phosphorus removal in a 100-m^3/d (18-gpm) pilot-plant operation, Barnard observed a soluble phosphorus concentration of 0.3 mg/L in the final aerobic basin. He recognized that phosphorus was being released in the designated second "anoxic" basin, which was actually experiencing anaerobic conditions (absence of both nitrate nitrogen and DO) and that it was being taken up in the final aerobic stage. This led him to conclude that biological phosphorus removal was possible in activated sludge systems provided that an aerobic stage was preceded by an anaerobic stage, where phosphorus release occurred. It was also noted that when a high level of phosphorus removal was reported in plug flow U. S. plants, phosphorus release occurred near the inlet of the aeration basin followed by phosphorus uptake along the length of the basin where the DO concentration increased.

In a later paper, Barnard (12) proposed the use of a separate anaerobic basin ahead of the Bardenpho nitrogen removal system or ahead of aerobic basins when nitrogen removal was not necessary. The former was called the Modified Bardenpho process and the latter the Phoredox process. Phoredox was derived from "phosphorus" and "redox potential," which is at a lower level in the anaerobic phosphorus release zone. Figure 17.1 shows phosphorus release and uptake characteristics of such biological phosphorus removal systems that employ sequential anaerobic–aerobic contacting (1).

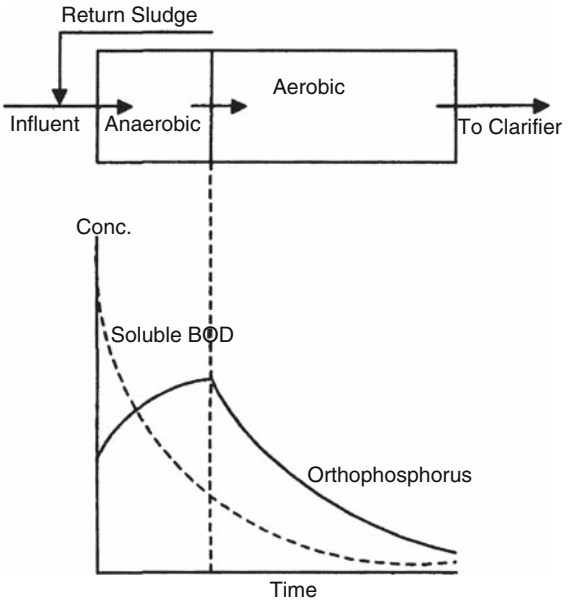


Fig. 17.1. Biological P and BOD removal due to Anaerobic–Aerobic contacting (*Source*: U. S. EPA).

Following Barnard's pilot-plant work, full-scale plants were modified at Johannesburg, South Africa, to investigate the feasibility of biological phosphorus removal. At the Alexander plant, surface aerators near the inlet of an activated sludge basin were turned off to create an anaerobic zone (13). An overall nitrogen removal of 85% and a total phosphorus removal of 46% were reported. At the Olifantsvlei plant, various combinations of surface aerators were turned off in the four-stage system and an effluent soluble phosphorus concentration of 0.9 mg/L was reported (14). On the basis of this work, a modified Bardenpho system was designed for a 150,000-m^3/d (39-MGD) facility at the Johannesburg Goudkuppies wastewater plant that became operational in 1978 (15). In the late 1970s, a modified Bardenpho plant was started up at Palmetto, Florida (16), and a portion of the Largo, Florida facility was converted to the Anaerobic/oxic (A/O) process (17), an anaerobic–aerobic biological phosphorus removal system.

The advantages and disadvantages of biological phosphorus removal processes are summarized below (1):

Advantages:

1. Sludge quantities generated by biological phosphorus removal processes are comparable to sludge production from conventional activated sludge systems.
2. Can be implemented directly at existing plug flow activated sludge plants with little or no equipment changes or additions, provided that the plant has sufficient capacity.
3. Can utilize existing sludge handling equipment for plants retrofitted with biological phosphorus removal process if phosphorus is not solubilized and returned to the plant during sludge handling.
4. Little or no chemicals or chemical handling equipment required except for PhoStrip process or for effluent polishing.
5. Phosphorus removal can be accomplished together with ammonia nitrogen or total nitrogen removal at virtually no additional operating cost with some of the processes.
6. For some of the processes, better control of filamentous organisms in the activated sludge system is possible.

Disadvantages:

1. In all but PhoStrip, phosphorus removal performance is controlled by the BOD:TP (total phosphorus) ratio of the wastewater.
2. Require highly efficient secondary clarifier performance to achieve effluent concentrations with 1 mg/L total phosphorus.
3. Not easily retrofitted into fixed film biological systems.
4. Potential for phosphorus release in sludge handling system. Recycle streams must be low in phosphorus content.
5. Standby chemical feed equipment may be necessary in case of loss of biological phosphorus removal efficiency.

2. BIOLOGICAL PHOSPHORUS REMOVAL MECHANISM

The theory for biological phosphorus removal is that anaerobic–aerobic contacting results in a competitive substrate utilization and selection of phosphorus-storing microorganisms. An understanding of the steps involved in the biological phosphorus removal mechanism provides a useful insight into the factors that can affect the performance of biological phosphorus removal systems.

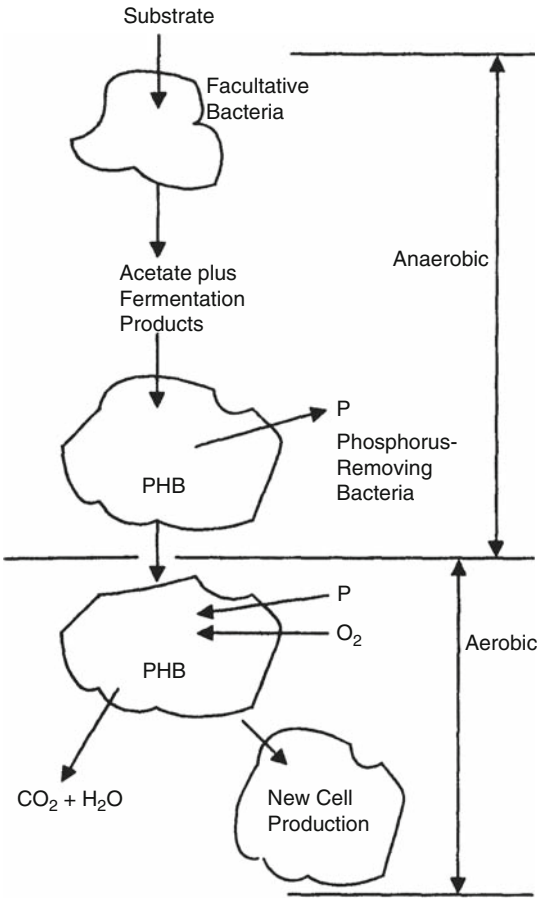


Fig. 17.2. Schematic of biological P removal mechanism (*Source*: U. S. EPA).

The proposed biological phosphorus removal mechanism (18, 19) is summarized in Fig. 17.2. Acetate and other fermentation products are produced from fermentation reactions by normally occurring facultative organisms in the anaerobic zone. A generally accepted concept is that these fermentation products are derived from the soluble portion of the influent BOD, and there is not sufficient time for the hydrolysis and conversion of the influent particulate BOD. The fermentation products are preferred and readily assimilated and stored by the microorganisms capable of excess biological phosphorus removal. This assimilation and storage is aided by the energy made available from the hydrolysis of the stored polyphosphates during the anaerobic period. The stored polyphosphate provides energy for active transport of substrate and for the formation of acetoacetate, which is converted to polyhydroxybutyrate (PHB). The fact that phosphorus-removing microorganisms can assimilate the fermentation products in the anaerobic phase means that they have a competitive advantage when compared with other normally occurring microorganisms in activated sludge systems. Thus, the

anaerobic phase results in a population selection and development of phosphorus-storing microorganisms. Rensink (20) has pointed out that *Acinetobacter* are relatively slow growing bacteria and that they prefer simple carbohydrate substrates. Thus, without the anaerobic phase, they may not be present at significant levels in conventional activated sludge systems.

During the aerobic phase, the stored substrate products are depleted (21) and soluble phosphorus is taken up, with excess amounts stored as polyphosphates. An increase in the population of phosphorus-storing bacteria is also expected as a result of substrate utilization. The above mechanism indicates that the level of biological phosphorus removal achieved is directly related to the amount of substrate that can be fermented by normally occurring microorganisms in the anaerobic phase and subsequently assimilated and stored as fermentation products by phosphorus-removing microorganisms, also in the anaerobic phase.

3. PROCESS DESCRIPTION

The recent developments leading to a better understanding of the conditions, causing excess biological phosphorus removal, help explain the earlier observations on excess phosphorus removal reported for full-scale facilities. It is apparent now that sufficient BOD was present and oxygen was limiting so that fermentation conditions likely occurred at the front end of the relatively long, narrow aeration basins of these plants. Since these observations, three major proprietary biological phosphorus removal processes that employ more definitive anaerobic fermentation zones have been commercialized. These processes are, in order of development, the PhoStrip process, the modified Bardenpho process (for both P and N removal), and the A/O process.

A summary of typical recommended design criteria for the biological P removal processes is shown in Table 17.1. The A/O process is generally designed as a high-rate activated sludge system. The A/O process shown in Fig. 17.3 is marketed in the United States by Air Products and Chemicals, Inc. (17) and is similar to the Phoredox concept described by Barnard (12), except that the anaerobic and aerobic stages are divided into a number of equal size complete mix compartments. Typically, three compartments have been used for the anaerobic stage and four for the aerobic stage. The key features of the A/O process are its relatively short design SRT (sludge retention time) and high design organic loading rates. This results in greater sludge production and more phosphorus removal per unit of BOD removed in the system. However, the use of further sludge stabilization methods, such as anaerobic or aerobic digestion, must consider the amount of phosphorus released during stabilization and the effect of recycle streams from the stabilization units on facility performance.

As shown in Table 17.1, the A/O process can also be used, where nitrification and/or denitrification are required. The modified flow scheme incorporates an anoxic stage for denitrification between the anaerobic and aerobic stages and is called the A^2/O process (see Fig. 17.3). The anoxic stage is also divided into three equal- size, complete mix compartments. Mixed liquor is recycled from the end of the nitrification stage to feed nitrate nitrogen into the anoxic stage for denitrification. Internal recycle flows of 100–300% have been used. Nitrate nitrogen removals of 40–70% can be accomplished this way.

Table 17.1
Typical design criteria for biological phosphorus removal processes

Phostrip		Modified Bardenpho		A/O		A/O plus nitrification	
Parameter	Value	Parameter	Value	Parameter	Value	Parameter	Value
AS system							
F/M, kg TBOD/kg MLVSS/d	–[a]	F/M, kg TBOD/kg MLVSS/d	0.1–0.2	F/M, kg TBOD/kg MLVSS/d	0.2–0.7	F/M, kg TBOD/kg MLVSS/d	0.150–.25
SRT, d[b]	–[a]	SRT,d[b]	10–30	SRT, d[b]	2–6	SRT, d[b]	4–8
MLSS, mg/L	600–5,000	MLSS, mg/L	2,000–4,000	MLSS, mg/L	2,000–4,000	MLSS, mg/L	3,000–5,000
HRT, h[c]	1–10	HRT, h[c]		HRT, h[c]		HRT, h[c]	
		Anaerobic	1–2	Anaerobic	0.5–1.5	Anaerobic	0.5–1.5
		Anoxic	2–4	Aerobic	1–3	Anoxic	0.5–1.0
		Nitrification (Aerobic 1)	4–12			Nitrification	3.5–6.0
		Anoxic 2	2–4				
		Aerobic 2	0.5–1.0				
Phostrip stripper							
Feed, % of inf. flow	20–30	Return sludge, % of inf. flow	100	Return sludge, % of inf. flow	25–40	Return sludge, % of inf. flow	20–50
SOT, h	5–20	Int. recycle, % of inf. flow	400			Int. recycle, % of inf. flow	100–300
Sidewater depth, m	6.1						
Elutriation flow, % of stripper feed flow	50–100						
Underflow, % of inf. Flow	10–20						
P release, g P\g VSS	0.005–0.02						
Reactor-clarifier							
Overflow Rate, $m^3/m^2/d$	48						
Ph	99.5						
Lime dosage, mg/L	100–300						

[a] Based on activated sludge system design.
[b] Average mass of solids in the system divided by average mass of solids wasted daily.
[c] Hydraulic retention time, volume by influent flow rate.

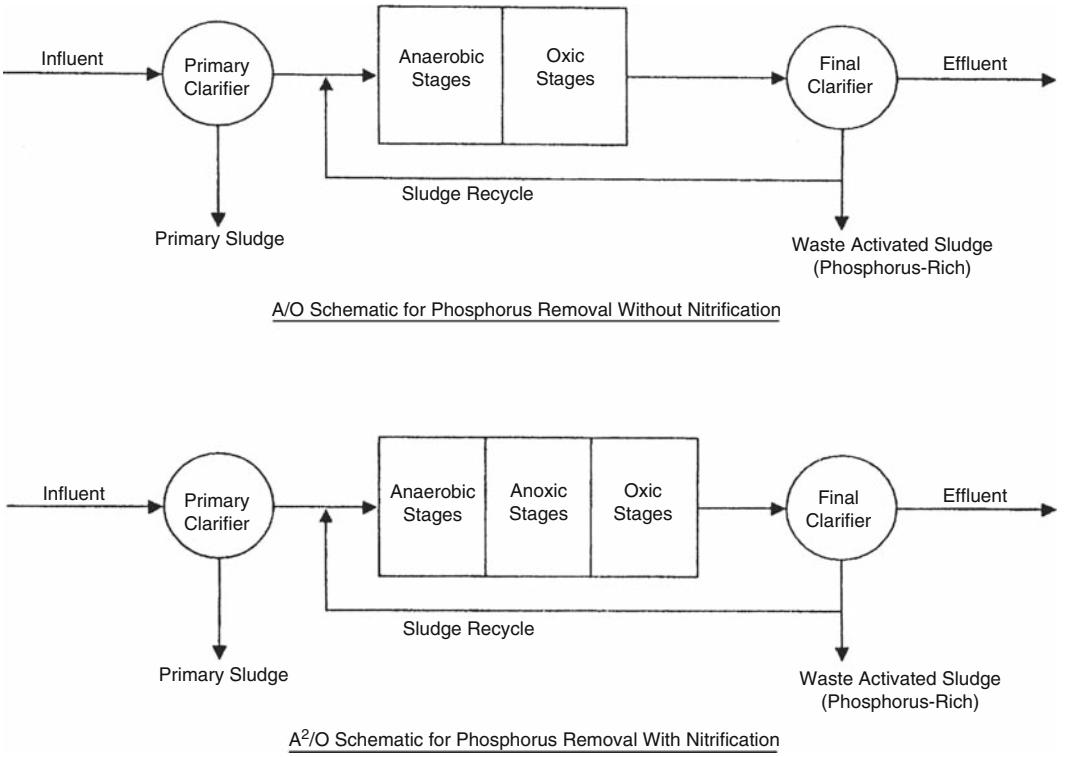
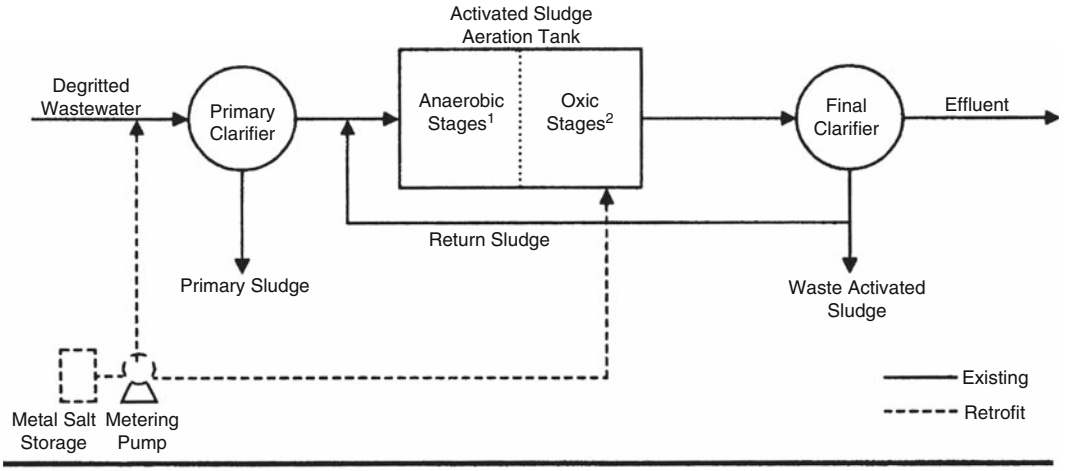


Fig. 17.3. A/O and A^2/O schematics for biological phosphorus removal (*Source*: U. S. EPA).

4. RETROFITTING EXISTING ACTIVATED SLUDGE PLANTS

Although the A/O process is most easily retrofitted to plug flow activated sludge tanks, it can also be adapted to most of the activated sludge flow regimes. The ease of retrofit is determined by the ability to delineate and convert a portion of the aeration tankage to an anaerobic zone. Here, anaerobic is defined as the absence of all DO and oxidized nitrogen, while anoxic refers to the conditions where DO is low or absent, but oxidized nitrogen is present. Space for construction of retrofit facilities is normally not required for the A/O process, and generally an A/O retrofit is more easily accomplished than a retrofit for PhoStrip. The A/O process is owned and marketed by Air Products and Chemicals, Allentown, PA.

A schematic of an activated sludge system retrofitted for biological phosphorus removal employing the A/O process is presented in Fig. 17.4. A critical design feature of the A/O process is the provision of sequential stages of two different environments for the biomass to cycle through (22, 23). Therefore, if an existing complete mix activated sludge system could not be retrofitted into a staged reactor by baffling, the A/O process would not be suitable. Installation of a chemical backup feed system in an A/O process retrofit is recommended to ensure that effluent TP (total phosphorus) concentrations of 1 and 0.5 mg/L can be consistently achieved. Because of the high phosphorus content of effluent solids, consideration of effluent



Legend

(1) Mixing by submersible pump or mixers. No DO is present.
(2) Conventional aeration devices for achieving satisfactory DO levels.

Final Effluent TP mg/L	Probable Need for Chemical Addition
2	None
1	Occasional
0.5	Continuous polish dose
0.2	Continuous polish dose

Fig. 17.4. Schematic of an activated sludge system retrofitted for the A/O process (*Source*: U. S. EPA).

filtration is also recommended if effluent concentrations of less than 1 mg/L TP must be obtained (24).

Retrofit with the A/O process is most easily accomplished in plug flow activated sludge tanks, but can also be adapted to most of the other activated sludge flow configurations. The ease of retrofit is determined by the ability to delineate and convert a portion of the tankage to an anaerobic stage. Here, anaerobic is defined as the absence of all DO and oxidized nitrogen, while anoxic refers to the conditions where DO is low or absent, but oxidized nitrogen is present. Space for construction of retrofit facilities is normally not required for the A/O process, and generally an A/O retrofit is more easily accomplished than a retrofit for PhoStrip (the other biological phosphorus removal process) (25, 26).

The A/O process induces the occurrence of a natural selection of phosphorus-accumulating microorganisms by providing alternate environments of anaerobic and aerobic (oxic) conditions. Wastewater and return sludge are mixed in the anaerobic stage. It is not necessary to cover this stage if suitable nonturbulent mixing is provided. For example, mixing can be accomplished by submersible pumps or impeller mixers in a manner that does not cause oxygen transfer from excessive exposure of liquid surface to air. The hydraulic detention time (HRT) of the anaerobic stage can vary from 1 to 2 h. During this time, soluble phosphorus

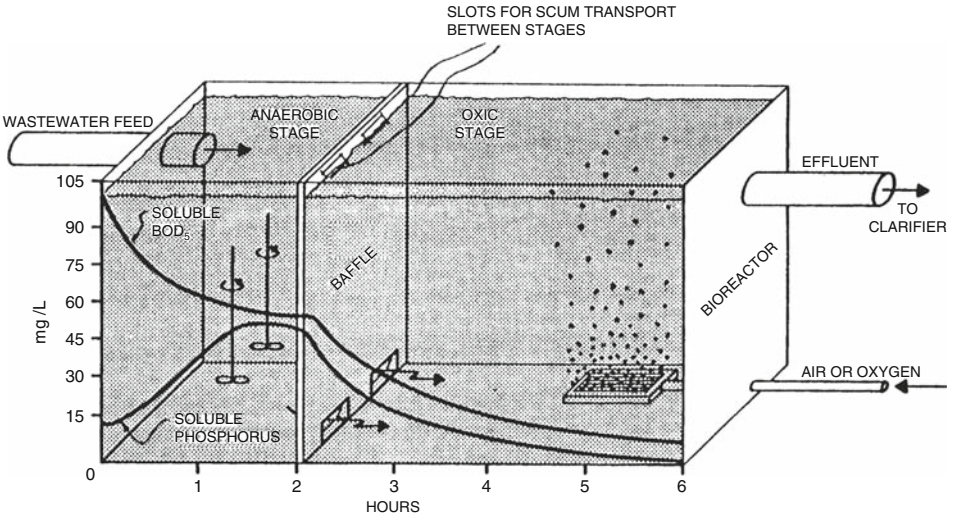


Fig. 17.5. Conceptual sequence of reactions in the A/O process (*Source*: U. S. EPA).

is released from the biomass into the bulk liquid and, concurrently, organic matter (BOD) is sorbed from the bulk liquid and stored by the biomass.

In the subsequent oxic stage with DO levels of 2 mg/L, the stored organics are biodegraded and new cellular growth occurs coincident with transport of soluble phosphorus into intercellular granules. The HRT of the oxic stage can vary from 2 to 4 h.

This sequence of anaerobic and aerobic events is shown in Fig. 17.5. These reactions are common to all biological phosphorus removal processes. These processes must be designed and operated to accommodate the anaerobic sorption of BOD and release of soluble phosphorus, followed by aerobic cellular synthesis and phosphorus uptake.

The efficiency of phosphorus removal is affected by the ratio of soluble BOD_5 (SBOD) to soluble phosphorus (SP) in the system influent. A ratio of influent SBOD to SP of 10–15 is necessary to achieve a 1-mg/L TP effluent concentration with main stream biological phosphorus removal processes such as A/O (27). To achieve a TP effluent concentration of 0.5 mg/L, the ratio would have to be about 20 to 25. The effect of internal recycle streams, such as digester supernatant (28), sludge thickener overflow, and filter press or vacuum filter filtrates on the influent SBOD-to-SP ratio must also be considered.

An evaluation of four full-scale biological phosphorus removal facilities (27) revealed that none of the facilities practiced anaerobic digestion of sludges. Chemical treatment of some sludge handling recycle streams was necessary to prevent previously removed phosphorus from re-entering the secondary system and adversely affecting attainment of effluent phosphorus limitations.

Final clarifier design (29, 30) and operation is important for biological phosphorus removal processes. A long sludge blanket residence time in a clarifier can lead to the development of anaerobic conditions in the blanket and cause leaching of soluble phosphorus from the

phosphorus stored in the biomass granules. Provisions for rapid sludge removal from the clarifier and reserve return sludge pumping capacity are practiced, where biological phosphorus removal is contemplated. Because of the potential release of phosphorus during settling of the waste activated sludge (WAS), use of dissolved air flotation (DAF) for the separation of biosolids from water is recommended (31–35).

4.1. A/O Process Performance

Two full-scale A/O plants have been in operation for more than two decades: Largo, FL, and Pontiac, MI. The operational data for the Largo and Pontiac facilities are summarized below.

Largo, FL. The Largo A/O system is a retrofit of a plug-flow activated sludge plant designed for a flow of 0.14 m^3/s (3.2 MGD). Anaerobic and aerobic detention times are 1.5 and 2.6 h, respectively. Influent TP averaged 8.9 mg/L during the performance test period, while the effluent TP averaged 1.85 mg/L. The effluent SP concentration during the same period averaged 0.51 mg/L. The plant was designed to achieve an effluent TP of 1.5 mg/L. Sludge handling consists of aerobic digestion followed by mechanical dewatering.

Pontiac, MI. The A/O system in Pontiac is a 0.15-m^3/s (3.5-MGD) retrofit of a plug-flow activated sludge system. Detention times for the anaerobic and aerobic stages are longer here, 2.1 and 7.7 h, respectively. TP was reduced from an average influent concentration of 3.7 mg/L to an average effluent concentration of 0.9 mg/L on a U. S. EPA demonstration project during a period when nitrification was being achieved and the main treatment process was receiving full in-plant recycle of sidestreams, including anaerobic digester supernatant (24). During the 1-year demonstration project, average effluent TP concentrations exceeded 1 mg/L during only two 2-week periods. The excursions were attributed to the effect of extremes in pH caused by industrial discharges. The plant has been successfully operating with seasonal nitrification and recycle of anaerobic digester supernatant. It was shown that only a fraction of the phosphorus removed biologically was released into the digester supernatant under anaerobic conditions. The mechanism by which phosphorus is trapped in the solids in the digester is being further studied, but it is currently hypothesized that phosphorus is being chemically precipitated in the presence of magnesium and ammonia in the digester to an insoluble compound called magnesium–ammonium–phosphate. The average performance of the Pontiac A/O system is summarized in Table 17.2 for a 45-day period with nitrification and full in-plant digester supernatant recycle and a 54-day period without nitrification, but with full in-plant digester supernatant recycle.

4.2. Cost for A/O Process Retrofit

Retrofit costs for the A/O process must be evaluated on a site-specific basis. Capital costs include construction of baffles to separate the various stages, removal of existing aeration devices in anaerobic stages, possible addition of aeration devices in aerobic stages, and installation of mixers in anaerobic stages. O&M costs include energy for internal recycle pumping, if required, and to operate the mixers. It has been reported that some savings in aeration power may be realized due to the decreased aerobic stage organic loading resulting from the BOD removal that occurs in the anaerobic stages (36).

Table 17.2
Pontiac A/O wastewater treatment facility performance data

	Influent		Effluent	
Parameter	With nitrification	Without nitrification	With nitrification	Without nitrification
Flow, MGD[a]	2.86	4.28	–	–
TBOD, mg/L	163	136	94	11
TSS, mg/L	140	136	7	10
TP, mg/L	3.7	2.6	0.9	0.7

[a] 1 MGD = 3.785 MLD = 43.8 L/s = 0.0438 m^3/s

Retrofit costs (in 2009 dollars) for the Pontiac project totaled $95,000 for conversion of a 0.15-m^3/s (3.5-MGD) plug flow activated sludge train to an A/O system. The cost has been adjusted from 1984–2009 U. S. Dollars using the U. S. Army Corps of Engineers Cost Index for Utilities shown in Appendix (37). More information on costs and cost comparison of different biological phosphorus removal processes at various plant sizes and residual phosphorus concentrations are given in Sect. 8.

5. A/O PROCESS DESIGN

5.1. A/O Operating Conditions

The ratio of influent SBOD to SP should be greater than 10 to achieve an effluent TP concentration of 1–2 mg/L, and high ratios are desirable since they are more conducive in achieving the release of phosphorus in the anaerobic stage. This minimum ratio is necessary to provide sufficient readily biodegradable substrate, to produce enough cell mass to trigger the phosphorus release/uptake mechanisms, and to ensure the absence of exogenous electron acceptors in the anaerobic stage. Additional operating conditions are as follow:

1. Wastewater temperature = 10–30°C
2. Anaerobic detention time = 1–2 h
3. Anoxic (when required) detention time = 1 h
4. Aerobic detention time 2.5 h without nitrification, 6 h with nitrification
5. MLSS = 2,000 mg/L in the summer; 3,500 mg/L in the winter
6. F/M loading = 0.15–0.6 kg TBOD/kg MLVSS/d
7. SBOD:SP > 10 to achieve an effluent TP concentration of 1–2 mg/L

If an A/O is being considered for a plant where operating conditions within the ranges presented cannot be achieved, a pilot study is warranted prior to full-scale design and construction.

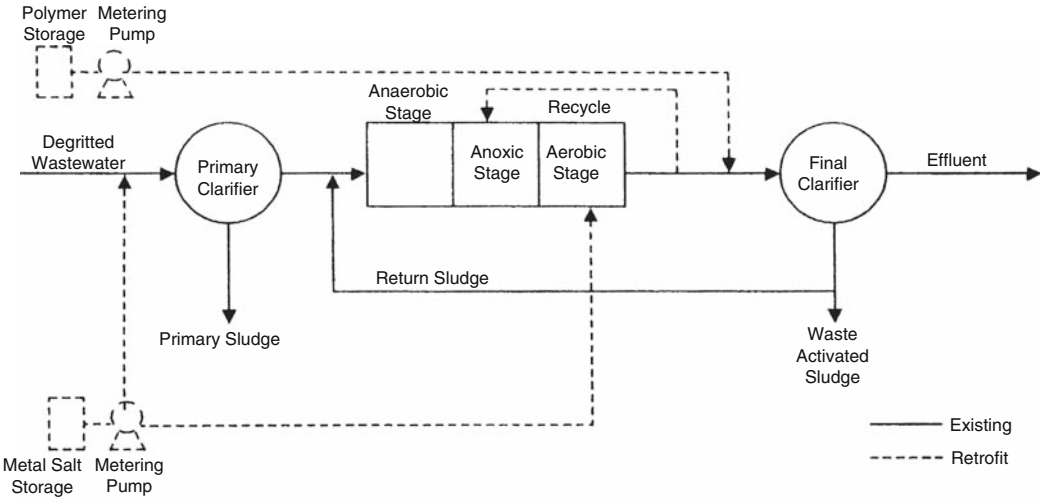
5.2. Design Considerations

Generic A/O flow diagrams for the cases without and with nitrification are illustrated in Fig. 17.3. An anoxic stage is normally required after the anaerobic and prior to the aerobic stages when nitrification is a process consideration. A flow scheme consisting of anaerobic, anoxic, and aerobic stages in series is generally referred to as the A^2/O process, a modification

of the basic A/O process designed to remove nitrogen and/or mitigate the negative effects of nitrification on biological phosphorus release in the anaerobic stage.

In the A^2/O scheme, some method of low-head pumping is required to recycle mixed liquor at a rate of approximately 100% of the influent flow rate from the last aerobic stage to the first anoxic stage (see Fig. 17.6). The anoxic stage serves to denitrify the oxidized nitrogen, thereby preventing competition with the microorganisms responsible for phosphorus leaching in the anaerobic stage. While the internal recycle does not prevent nitrate nitrogen from entering the anaerobic stage, it does reduce the nitrate concentration in the return sludge stream.

The A/O process has not been operated with nitrification on a full-scale basis at total detention times less than 8 h. If sufficient total detention time is available, existing tankage can be retrofitted with baffles to delineate the anaerobic, aerobic, and, if necessary, anoxic stages. It is desirable to maintain plug flow as much as possible to ensure good contact of microorganisms with substrate. For this reason, three anaerobic stages, four aerobic stages, and, if necessary, three anoxic stages are normally used (see Fig. 17.7). The exact configuration is a site-specific consideration. Excess tankage may be available at some plants such as those employing the extended aeration process. In these cases, a portion of the tankage may be blocked off and taken out of service.



Final Effluent TP	Probable Need for Chemical Addition	Final Clarifier SOR*
mg/L		gal/ft²/d
2	None	800
1	Occasional	600
0.5	Continuous polish dose	500
0.2	Continuous polish dose	500

* at peak sustained flow

Approximate final nitrogen concentrations (mg/L):

Organic-N:	3.5
NH_4-N:	1
NO_2-N:	0.1
NO_3-N:	6

Fig. 17.6. Schematic of an activated sludge system retrofitted for the A^2/O process (*Source*: U. S. EPA). (Conversion factor: 1 gal/ft^2/d = 0.0408 m^3/m^2/d)

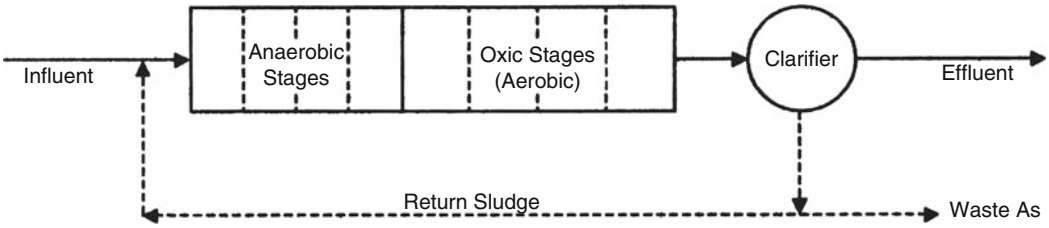


Fig. 17.7. A/O process with multiple anaerobic and oxic stages (*Source*: U. S. EPA).

A variety of baffle materials are available including concrete, different types of woods and plyboard, and plastic coated fabrics. Open slots should be left at the bottom of these baffles to allow movement of mixed liquor. Where there are several baffles in sequence, the slots should be constructed at alternating corners to enhance plug flow. In addition, slots should be provided at the top of each baffle to prevent the buildup of scum.

Aeration devices in the anaerobic and anoxic stages must be removed or prevented from operating since it is extremely important that air not be introduced to these stages. Aeration capacity in the remaining aerobic stages may be insufficient, especially in extended aeration type systems, where long detention times are being retrofitted to shorter ones. In these cases, aeration devices can be moved from anaerobic stages to aerobic stages when possible, or auxiliary aeration may be required. Oxygen demand in the aerobic stages is similar to that for conventional activated sludge or 1.5 kg O_2/kg TBOD removed. DO concentrations in excess of 2 mg/L should be maintained at all times in the aerobic stages.

Some form of mixing is required in the anaerobic and anoxic stages. The mixing must be such that oxygen transfer through excessive exposure of liquid surface to air is minimized. Submersible pumps can be used in smaller tanks or stages. Vertical mechanical mixers are appropriate where the stage length and width dimensions are similar and the depth is shallow since excessive shaft vibration can occur when the shaft is long. For stages that are much longer than they are wide, or for large, deep stages, side-mounted submerged propeller-type mixers are desirable. The goal of the mixing is simply to maintain the MLSS in suspension with less than a 10% variation in concentration. Mixing power requirements in the anaerobic and anoxic zones can be estimated at 20 kW/1, 000 m^3 (0.75 hp/1, 000 ft^3) although this depends heavily on tank configuration.

The return activated sludge rate is typically between 20 and 75% of the influent flow rate. When operating at the lower end of this range, it is important that appropriate valving be provided to accurately control return sludge rates for proportional pacing with low influent flows. The sludge blanket depth in the secondary clarifier should be maintained at less than 0.6 m (2 ft) to prevent development of anaerobic conditions and subsequent leakage of phosphorus into the secondary effluent. Secondary, clarifier surface overflow rates (SORs) can range between 24 and 26 $m^3/m^2/d$ (600 and 650 gpd/ft^2).

The organic loading rate is an important consideration in successful operation of the A/O process. Higher organic loading rates result in higher sludge yields and, therefore, greater removals of phosphorus since the only exit for phosphorus in the A/O process is

through the WAS. Although higher rates have been successfully used, the recommended volumetric organic loading rate for concurrent nitrification is 0.15 kg TBOD/kg MLVSS/d or 0.08 kg SBOD/kg MLVSS/d. Higher volumetric organic loading rates up to 0.6 kg TBOD/kg MLVSS/d may be applied when nitrification is not required. When the method of sludge handling includes anaerobic digestion or other operations where the sludge is subjected to anaerobic conditions, at least a portion of the biologically removed phosphorus will be released in a soluble form. This can then find its way back to the influent of the plant through recycle streams.

The effects of recycle streams such as digester supernatant, sludge thickener overflow, and filter press or vacuum filter filtrates on mainstream process operation and performance must also be carefully considered. These streams may carry a large loading of phosphorus back to the influent and may upset the required influent BOD-to-phosphorus ratio. Segregation of the recycle streams may be necessary in extreme cases.

5.3. Attainability of Effluent Limits

An effluent limit of 2 mg/L TP should be attainable for most wastewaters where an A/O is used. An effluent limit of 1 mg/L TP could be attainable most of the time with the A/O process and perhaps all of the time at some locations. However, a backup feed system for dosing selected chemicals to the aerobic zone is recommended for any necessary polishing (see Figs. 17.4 and 17.6).

In achieving effluent limits of 0.5 mg/L TP or less, the influent SBOD-to-SP ratio becomes more important, with a desired ratio of 20–25. Provisions for chemical polishing in the aerobic zone would be necessary to achieve an effluent TP concentration of 0.5 mg/L, and a tertiary chemical polishing stage plus tertiary filtration would be required to achieve an effluent TP concentration of 0.2 mg/L (see Figs. 17.4 and 17.6).

5.4. Oxygen Requirements for Nitrification

When a biological phosphorus removal process must also be able to achieve nitrification, oxygen supply capability must be considered. Calculation of the consumption of oxygen by biological nitrification shows that for each 1 mg of ammonium nitrogen oxidized to nitrate nitrogen, 4.5 mg of oxygen will be required. This is in addition to the oxygen demand for carbonaceous oxidation and the oxygen required for meeting the endogenous demand of biomass.

In any case, the anticipated peak oxygen requirement anticipated from these combined demands should be evaluated to make sure that the capability of the aeration system is sufficient to transfer this amount of oxygen.

6. DUAL PHOSPHORUS REMOVAL AND NITROGEN REMOVAL A^2/O PROCESS

If a facility must remove both phosphorus and nitrogen to meet effluent requirements, the decisions will have to be carefully tailored to the numerical limits imposed. Numerous process options are available for controlling both nitrogen and phosphorus with a wide spectrum

of overall efficiencies. An effluent specification that had stringent limits, such as 0.2 mg/L TP and 3 mg/L total nitrogen, would dictate consideration of a multistage biological process supplemented with chemical additions. A less stringent effluent requirement, such as 2 mg/L TP and 10 mg/L total nitrogen, would lead to consideration of managed biological systems. Between these two extremes are numerous options that could be evaluated.

To effectively remove both phosphorus and nitrogen, any process must be operated to control a series of transformations. Influent phosphorus forms must be converted to orthophosphate for efficient insolubilization by chemicals or incorporation into cellular material.

For the occurrence of nitrogen removal, the various unoxidized forms of nitrogen in the influent must first be transformed into nitrate nitrogen. Then, conditions must be arranged for biological denitrification to convert the nitrate nitrogen to nitrogen gas. For this conversion, microorganisms utilize hydrogen bound in organic materials to combine with oxygen from the nitrate radical. This is an oxidation/reduction reaction with water, carbon dioxide, nitrogen gas, and hydroxyl ions as end products. Using methanol as an example of organic matter, the reaction is:

$$6NO_3^- + 5CH_3OH \rightarrow 7H_2O + 5CO_2 + 3N_2 + 6OH^- \quad (1)$$

The velocity of this reaction is dependent on the type of organic substrate provided. Materials such as methanol are very soluble in wastewater and readily utilized by acclimated denitrifying organisms. In addition, the chemical is available in pure form and can be dosed into a denitrification process in a known ratio to nitrate nitrogen. Managed biological processes depend on different types of organics. Some utilize organics (BOD) present in influent wastewater; others rely on organics liberated by endogenous hydrolysis of cellular organics present in biomass. The quality and quantity of these organics are largely unknown and can be highly variable. The extent of biological utilization of these materials is also subject to variability. Generally, these types of organics have slower reaction velocities than materials such as methanol. However, no implicit cost is associated with these in-plant sources of organics.

The trade-off that must be evaluated between externally added materials like methanol and in-plant organics is the cost of chemical and dosing equipment for the former vs. biological reactor size and recycle pumping costs for the latter, coupled with the effluent total nitrogen residual and degree of compliance required. Retrofitting for removal of both nitrogen and phosphorus at any facility that was near design hydraulic and organic loadings, no matter which option was chosen, would no doubt involve capital construction.

Many examples of multistage, chemically assisted processes – a subject that is outside the scope of this chapter which deals with biological removals – are provided elsewhere (38–43). The data base for this technology is well established from both design and operational standpoints. The array of process options is large because various combinations of suspended and attached growth biological processes, operated in series, have been constructed. The most complex are systems with separate reactors for carbonaceous oxidation, nitrification, and denitrification, each with its own clarifier. Other configurations combine carbonaceous oxidation and nitrification in a single reactor and have a separate stage for denitrification. The denitrification reactor has been designed both as a postdenitrification and a predenitrification

process. In some systems, where an attached growth process is included in the design, the provision for clarification after this process may not be necessary.

Currently, a large reservoir of full-scale operational experience is available with biological processes that cycle biomass through managed environments to achieve both nitrogen and phosphorus removal to meet prescribed effluent limitations.

Kang et al. (44) states that three full-scale facilities (Lansdale, PA; Reedy Creek, FL; and DePere, WI) practicing dual biological phosphorus removal and nitrification did exhibit significant degrees of overall nitrogen removal. This was attributed to unintentional denitrification of nitrate nitrogen in recycle streams entering anaerobic zones of the processes.

A large number of process configurations have been evaluated for biological phosphorus and nitrogen removal. The three that have received considerable attention in the United States are the Anaerobic/Anoxic/Oxic (A^2O) process, the Bardenpho process, and the modified PhoStrip process. These processes will be presented and discussed in subsequent sections.

As notedearlier, for biological phosphorus removal, the wastewater should have a SBOD-to-SP ratio of at least 10–15. With the dual process approach, incorporating denitrification, the ratio of TBOD to total Kjeldahl nitrogen (TKN) also becomes important. Evans and Crawford (45) recommend that the ratio of TBOD to TKN be 5–10. It is important with managed nutrient control processes, which are not provided with supplemental organic compounds, that the influent wastewater contain enough organic matter to enable the denitrification reaction between nitrate and organics to occur. If the TBOD-to-TKN ratio were lower, greater concentrations of nitrate nitrogen would appear in the final effluent.

It should be noted in the following process flow diagrams that all of these dual control systems recover some of the oxygen contained in the nitrate radical via recycle of internal streams into an anoxic stage. Also important to note is the fact that the last reactor stage in all these systems is an aerobic (oxic) stage where nitrification occurs. Theoretically, this indicates that unless high internal recycle rates and extensive baffling are employed along with long HRTs, these systems cannot achieve as low final effluent nitrate nitrogen content as a multistage system with a chemically supplemented terminal denitrification reactor.

Both biological nitrification and biological denitrification transformations are affected by wastewater temperature. Data on temperature coefficients for multistage systems from pilot- and full-scale studies are included in the U. S. EPA Process Design Manual (41). However, temperature data for dual biological systems in a variety of options are not extensive.

In dual biological phosphorus and nitrogen removal applications, the biomass has to be managed to balance two opposing microbial population selection objectives. Biological phosphorus removal is dependent on excess sludge production by a group of organisms that can survive extremes of DO concentration. Increased rates of sludge production are favored by a short SRT.

Since denitrification must be preceded by nitrification, conditions for survival of nitrifiers must be provided. These organisms require an aerobic environment and have a low growth rate, which mandates a long SRT.

At the present time, owing to the lack of a large data base on the influence of wastewater quality and component ratios, the magnitude of internal recycle streams necessary to achieve

a given effluent residual, temperature effects on dual processes and the need to balance SRT, it is recommended that any design for these systems be guided by pilot plant studies.

6.1. Phosphorus and Nitrogen Removal with the A^2/O Process

The A^2/O process configuration is presented in Fig. 17.6. It is similar to the A/O process (Fig. 17.4), except that an anoxic stage is inserted between the anaerobic stage and the oxic stage.

The system must be designed and operated to obtain phosphorus leaching in the anaerobic stage and subsequent biological cellular uptake in the following aerobic stage. The SRT of the biomass must be selected to ensure a steady-state population of nitrifiers at the design temperature. The detention time of the aerobic stage must be sufficient to accomplish nitrification and organic oxidation in this, the only aerated portion of the process.

Nitrified mixed liquor is recycled to the anoxic stage where biological denitrification occurs in response to organics (BOD) entering this stage from the anaerobic stage. By recycling mixed liquor from the aerobic to the anoxic stage for denitrification, the negative influence of nitrate nitrogen on phosphorus leaching in the anaerobic stage is somewhat alleviated as only nitrate contained in the return sludge enters the anaerobic stage. Thus, in the A^2/O process, denitrification can occur in both the anoxic and anaerobic stages.

The influent to the anaerobic stage must contain organics for two purposes: first, to serve as substrate to be sorbed by the phosphorus-accumulating organisms and, second, to serve as organic substrate for anaerobic stage denitrification of return sludge nitrate, which in turn reduces the negative influence of nitrate on phosphorus leaching. Enough organic material must pass through the anaerobic stage to the anoxic stage, however, so that reduction of nitrate in the recycle mixed liquor is not inhibited. Consideration may be given to bypassing all or a portion of the raw wastewater around the primary clarifier to increase the organic concentration entering the anaerobic stage if the influent wastewater has a low organic content.

Depending on the magnitude of the two recycle streams, the A^2/O process recovers some fraction of the oxygen content of the nitrate radical. Additionally, the denitrification reactions in the anaerobic and anoxic stages will create alkalinity to help offset downstream alkalinity loss because of nitrification and metal salt addition if required.

Operational control must be utilized to manage reactor influent organic concentration; select the rates of the two recycle streams, and maintain proper environmental conditions within each stage and appropriate sludge wasting schedules. It should be noted that as the magnitude of these internal recycles increases, the more closely the bioreactor approaches a complete mix process, with increasing loss of environmental control of the separate stages.

The effect of in-plant recycles, such as sludge processing and handling supernatants or filtrates, needs to be evaluated regarding their impact on organic, phosphorus, and nitrogen loadings received by the bioreactor.

To achieve an effluent TP concentration of 2 mg/L with the A^2/O process, metal salt addition would probably not be needed. To achieve 1 mg/L might require occasional polish dosing. To achieve 0.5 or 0.2 mg/L would probably require continuous polish dosing.

6.2. Phosphorus and Nitrogen Removal with the Bardenpho Process

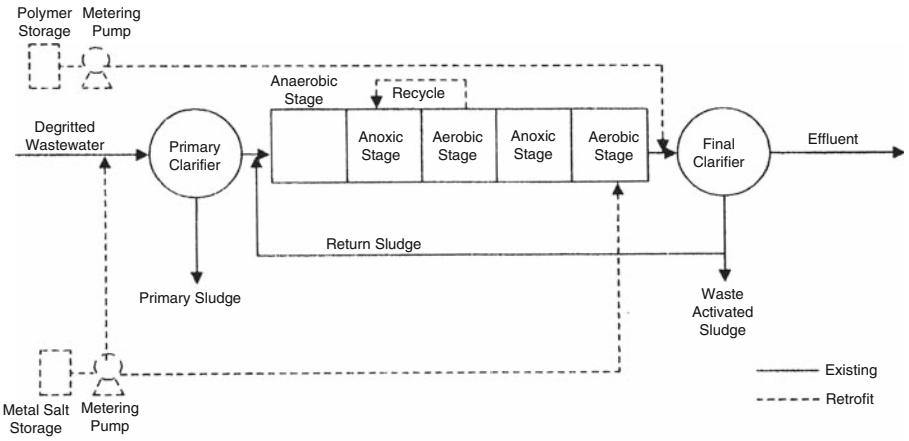
Inspection of Fig. 17.8 shows that the Bardenpho process has a similar configuration to the A^2/O process; however, it is segmented into a greater number of stages.

The lead stage is an anaerobic stage where phosphorus leaching from the microorganisms must occur. This is followed by four alternate stages that are managed to provide anoxic and aerobic environments. The first anoxic stage is the site of the major denitrification reaction. In the first aerobic stage, biological phosphorus cellular uptake, oxidation of ammonium nitrogen, and oxidation of organics occur.

The subsequent anoxic and aerobic stages are essentially polishing stages to provide low effluent residual total nitrogen and efficient organic removal. Any denitrification occurring in the second anoxic stage is because of the endogenous oxygen demand of the mixed liquor since there is no direct input of organics to this stage. Thus, three locations exist in the Bardenpho system where denitrification can occur: the anaerobic, first anoxic and second anoxic stages. Nitrification and organic oxidation can occur in the two aerobic stages.

As with the A^2/O process, the Bardenpho process has an interstage recycle between the first aerobic and anoxic stages for denitrification and reduction of the influence of nitrate nitrogen on phosphorus leaching in the anaerobic stage. The anaerobic stage receives only nitrate contained in the return sludge.

The highly baffled configuration approaching plug flow and the presence of the two internal recycles are conducive to the reuse of oxygen from the nitrate radical. Operating and environmental constraints for managing biological phosphorus and nitrogen removal discussed in



Final Effluent TP	Probable Need for Chemical Addition	Final Clarifier SOR*
mg/L		gal/ft^2/d
2	None	800
1	Occasional	600
0.5	Continuous polish dose	500
0.2	Continuous polish dose	500

* at peak sustained flow

Approximate final nitrogen concentrations (mg/L):

Organic-N:	3.5
NH_4-N:	1
NO_2-N:	0.1
NO_3-N:	5

Fig. 17.8. Schematic of activated sludge system fitted for the Bardenpho process (*Source*: U. S. EPA). (Conversion factor: 1 gal/ft^2/d = 0.0408 m^3/m^2/d)

the section for the A^2/O process apply equally well to the Bardenpho process. Likewise, the influence of in-plant recycle streams must be evaluated.

Some descriptions of the Bardenpho process label the first stage in the process of a fermentation zone instead of an anaerobic stage. In reality, the biological transformations that occur in this reactor – whether labeled fermentation zone or anaerobic stage – are the same. The fermentation zone concept originated at facilities where the wastewater was weak in organic content. The purpose of this fermentation zone was to provide an anaerobic operation, where particulate organics in influent wastewater could be hydrolyzed (fermented) to short-chain fatty acids, such as acetate (46). As discussed earlier, soluble organics must be present in the initial anaerobic stage for sorption by the phosphorus-accumulating microorganisms.

The need for the presence of short-chain fatty acids in the anaerobic stage of all biological phosphorus uptake processes, not just the Bardenpho process, has been established (46). Various operational approaches have been applied to ensure that these materials are present. These include:

1. Adding increments of primary sludge to the anaerobic stage (47)
2. Recycling in-plant streams such as thickener overflow to the anaerobic stage (48)
3. Infrequent mixing of the anaerobic stage to allow sludge deposition and subsequent hydrolysis of the sludge (49)
4. Provision of an off-line fermentation reactor for biological hydrolysis of primary sludge to produce fatty acids to be dosed into the anaerobic stage (50)
5. Addition of anaerobic digester supernatant to the anaerobic stage (27)

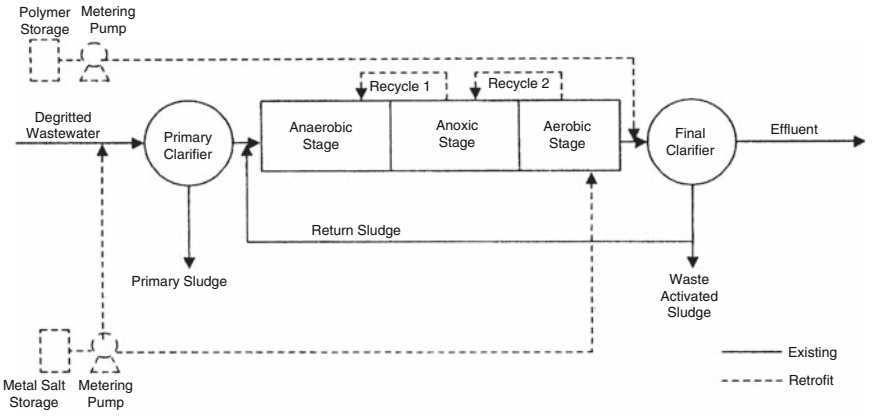
For the Bardenpho process, Fig. 17.8 indicates that attainment of an effluent TP concentration of 1 mg/L may require an occasional supplemental dose of a metal salt and that a continuous polish dose of metal salt will probably be required to attain an effluent TP concentration of 0.5 mg/L or less. Attainment of an effluent total nitrogen concentration of 1.5 mg/L has been reported for a 0.07-m^3/s (1.7-MGD) municipal facility. The effluent TP concentration was 3 mg/L (49).

6.3. Phosphorus and Nitrogen Removal with the University of Capetown Process

The UCT process was developed at the University of Capetown, Capetown, South Africa (50). A flow schematic is given in Fig. 17.9.

The process is akin to the A^2/O and Bardenpho processes. However, two interstage recycles are incorporated in the process flowsheet instead of one. As with the A^2/O and Bardenpho processes, mixed liquor is recycled from the aerobic stage to the anoxic stage, but to protect the anaerobic stage from nitrate inhibition of phosphorus leaching, an engineering modification was made. Return sludge is directed into the anoxic stage instead of the anaerobic stage and then mixed liquor from the anoxic stage is recycled to the anaerobic stage.

In evaluating this process for possible retrofit applications, careful consideration should be given to the detention times of the various stages since a high degree of recycling within the reactor could greatly alter the maintenance of proper stage environmental conditions and substrate utilization rates.



Final Effluent TP	Probable Need for Chemical Addition	Final Clarifier SOR*
mg/L		gal/ft^2/d
2	None	800
1	Occasional	600
0.5	Continuous polish dose	500
0.2	Continuous polish dose	500

* at peak sustained flow

Approximate final nitrogen concentrations (mg/L):

Organic-N:	3.5
NH_4-N:	1
NO_2-N:	0.1
NO_3-N:	5

Fig. 17.9. Schematic of activated sludge system fitted for the UCT process (*Source*: U. S. EPA). (Conversion factor: 1 gal/ft^2/d = 0.0408 m^3/m^2/d)

Metal salt addition would probably not be needed to achieve an effluent TP concentration of 2 mg/L. To achieve 1 mg/L might require occasional dosing. To achieve 0.5 or 0.2 mg/L would require a continuous polish dose.

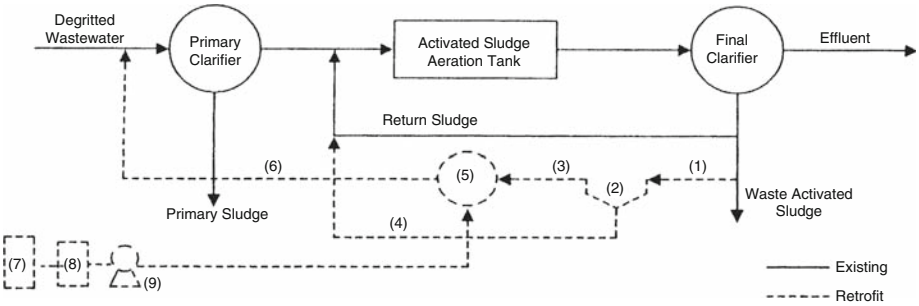
6.4. Phosphorus and Nitrogen Removal with the Modified PhoStrip Process

The original sidestream phosphorus removal, PhoStrip, process is shown in Fig. 17.10. The modification of the process that provides biological denitrification of the nitrate nitrogen contained in the return sludge flow is presented schematically in Fig. 17.11.

Figure 17.10 represents an activated sludge system that has been designed to include the PhoStrip process. A portion of the return sludge stream is subjected to anaerobic conditions in a reactor called an "anaerobic stripper." The portion of return sludge sent to the stripper can vary from 15 to 30% of total plant flow (51). The purpose of the stripper tank is to provide conditions conducive to the release of intercellular phosphorus from the microorganisms in the return sludge (phosphorus stripping).

The SRT of the return sludge solids in the stripper tank can vary from 5 to 20 h, and the HDT can vary from 1 to 10 h. The return sludge solids in the underflow from the stripper tank, which have now been partly stripped of intercellular phosphorus, are returned to the aerobic aeration tank to biologically insolubilize phosphorus from the main stream flow and then be recycled back to the anaerobic stripper.

Overflow from the stripper is treated with lime in a precipitation tank to chemically insolubilize the stripped phosphorus. Lime dosages of 100–150 mg/L are typically used to



Legend

(1) Portion of return sludge going to anaerobic stripper.
(2) Anaerobic stripper tank for leaching of phosphorus.
(3) Stripper tank overflow.
(4) Stripper tank underflow returned to activated sludge aeration tank.
(5) Tank containing lime slurry to precipilate phosphorus leached from return sludge in anaerobic stripper. Lime dose (CaO) = 20-25 mg/L based on plant influent flow.
(6) Insolubilized phosphorus returned to primary clarifier for co-settling with primary sludge.
(7) Lime storage.
(8) Lime slurry tank.
(9) Pump for transfer of lime slurry to phosphorus precipitating tank.

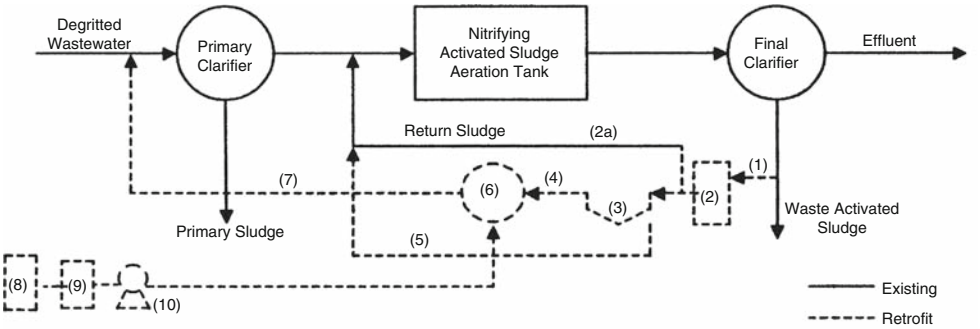
Fig. 17.10. Schematic of activated sludge system fitted for the PhoStrip process (*Source*: U. S. EPA).

increase the pH to 9.0–9.5. This equates to about 20–25 mg/L of lime based on plant influent flow. Some facilities route the precipitation tank contents directly to the primary clarifier as shown in Fig. 17.10. Once insolubilized by lime, the phosphorus does not resolubilize in the primary clarifier. The lime-phosphorus sludge co-settles with the primary sludge. Other facilities employ a reactor-clarifier in lieu of a precipitation tank and route only reactor-clarifier overflow to the primary clarifier. The underflow lime-phosphorus sludge is disposed of separately.

The aeration tank HDT should be between 4 and 10 h for effective PhoStrip operation. Detention time in extended aeration plants may, therefore, need to be shortened by blocking a portion of the tankage. This will also result in a higher organic loading rate, which is desirable for biological phosphorus removal. On the other hand, aerobic contact time in the contact stabilization process may be too short, necessitating the use of all or a portion of the sludge reaeration volume as additional contact volume. Typically, this can be accomplished with minor modifications.

The key process feature that determines the efficiency of the PhoStrip concept is the differential phosphorus content of the return sludge solids entering and leaving the anaerobic stripper. This phosphorus differential times the mass of solids passing through the stripper is equivalent to the amount of phosphorus removed from the main stream wastewater flow by cycling a portion of the return sludge through the stripper.

The other noneffluent outlet for phosphorus in a PhoStrip process is the WAS removed from the system. The phosphorus removed at a PhoStrip facility, therefore, is accomplished through a combination of stripping of return sludge and sludge wasting.



Legend

(1) Portion of return sludge going to anoxic tank.
(2) Anoxic tank for return sludge flow
(2a) Portion of denitrified return sludge flow.
(3) Anaerobic stripper tank for leaching of phosphorous.
(4) Stripper tank overflow.
(5) Stripper tank underflow returned to activated sludge aeration tank.
(6) Tank containing lime slurry to precipitate phosphorus leached from return sludge in anaerobic stripper. Lime dose (CaO) = 20-25 mg/L based on plant flow
(7) Insolubilized phosphorus returned to primary for co-setting with primary sludge.
(8) Lime storage.
(9) Lime slurry tank.
(10) Pump for transfer of lime slurry to phosphorus precipitating tank.

Final Effluent TP	Probable Need for Chemical Addition	Final Clarifier SOR*
mg/L		gal/ft^2/d
2	None	800
1	None	600
0.5	None	500
0.2	Occasional	500

* at peak sustained flow

Approximate final nitrogen concentrations (mg/L):

Organic-N:	3.5
NH_4-N:	1
NO_2-N:	0.1
NO_3-N:	6

Fig. 17.11. Schematic of activated sludge system fitted for the modified PhoStrip process (*Source*: U. S. EPA). (Conversion factor: 1 gal/ft^2/d = 0.0408 m^3/m^2/d)

The PhoStrip process can achieve 2- and 1-mg/L TP effluent concentrations without effluent polishing. A 0.5-mg/L TP effluent concentration is possible when effluent suspended solids concentrations are low (51). To achieve an effluent limit of 0.2 mg/L TP with PhoStrip, filtration and split dosing of chemicals should be considered.

The modification of the PhoStrip process to remove both P and N (52) retains all the features of the original PhoStrip process as described earlier. For implementing removal of nitrogen, the main stream activated sludge system would have to be operated to achieve nitrification. The nitrate in the return sludge flow is directed to an anoxic reactor ahead of the anaerobic stripper tank.

The denitrification rate is dependent on the hydrolysis of organics due to endogenous respiration since a nitrified return sludge stream has very little soluble organics present. Consideration could be given to providing a bypass line to introduce primary effluent directly into the anoxic reactor to increase the denitrification rate.

The anoxic reactor is sized larger than the quantity of flow required for the anaerobic stripping of return sludge for phosphorus removal. A portion of the anoxic denitrified flow is routed directly back to the aeration tank. Denitrification of the return sludge flow in this modification also serves to protect the anaerobic stripper phosphorus leaching reaction from the inhibiting action of nitrate.

Theoretically, Fig. 17.11 indicates that the denitrification removal capability of this modification would be limited to the percentage of return sludge flow. For instance, if the return flow were 50% of the influent plant flow, the amount of nitrate nitrogen that could be denitrified would be 0.5/1.5, or 33%. However, it has been reported (52) that a pilot plant operated in this mode denitrified 70% of the nitrate nitrogen present. The incremental removal was attributed to coincidental denitrification in the nitrifying activated sludge system. This agrees rather well with results presented elsewhere (27), where a full-scale PhoStrip plant producing a nitrified effluent with no anoxic reactor achieved an overall nitrogen removal of 31%.

Metal salt addition would probably not be needed to achieve effluent TP concentrations of 2, 1 or 0.5 mg/L. To achieve 0.2 mg/L an occasional polish dose might be required.

7. SLUDGES DERIVED FROM BIOLOGICAL PHOSPHORUS PROCESSES

7.1. Sludge Characteristics

Sludges derived from biological phosphorus removal systems exhibit properties similar to conventional biological sludges. The only possible exception is sludge derived from the PhoStrip process in which a portion of the total sludge results from lime addition to the anaerobic stripper vessel. Even with the PhoStrip process, the volume of lime sludge is relatively small when compared with the combined volume of primary and WASs.

Because of the mechanism of excess phosphorus uptake in biological phosphorus removal systems, resulting WASs tend to have higher phosphorus concentrations than conventional sludges. Typical phosphorus concentrations in WAS from the Bardenpho and A/C processes are 4–6% by weight vs. 2–3% for conventional WASs (1).

Resolubilization of phosphorus during anaerobic storage of lime-precipitated sludge in the PhoStrip process is unlikely to occur since the phosphorus is bound to the calcium ion. However, with A/O, Bardenpho, and other similar biological phosphorus removal systems, it is recommended that WAS be kept aerobic in order to prevent phosphorus solubilization.

7.2. Sludge Generation Rates

Sludge generation rates from biological phosphorus removal systems are not expected to be significantly different than for conventional activated sludge systems, and solids production will vary with wastewater characteristics and operational parameters such as SRT.

Theoretically, some increase in sludge production would be expected for biological phosphorus removal systems due to the increased mass of phosphorus taken up by the organisms. This will be dependent on the phosphorus content of the WAS. The theoretical WAS yield would increase by 8.5% if the phosphorus content of the WAS increased from 2 to 4% by weight. If the phosphorus content increased to 5% by weight, the theoretical mass of WAS production would increase by 13%. It should be noted that on a volumetric basis, the increased

sludge mass may be counteracted by an improvement in settling characteristics as SVI values of less than 80 mL/g have been reported for the Modified Bardenpho and A/C processes (1).

7.3. Sludge Management

Thickening: Because of the potential release of phosphorus during gravity thickening of WAS from biological phosphorus removal systems, use of DAF thickening is recommended (53). This would apply to the purely biological systems, and would not be a concern with lime sludges derived from treatment of the anaerobic stripper supernatant in the PhoStrip process. Lime sludges can be combined with other sludges and handled by conventional sludge handling processes (28) without special consideration for phosphorus release. With other biological systems, however, the choice of sludge handling processes must account for potential phosphorus resolubilization if thickened, stored, or stabilized in the absence of oxygen.

Stabilization: Phosphorus resolubilization would be anticipated during anaerobic digestion. However, at Pontiac, MI, significant levels of phosphorus in anaerobic digester supernatant were not observed, possibly due to formation of an ammonium–magnesium–phosphate precipitate in the digester.

Phosphorus release may also be possible during aerobic digestion due to destruction and lysing of biological solids. Consideration may have to be given to chemical treatment of digester supernatants for phosphorus removal in order to minimize return of phosphorus to the head of the plant. Further studies are needed to assess the magnitude of phosphorus release during stabilization of biological phosphorus removal sludges.

In some biological phosphorus removal systems employing long solids retention times, phosphorus-laden sludges are subjected to dewatering without separate stabilization. The acceptability of this practice is dependent on regulations as to whether such sludges are considered stabilized or whether separate stabilization is required prior to land disposal.

Conditioning: Sludges from biological phosphorus removal systems are expected to have similar conditioning requirements to conventional nonphosphorus sludges. Blending of lime sludge with other sludges in the PhoStrip process may reduce overall conditioning requirements.

Dewatering: Sludges from biological phosphorus removal systems are expected to have dewatering characteristics similar to those from conventional activated sludge systems. The lime sludge from the PhoStrip process is not expected to adversely affect dewatering, and, based on experience with dewatering of lime sludges alone, may improve dewatering characteristics when blended with other sludges. Design criteria for conventional primary and WASs should be used to size dewatering equipment for sludges from biological phosphorus removal processes if pilot- or full-scale performance data are not available.

Incineration: No unique problems are associated with incineration of sludges from biological phosphorus removal systems. Sludges from biological phosphorus removal processes will have volatile solids contents and BTU values similar to those of conventional biological wastewater treatment sludges. PhoStrip sludges, if lime sludge from treatment of the stripper supernatant is included, may have slightly lower volatile solids contents and BTU values due

to the addition of inert solids from the lime addition step. However, the overall impact is expected to be small.

Disposal: Sludges from biological phosphorus removal systems can be disposed of in the same manner as sludges from conventional biological systems. Higher phosphorus contents may make sludges from biological phosphorus removal systems particularly attractive for agricultural utilization.

8. CAPITAL AND O&M COSTS

The costs for biological phosphorus removal processes are sensitive to wastewater characteristics, treatment level needs, and existing equipment and site considerations (1). Where only phosphorus removal is required and nitrification is not occurring, reasonable retrofit treatment alternatives include chemical addition to existing biological systems and the PhoStrip and A/O processes. If nitrification is occurring or is required, the UCT process and the A/O process with an anoxic zone and internal recycle (i.e., the A^2/O process) are candidates as well. An anoxic zone for partial denitrification is not strictly needed to achieve nitrification with the A/O process, but it is recommended to minimize the amount of nitrate nitrogen recycled to the anaerobic zone in the return sludge and its adverse effect on biological phosphorus release in that zone. For both phosphorus removal and a high level of nitrogen removal, the Modified Bardenpho process is a viable alternative along with a variety of advanced treatment designs using chemical addition for phosphorus removal.

An analysis was performed to compare the cost of biological phosphorus removal to that for chemical addition to activated sludge for retrofitting existing facilities (54). Effluent total phosphorus limits of 1.0 and 0.3 mg/L were considered. Nitrogen removal was not a requirement in the analysis. The analysis concluded that chemical addition to activated sludge was more cost-effective to meet a 1.0-mg/L total phosphorus effluent and was also more cost effective for meeting an effluent total phosphorus concentration of 0.3 mg/L for flows of up to 4, 500 m^3/d (1.2 MGD). The A/O process was determined to be more cost effective for flows of 13, 600 m^3/d (3.6 MGD) or more.

On the other hand, the cost of the A/O retrofit for Pontiac, MI, was well below these cost predictions. The PhoStrip process was selected for the Reno-Sparks 150,000-m^3/d (40-MGD) phosphorus removal retrofit after it was estimated that a total annual cost savings of $550,000 would be realized when compared with chemical addition to activated sludge (55). It appears, therefore, that the potential for realizing retrofit cost savings with biological phosphorus removal will likely be very site specific.

A key economic factor in the above cost analysis and in other cost analyses is the decision to include polishing filters to meet effluent phosphorus concentrations of less than 1.0 mg/L for the A/O, UCT, and Modified Bardenpho systems. This would also apply to operationally modified activated sludge processes. Some chemical addition may be required in the above processes where unfavorable BOD:P ratios exist. Cost considerations for external acetate production may also have to be developed. Previous plant performance data indicate that effluent filtration may not always be required. This will be a function of the influent BOD:P

Table 17.3
Capital and O&M cost[a] comparison for biological phosphorus removal (2009 dollar value = cost × 1.61)

Alternative	Costs	Plant size, m^3/d 1,890	18,900	189,200
Case 1: Phosphorous removal (effluent TP = 1 mg/L)				
1-stage AS with alum addition	Capital, $	2,774,000	10,851,000	55,568,000
	O&M, $/year	218,000	868,000	5,611,000
	Total present worth, $	4,782,000	18,846,000	107,248,000
Phostrip	Capital, $	3,801,000	12,602,000	59,073,000
	O&M, $/year	273,000	744,000	3,956,000
	Total present worth, $	6,315,000	19,455,000	95,509,000
A/O (4-h detention) with effluent filters	Capital, $	3,370,000	13,257,000	63,472,000
	O&M, $/year	227,000	836,000	4,545,000
	Total present worth, $	5,461,000	20,957,000	105,333,000
Case 2: Phosphorus removal (effluent TP = 2 mg/L)				
1-stage AS with alum addition	Capital, $	2,762,000	10,821,000	55,350,000
	O&M, $/year	213,000	835,000	5,276,000
	Total present worth, $	4,724,000	18,512,000	103,944,000
Phostrip	Capital, $	3,801,000	12,602,000	59,073,000
	O&M, $/year	273,000	744,000	3,956,000
	Total present worth, $	6,315,000	19,455,000	95,509,000
A/O (4-h detention) with effluent filters	Capital, $	2,813,000	10,819,000	52,314,000
	O&M, $/year	197,000	692,000	3,820,000
	Total present worth, $	4,627,000	17,193,000	87,498,000
Case 3: Phosphorus removal plus nitrification (effluent TP = 2 mg/L; NH_4-N = 1 mg/L)				
2-stage AS with alum addition	Capital, $	3,370,000	12,820,000	63,381,000
	O&M, $/year	245,000	921,000	5,793,000
	Total present worth, $	5,627,000	21,303,000	116,737,000
A/O (6-h detention) with effluent filters	Capital, $	3,142,000	11,942,000	59,169,000
	O&M, $/year	210,000	764,000	4,264.000
	Total present worth, $	5,076,000	18,979,000	98,442,000
Case 4: Phosphorus removal plus nitrification and denitrification (effluent TP = 2 mg/L; TN 3 mg/L)				
2-stage AS with alum addition	Capital, $	3,869,000	14,553,000	72,777,000

(Continued)

Table 17.3
(Continued)

Alternative	Costs	Plant size, m^3/d 1,890	18,900	189,200
	O&M, $/year	296,000	1,200,000	8,059,000
	Total present worth, $	6,595,000	25,605,000	147,004,000
A/O (6-h detention) with effluent filters	Capital, $	3,321,000	13,553,000	77,472,000
	O&M, $/year	205,000	756,000	4,552,000
	Total present worth, $	5,209,000	20,516,000	119,398,000

[a] Total present worth calculated assuming a 20-year life and a discount factor of 8–7/8%

ratio or availability of fermentation products, the secondary clarifier design, the system SRT, and other parameters that affect activated sludge flocculation and clarification properties.

Cost curves for new plants have been presented in a report entitled Emerging Technology Assessment of Biological Removal of Phosphorus (56). Table 17.3 summarizes the updated capital and O&M costs developed for four basic cases. The updated costs are based on U. S. ACE, *Civil Works Construction Cost Index* of 570.38 (Year 2009). To obtain 2009 dollar value using Table 17.3, multiply the 1,987 costs by a factor of 570.38/353.35 = 1.61 (37)

Case 1: Phosphorus removal only with a required effluent total phosphorus concentration of 1 mg/L. A comparison is made between a single-stage activated sludge system with alum addition, a PhoStrip system, and an A/O system. Effluent filtration is assumed with the A/O system.

Case 2: Same as Case 1 except the required effluent total phosphorus concentration is 2 mg/L. Without effluent filtration, in this case, the A/O system is shown to be most cost-effective.

Case 3: Same as Case 2 with the addition of nitrification. In this case, a two-stage nitrification system with alum addition is assumed for the conventional alternative and is compared to a single-sludge A/O system. The two-stage system has a much higher capital cost.

Case 4: Same as Case 3 with the addition of denitrification to achieve an effluent total nitrogen concentration of 3 mg/L In this case, a three-stage activated sludge system with alum addition is compared to a Modified Bardenpho system. The three-stage system has significantly higher capital and operating costs.

In summary, the cost comparisons illustrate that the biological phosphorus removal alternatives may be competitive with conventional chemical methods (1, 22, 57, 58). The use of effluent filtration is a critical economic factor and any final cost comparison will be extremely site specific and affected by wastewater characteristics.

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APPENDIX

U.S. Army Corps of Engineers Civil Works Construction Yearly Average Cost Index for Utilities (37)

Year	Index	Year	Index
1967	100	1989	383.14
1968	104.83	1990	386.75
1969	112.17	1991	392.35
1970	119.75	1992	399.07
1971	131.73	1993	410.63
1972	141.94	1994	424.91
1973	149.36	1995	439.72
1974	170.45	1996	445.58
1975	190.49	1997	454.99
1976	202.61	1998	459.40
1977	215.84	1999	460.16
1978	235.78	2000	468.05
1979	257.20	2001	472.18
1980	277.60	2002	484.41
1981	302.25	2003	495.72
1982	320.13	2004	506.13
1983	330.82	2005	516.75
1984	341.06	2006	528.12
1985	346.12	2007	539.74
1986	347.33	2008	552.16
1987	353.35	2009	570.38
1988	369.45		

18
Treatment of Septage and Biosolids from Biological Processes

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CONTENTS

Abstract This chapter deals with processes for biosolids dewatering and septage treatment. Septage is the liquid and solid material pumped from a septic tank or cesspool when it is cleaned. A selection of recent methods that show promising applications is presented. These include: Expressor Press, Som-A-System, CentriPress, screw press, Sun Sludge system, wedgewater bed, vacuum assisted bed, reed bed, biosolids freezing bed, biological flotation, and septage treatment and management systems. When septage is to be ultimately treated at a wastewater treatment plant or independent septage treatment facility, a receiving station is

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required in order to provide preliminary treatment and equalization. The septage treatment and management options discussed include septage addition to biological wastewater treatment plants, septage land application, septage lagoon disposal, septage composting, and odor control.

Key Words Biosolids • septage • dewatering • land application • lagoons • composting • odor control • Expressor Press • Som-A-System • CentriPress • screw press • Sun Sludge system • wedgewater bed • vacuum assisted bed • reed bed • biosolids freezing bed • biological flotation • septage addition to biological wastewater treatment plants • septage management systems • pretreatment • primary treatment • secondary treatment • aerobic digestion • anaerobic digestion • mechanical dewatering • sludge drying beds • costs.

1. INTRODUCTION

This chapter deals with the current processes for biosolids dewatering and septage treatment. Septage is the liquid and solid material pumped from a septic tank or cesspool when it is cleaned (1). The main objective of biosolids dewatering is to remove water or moisture content, thereby reducing the residual volume (2). The end product is a sludge cake or powder, which possesses solid characteristics and no longer considered as a liquid. In this treatment, there will be substantial reduction of cost of subsequent treatment and disposal. In most applications, the ultimate "percent solid content" of dewatered biosolids is set by the requirements for subsequent treatment and disposal options. The percent solid content for dewatered biosolids is alwayssignificantly higher than the percent solid content of thickened biosolids.

The combination of processes used for biosolids treatment prior to dewatering, transport, and disposal varies widely in many countries and also from plant to plant. Generally, the dewatering process is preceded by one of the following stabilization processes: anaerobic or aerobic digestion; thickening by gravity, centrifugation, air floatation, chemical (alkaline treatment) or heat treatment (2). In some cases, raw biosolids, particularly raw primary sludge, maybe dewatered directly without prior thickening, although the handling and the method of final disposal would have to be considered carefully (3). It is a common practice to further treat the dewatered biosolids by means of stabilization using methods such as composting. If volume and organic reduction is the target, technologies of incineration and gasification are becoming a popular choice; otherwise, the dewatered biosolids may be ultimately reused by spreading on agricultural or landscaped areas or disposed off by trucks to either a landfill or designated area for land spreading (2).

A number of biosolids dewatering techniques are currently being used by many wastewater treatment plant operators. The selection of any biosolids-dewatering system depends on the characteristics of biosolids to be dewatered, available space, and moisture content requirements of the biosolids cake for ultimate disposal. When land is available and the biosolids quantity is small, natural dewatering systems are more attractive. These include drying lagoons and drying beds (4). The mechanical dewatering systems are generally selected in places where land is not available. Common mechanical biosolids dewatering systems are more appropriate for larger plants to maximize space requirement and also to ease handling

operation. The mechanical systems include vacuum filter (5), centrifuge (6), filter press (7), and belt filter press (8).

Some biosolids, particularly those that are aerobically digested, are not readily amendable to mechanical dewatering. These biosolids can be dewatered on sand beds with good results. When particular biosolids must be dewatered mechanically, it is often difficult or impossible to select the optimum dewatering device without conducting bench-scale or pilot studies. Trailer mounted, full size equipment is available from several manufacturers for field testing purposes (9, 10). Advanced biosolids treatment processes using thermal and thermochemical processes, or chemical oxidation have been developed to improve biosolids dewatering and to facilitate handling and ultimate disposal (2, 4).

When evaluating or selecting a dewatering process, one must keep in mind the influence of the prior wastewater and biosolids treatment processes as well as the subsequent use or disposal practices. The choice of a reuse strategy or disposal process is in turn strongly influenced by local, state, and federal regulations. A dewatering process cannot be evaluated without considering the other processes involved in the overall wastewater/solids handling system. This evaluation or selection can be a complex procedure because of the large number of possible combinations of unit processes available for wastewater treatment and biosolids thickening, stabilization, conditioning, dewatering, and ultimate use/disposal.

Above all, the design engineer must ensure that capacity limitations in the biosolids processing system are not the direct cost of impaired effluent quality. That is, the design should provide for sufficient standby capacity or an alternative mode of biosolids handling, whereby solids can be removed from the wet-end processing in an orderly manner – even if the primary means of biosolids disposal is unavailable or has failed in some manner. This criterion applies equally well to small and large plants, whether utilizing mechanical or non-mechanical means of biosolids disposal (2, 12).

There are many developments in the technology of biosolids dewatering and septage management. A selection of recent methods that show promising applications is presented in the following sections. This review highlights some of the systems used by municipalities including Expressor Press, Som-A-System, CentriPress, screw press, Sun Sludge system, wedgewater bed, vacuum assisted bed, reed bed, biosolids freezing bed, biological flotation, and septage management systems.

2. EXPRESSOR PRESS

One of the leading manufacturers of dewatering equipment had developed a belt press using some modification to incorporate twin belt mechanism for use primarily in the industrial market. Substantial tests have been conducted with municipal biosolids and various kinds of fibrous industrial waste sludges. The device, named the Expressor® or Expressor Press, consists of, in its basic form, two or three S rolls (wrap-around) and a series of five P rolls (direct) on which the pressure can be individually varied. An Expressor Press with this configuration is shown in Fig. 18.1 (12).

In a second configuration, a unit called the Hybrid Expressor Press contains a gravity drainage section, four or five S rolls, and the five variable pressure P rolls. Depending on the

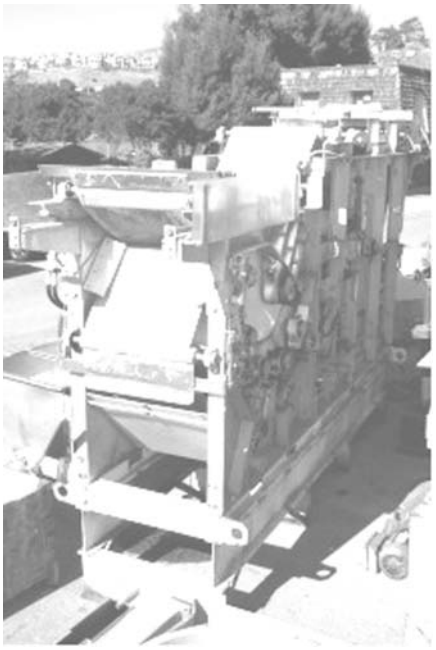


Fig. 18.1. Photograph of a typical expressor belt press.

model being considered, the P roll pressure can be varied from zero above the belt tension up to 200 kg/cm (1,000 lb/in.). This new unit is capable of producing a very dry cake from the most difficult sludges with the use of press aids. A variety of press aids have been employed, but the most widely investigated material has been sawdust. The unit can produce an autogenous cake from waste activated sludge using between 50 and 125% sawdust by dry weight, based on the content of sludge solids. The water displacement by the press aid varies from the slightly over one to as much as 3 kg H_2O/kg press aid added. The water displacement is based on the kg H_2O/kg sludge solids with and without press aid. The cake produced varies from 30 to 40% solids and, in some instances, runs somewhat higher than 40% (12).

Other press aids have been tested, including sand, soil, finely divided paper, fly ash, and coal fines. All work to some degree to increase the cake resistance to shear in the P rolls and hence permit higher pressure and in turn, higher solids content. Press aids in the 30–80 mesh region seem to be the most effective. With materials not particularly resistant to shear, such as paper and fiber, the particle size seems to have little impact on final sludge solids.

The press has also been tested on primary sludges and on mixture of primary and waste-activated sludge from a pulp and paper manufacturing facility. Typical cake concentrations varied from 40 to 47% solids without a press aid. Wastes from the manufacture of pulp and/or paper would seem to work particularly well with this equipment because of the fibrous nature of the primary sludge. Also of interest is the ability of the press to produce an alum sludge cake of 40–60% solids using soil as a press aid in one test and sawdust in another. In each case, the press aid used was approximately 100% of the weight of dry solids of the alum sludge.

Determination of the pressure profile is a function of the biosolids, the biosolids blend and the quantity and nature of the press aid used. On primary and waste activated sludge in the normal proportions (i.e., approximately 50–50) and the pure waste activated sludge, the P rolls pressures are usually tapered and will vary from 10 kg/cm (56 lb/in.) on the first roll to 60–250 kg/cm (336–1,401 lb/in.) on the last roll (12).

The biosolids in the Portland Columbia River Wastewater Treatment Plant were dewatered during a demonstration study at that facility. The activated sludge feed varied from 2.5 to 3.5% solids, and each test was run at approximately 100% of the press aid by weight. Sawdust additive yielded a cake in the range of 30 to 40% solids, while the paper press aid produce a cake from 35% to somewhat over 40% solids. Solids capacity of the press varies from 225 to 600 kg/m/h (102–272 lb/ft/h) and an acceptable hydraulic feed rate ranges from 1.6 to 3.2 L/s (25–51 gpm) on a 1-m (39-in.) wide machine. The basic press has been investigated for further dewatering of cake derived from other dewatering equipment (12).

3. SOM-A-SYSTEM

The Som-A-System Screw Press consists of a vertical, rotating screw enclosed by dual stainless steel screens. The screens and screw are encased in stainless steel housing with a removal cover on each side. Tiny perforations in the inner screen allow only water to escape. The outer screen has larger holes and easily collects the pressate, which sprays inside the housing and drains into a receptacle. Brushes are located along the edge of the screw to sweep the cake that builds up on the screen, allowing a clear opening for the pressate to escape (12).

The feed enters at the bottom of the screw press. A buildup of biosolids cake on the screw is recommended to get good dewatering. As the pressate drains, the cake becomes progressively drier and is pushed to the top, where it is discharged into a waiting dump truck or hopper. A back pressure system is located below the discharge chute and gives the cake a final squeeze before discharge. One plant, however, remove this cone, which collected hairballs, with no adverse effect to its operation or dewatering results. The Som-A-Press is shown in Fig. 18.2.

Biosolids that floc easily and are fibrous are the most conducive to a screw press operation. Feed concentration is critical to achieving high cake solids. The higher the feed concentration, the higher the cake solids and the unit capacity (kg/h). Table 18.1 reports feed solids, cake solids, and solids recovery from several different plants using the Som-A-System. The key to the action of the unit is bridging of the holes in the screen, as the bulk of the particles in the biosolids will be finer than the holes in the screen. Consequently, a proper biosolids conditioning is essential using this system. Table 18.2 presents the polymer usage of several plants using the Som-A-System.

A slow screw speed will yield a better cake, although it will also decrease the throughput. High flow rates and screw speed generally result in a discharge of wet sludge. A variable speed pump regulates the feed rate to the screw press. At Pinetop, AZ the plant generally keeps the feed rate at 2.5 L/s (40 gpm), near the maximum. Biosolids that have been aerobically digested at a 20 days detention will easily yield an acceptable 12–15% cake at a feed rate of 2.5 L/s (40 gpm). However, if the biosolids had a lower detention time, a feed rate of 2.5 L/s (40 gpm) would produce a wetter cake (12).

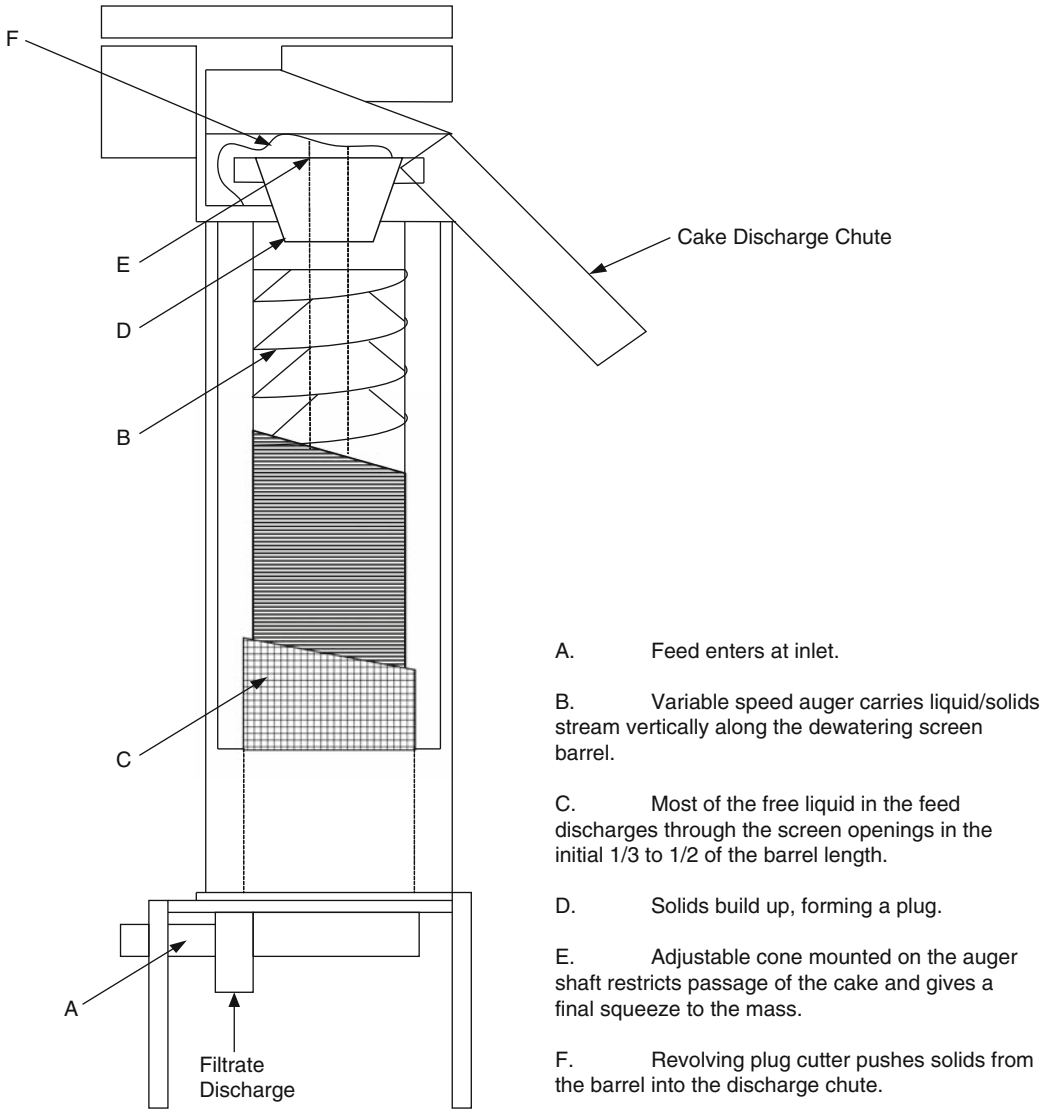


Fig. 18.2. Functional schematic of Som-A-Press (12).

In terms of operation and maintenance, the Som-A-System is found to be very simple. Depending on the biosolids, the press can normally be operated with only periodic checks. Many plants simply turn the machine on in the morning and periodically check the feed solids, the cake solids, and the level of biosolids in the waiting dump truck. Some operations require more attention to the feed biosolids to ensure that proper concentration is achieved and that water is not being fed to the press. The unit is also relatively easy to disassemble. General maintenance involves routine lubrication and washing the screens to prevent buildup

Table 18.1
Som-A-System operating data (12) (Conversion factor: 1 gpm = 3.785 Lpm)

Plant	Sludge	Average plant flow MGD	Feed solids TSS (%)	Feed rate gpm	Cake solids (%)	Solids recovery (%)
Camden, NY	Aerob. Digested	0.6	1–2	10–24	10	85
Churchville, NY	Aerob. digested	0.11	2.5–3.5	10.5	12.3	–
New Canaan, CT	Aerob. digested	0.25	1.0–1.5	30–40	12–17	84–94
Danville, VA	WAS/Stab. scum	16.2	5–6	15	21–23	86
	from DAF		8	30–40	28–30	90
Pinetop, AZ	Aerob. digested	0.4	2	40	12–15	88–90
Sunriver, OR	Aerob. digested	0.5	0.5–0.75	35–40	7–12	85[a]
Frisco, CO	Aerob. digested	1.0	2	15–18[b] 40[c]	11	–
Provo, UT	Anaer. digested	1.5	2	30	7–15	87–94

[a]Normally the solids recovery runs 90–94%.
[b]Undersized polymer pump limits feed rate 15–18 gpm – new pump ordered.
[c]With larger pump, expect to run presses at 40 gpm.

Table 18.2
Som-A-System chemical conditioning data (12) (Conversion factor: 1 lb = 0.4536 kg; 1 ton = 2000 lb = 907.2 kg = 0.9072 metric ton)

Plant	Polymer			
	Name	Dosage	$/lb	$/ton DS
Camden, NY	Percol 767	5–20	–	–
Churchville, NY	Percol 757	1.57	2.80	4.39
New Canaan, CT	Percol 757	6–9	3.25	24.38
Danville, VA	Cationic	21	0.92	19.32
Pinetop, AZ	Percol 757	8–10	–	27.00
Sunriver, OR	Allied CC4450	21	1.65	34.65
Frisco, CO	Percol 757	12	2.70	32.40
Provo, UT	Percol 763	21	–	–

of biosolids, which can prematurely wear the brushes. Repairs reported by plants have been limited to replacement of inner and outer screens and brushes after approximately 1,500 h (12).

The low capital cost of this screw press is a primary attraction and comparative economic evaluation points favorably to it. It is ideal for operations with limited space requirements, since the system occupies, at a maximum, approximately 3 m^2 (32 ft^2) of floor space. On the other hand, potential drawbacks include low unit capacity, higher polymer dosage and lower cake solids. Capacity of the presses can be a deterrent because the small throughput demands a multiplicity of units, which can be more difficult to control. One of the plants expressed disappointment on the amount of polymer required, and were attempting some experiments in an effort to reduce the quantity.

4. CENTRIPRESS

Based on observed field demonstrations, there have been significant improvements in the capabilities of a newly designed solid bowl continuous flow centrifuge. The improvements were in the area of cake solids concentration. During testing, the centrifuge was operated in parallel with a filter press system. The new centrifuge design, called the CentriPress, produced high cake solids as much as the filter press system (12).

A model, 45 cm diameter ×135 cm long (18 in. × 53 in.), centrifuge at the Marienfelde STP was operating on digested primary and waste activated sludge. These same biosolids were fed to 91.5 cm diameter × 274 cm long (36 in. × 108 in.) centrifuge which was dewatering the plant biosolids to a cake product of approximately 22% TS. The CentriPress was producing a granular cake of 30–32% TS. The "standard" centrifuge produced a cake with 60% higher moisture content. Both centrifuge installations were recovering in excess of 90% of the feed solids.

A larger unit, 91.5 cm diameter × 274 cm long (36 × 108 in.), is operating at Vienna, Austria WWTP. This unit is dewatering a heated primary and waste activated sludge to a cake solids content of 40–42% TS. Results from the centrifuge are comparable to those produced by a recessed plate filter press. The manufacturer had taken orders for Europe for the new machine.

Test runs using a Humboldt-Wedag CentriPress were performed by the Metropolitan Sanitary District of Greater Chicago (MSDGC) at the West-Southwest STP. This plant employs high-speed centrifuges for dewatering digested primary and waste activated sludge, which has an original solids ratio of 0.21 PS: 0.79 WAS. The existing centrifuges produce a cake of 14–16% TS. The tests were conducted using two types of cationic polymers as shown in Table 18.3.

One of the polymers was not cost effective for the plant's digested biosolids. The tests used different feed rates and differential speeds, with the polymer adjusted to maintain the TSS recovery in the range of 85–95%. The key results of Table 18.3, using American Cyanamid 2540C polymer are shown as follows (12):

Average and Range

- Cake Solids, % 29.4, 26.2–33.9
- Solids Recovery, % 92.7, 78.4–97.9
- Polymer Dosage, kg/MG 7.45, 3.23–15.93
- $/MG 3.37, 5.86–29.22. Here 1 MG = 3.785 ML = 3784 m^3

Figure 18.3 shows the effects of polymer dosage on the solids recovery of the CentriPress. About 5 kg/metric ton (10 lb/ton) of cationic polymer was required to maintain the solids recovery in excess of 90% TS. Table 18.3 does not indicate that higher dosages of polymer were beneficial to improve cake solids, although recoveries above 95% were achieved.

The use of low differential speeds appears to be the key to achieving good cake solids. As shown in Fig. 18.4, there was a good correlation between cake solids and centrifugal force at about 2, 600 G (12).

Table 18.3
Results of Chicago WSW CentriPress Study (12) (Conversion factors: 1 gpm = 3.785 Lpm = 0.0631 L/*s*; 1 ton = 2000 lb = 907.2 kg = 0.9072 metric ton; 1 lb = 0.4536 kg)

Run	Machine data		Sludge data				Polymer data		Performance			
	G-force, g's	Diff. speed	Feed rate (gpm)	Feed conc., (%)	Feed solids, ton/d	Volatiles (%)	Flow rate (gpm)	Polymer (Dry), lb/ton	Cake solids (%)	Centrate solids (%) TSS	Capture (%)	Cost, $/ton
1[a]	2,300	3	27	4.18	6.78	48.3	3.45	14.67	29.1	4,200	91.27	11.24
2	2,300	2	27	4.19	6.79	48.0	3.09	13.12	26.3	1,700	96.57	10.06
3	2,300	1.8	25	4.00	6.01	47.0	3.28	15.73	29.6	5,000	89.00	12.06
4	2,300	2	31	4.09	7.61	46.7	4.10	8.41	29.2	1,000	97.89	6.45
5	2,600	2	26.5	4.16	6.62	48.4	3.9	10.61	33.2	3,200	93.21	8.74
6	2,600	2	26.5	4.10	6.52	48.9	3.9	10.28	33.9	2,700	94.17	8.88
7	2,600	3.5	32	3.96	7.61	48.7	3.73	9.42	29.7	7,200	83.85	7.76
8	2,600	5.5	32	4.07	7.82	49.0	2.63	6.46	26.2	10,000	78.42	5.33
9	2,600	5.5	32	4.10	7.89	49.2	4.58	11.15	27.4	4,200	91.15	9.19
10	2,600	2.2	16	4.12	3.96	48.6	3.27	19.83	30.2	1,900	95.99	16.34
11	2,600	2.8	16	3.80	3.65	50.1	2.67	17.57	31.1	2,400	94.41	14.48
12	2,600	7.5	50	4.13	12.43	48.8	5.57	10.76	29.8	1,400	97.07	8.87
13	2,600	5.8	50	4.11	12.34	48.1	5.57	11.93	28.4	3,700	92.20	9.83
14	2,600	2.5	22	4.23	5.59	50.7	3.91	21.84	28.5	2,900	94.10	18.20
15	2,600	2.5	22	4.25	5.62	50.5	4.4	24.45	28.4	1,500	96.98	20.37
16	2,600	2.7	22	4.20	5.55	50.1	3.2	31.85	28.9	1,300	97.34	26.56
17[b]	2,600	2.5	22	4.25	5.62	49.4	2.73	33.25	29.2	2,900	94.11	76.48
18	2,600	2.8	22	4.25	5.62	49.4	2.73	33.25	32.0	1,700	96.51	76.48
19	2,600	2.9	22	4.00	5.28	49.8	2.4	31.14	29.4	1,100	97.61	71.58
20	2,600	2.2	22	4.10	5.42	49.2	2.4	30.31	31.3	2,200	95.30	69.71
21	2,600	2	22	4.08	5.39	50.6	3.57	45.34	32.8	1,300	99.20	104.28
22	2,600	2	22	4.16	5.50	50.5	3.50	42.8	34.8	2,200	95.31	98.44
23	2,600	5	26.5	4.09	6.51	49.3	3.50	36.16	30.0	2,400	94.84	83.17
24	2,600	1.5	18	4.05	4.38	49.1	2.5	38.39	30.8	1,000	97.85	88.30
25	2,600	1.2	18	4.08	4.41	49.4	2.9	44.23	29.7	1,200	97.45	101.73

[a]Tests 1 through 16 used American Cyanamid 2540C polymer.
[b]Tests 17 through 25 used Allied Chemical Percol 778F525.

5. HOLLIN IRON WORKS SCREW PRESS

This Korean screw press was evaluated for dewatering biosplids from liquid to cake, and for second stage (cake to drier cake), operations have also been evaluated. The Hollin Iron Works (HIW) screw press, shown in Fig. 18.5, is continuously fed with biosolids being conditioned by polymer.

Once inside the unit, the biosolids receive gradually increasing pressure as they progress through the screw press. The maximum pressure before discharge may exceed 10 kg/cm^2 (147 lb/in^2). In some instances, the dewatering may be enhanced by heating (a

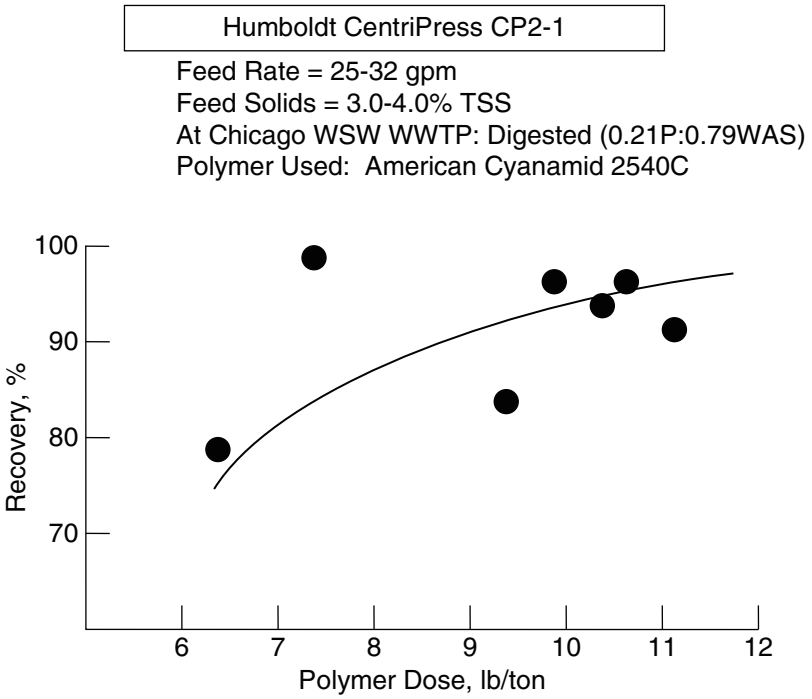


Fig. 18.3. Effect of polymer dose on solids recovery (12). (Conversion factors: 1 gpm = 0.0631 L/s; 1 lb = 0.4536 kg; 1 ton = 907.2 kg)

normal experience with screw presses) prior to the dewatering. This screw press is said to be relatively simple and easy to maintain. Also, the low operating speed helps keep repair costs to a minimum. HIW reports that there are over 100 units in operations (or installed) for various types of wastewater treatment and are providing satisfactory service. Some results reported are shown in Table 18.4.

More data and a better definition of the feed biosolids are required to fully evaluate the possibility of the screw press replacing conventional dewatering equipment. Past excessive secondary solids losses must be evaluated as a function of the cake solids content produced.

The MSDGC tested a pilot HIW screw press. The unit was tested on primary and anaerobically digested biosolids at the West-Southwest STP. The test was performed over a period of 2 days and approximately twelve separated runs were undertaken. Biosolids flow rate, dilute polymer concentration, and polymer flow were varied. With an average biosolids feed concentration of 4.5%, the test unit attained the following average results.

Cake concentration: 17.5%
Solids recovery: 94.5%
Pressate concentration: 3,720 mg/L (0.43 lb/gal)
Polymer usage: 8 dry kg polymer/dry metric ton solids (16 lb/dry ton)

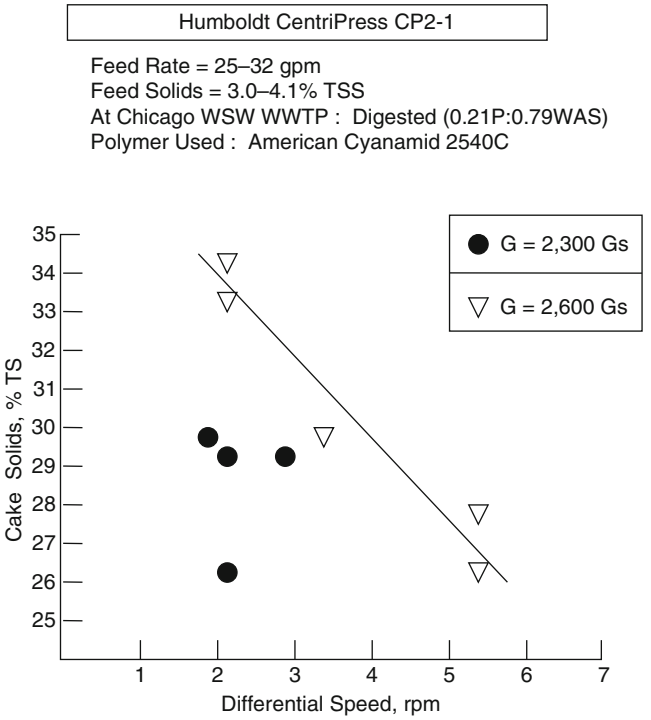


Fig. 18.4. Cake solids vs. differential speed (12).

Based on this pilot test results, the MSDGC decided to purchase a full-sized screw press. MSDGC anticipates that a full-size screw press may be a cost effective alternative to centrifugation due to the following considerations (12):

- Low initial cost
- Lower electric power consumption
- Equal to or higher cake concentrations
- Slow operating speed (low G force)
- Lower maintenance cost
- Comparable polymer cost

Krofta and Wang (48, 49) invented a combined dissolved air flotation (DAF) and screw press (SP) process equipment for simultaneous sludge thickening and dewatering. The combined DAF-SP process unit was installed at the Lenox Water Treatment Plant, Lenox, MA, USA for successful operation (48, 49). In the Lenox plant's full scale operation, the DAF chamber at the bottom floats the sludge to the water surface and thickens it. The SP which sits on the top of DAF chamber picks up the thickened sludge and further dewaters it. The theory and principles, and operational data of DAF-SP sludge thickening-dewatering can be found from the literature (18, 48–51).

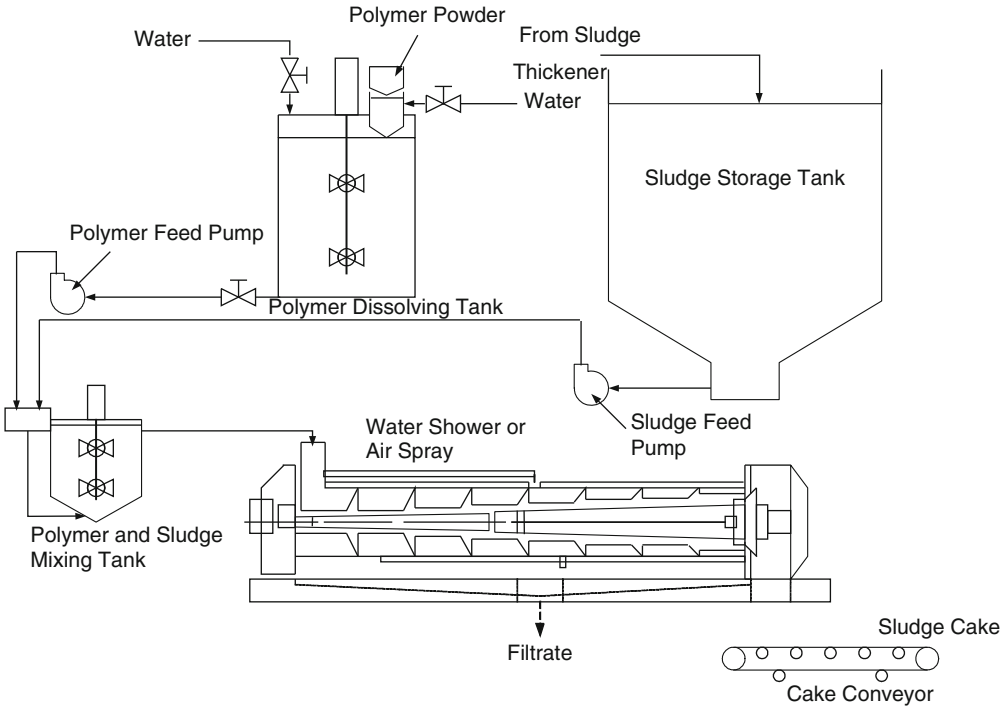


Fig. 18.5. Schematic of the screw press dewatering system (Courtesy of HIW) (12).

Table 18.4
Test Result for HIW Screw Press (12)

Sludge	P/S ratio	Feed solids (%)	Cake solids (%)	Solids recovery (%)	Polymer dosage (kg/mg)
Digested A	10/90	4.65	20.9	93.0	9.1
Digested B	10/90	4.93	25.3	97.8	7.6
Primary A	NR	2.85	20.5	95.0	13.4
Primary B	NR	2.37	21.2	95.9	16.0
Paper Mill 1	0/100	3.45	48.6	99.0	1.0
Paper Mill 2	60/40	4.08	44.6	98.9	NR
Paper Mill 3	50/50	2.95	42.3	98.9	NR
Paper Mill 4	0/100	2.4	23.0	95.4	NR

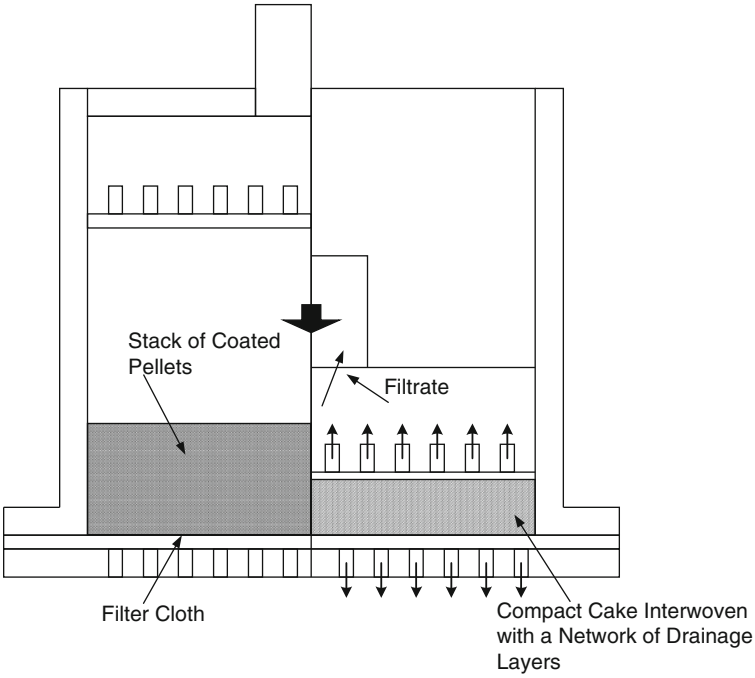


Fig. 18.6. Sludge press function of hi-compact method (Courtesy of Humboldt-Wedag) (12).

6. SUN SLUDGE SYSTEM

The Sun System (Hi-Compact) for biosolids dewatering was developed in Japan, and has been licensed for marketing and manufacture for Europe and the United States. The principle of the process is to develop a structured material from a cake of poor dewatering characteristics, and to form liquid channels. The cake is then subjected to high pressures. To that end, dewatered wastewater biosolids are reduced to pellets which are subsequently coated by a powdery layer such as ash or pulverized coal. Compressing a stack of these pellets results in a compact block interwoven with a network of drainage layers; the water being removed by pressing flows through a line of least resistance to the nearest drainage layer as shown in Fig. 18.6.

In the system, biosolids are first dewatered by conventional dewatering equipment such as vacuum filters, centrifuges, or continuous belt filters to a 20–25% solids concentration. This material is then conditioned in a unit called a disintegrating palletizer, which first breaks and forms the biosolids cake into small particles with a dry powder, forming biosolids-like pellets. The dry additive used should be mostly water insoluble and should not break up at the high pressures used. Materials such as diatomaceous earth, gypsum, calcium carbonate, incinerator ash, coal powder, bone meal, dried pulp, sawdust, and soil have been used, either alone or in combination with each other. The conditioners should be added in the ratio of 40–60% by weight per unit dry weight of the original biosolids cake. The effective biosolids

pellet's diameter should not be greater than 20 mm (0.8 in). Best performance occurs when the effective diameter of the pellets is between 3 and 5 mm (0.1–0.2 in). Also, the conditioning agents should coat only the surface and should not be kneaded into the biosolids pellets for maximum effectiveness (12).

The conditioned biosolids cake particles are conveyed to a hydraulic press where additional water is removed, and a cake of greater than 40% solids is produced. The palletized biosolids are pressed between two sheets of filter cloth that cover thick plates that have a number of perforations of 2–10 mm (0.1–0.4 in) in diameter. Compression is carried out in two steps. The initial compression step is usually at 15–25 kg/cm^2 (210 lb/in^2) for 45 s followed by a pressure of 30 kg/cm^2 (430 lb/in^2) for 5 min (12). In practice, the compression has occurred at 15 kg/cm^2 (210 lb/ft^2) for 45 s, followed by a pressure of 30 kg/cm^2 (430 lb/in^2) for 1 min. The palletized biosolids cake is compressed by hydraulic cylinder to form a disc-shaped solid with a 40–55% solids concentration. As an example, a mixture of primary and waste activated sludge having a 2% solid concentration could first be dewatered by a belt filter press to a solids concentration of 25% and then, with the Sun Sludge System, could be further dewatered to a solids concentration of 55%.

The Ashigara Works of Japan has successfully been using this system for waste activated sludge treatment since 1982. The excess biosolids are dewatered by a belt press to a water content of 80%, then palletized and conditioned with incinerator ash and further dewatered to a water content of 50% or less. Ash is added in the ratio of 10–15% by weight of the amount of belt press cake (or 50–75% of the dry solids). Biosolids cake is incinerated and heat is recovered (12).

A field demonstration of the process produced a cake of 55% from a 32% cake mixed with biosolids ash (50% by dry solids weight) every 3 min. The unit was a pilot scale producing in excess of 1,000 kg cake/h (2,204 lb/h). This would be equivalent to 370 kg/h (816 lb/h) of wastewater sludge solids. In this demonstration, pressures up to 60 kg/cm^2 (853 lb/in^2) were employed and the press time was shortened to about 3 min.

While the product from the press is very hard, it is also quite friable. It can be easily fragmented into particles which are dry to the touch and can easily be transported pneumatically. The palletizing/pressing operation reduced the moisture content from 3.5 kg H_2O/kg TS to1.3 kg H_2O/kg TS (biosolids only basis). The feed would have been suitable for boiler feed and would produce an equilibrium temperature of about $1,090^\circ C$ ($2,000^\circ F$). While the process mechanics looks favorable, the machine design capable of long-term operation at 50–60 kg/cm^2 (710–850 lb/in^2) needs further evaluation.

7. WEDGEWATER BED

Wedgewater, or wedgewire beds, are often constructed with an interlocking synthetic filter media placed on a concrete basin with an underdrain system. Polymer is always added to the biosolids before placement on the media surface. Wedgewater bed systems can produce biosolids with a final solids content of about 8–12% in 24 h and up to 20% is given additional drying time. Beds are usually uncovered, but may be covered to protect biosolids from excessive precipitation. The process is best suited for smaller treatment plants, 1, 893 m^3/d (0.5

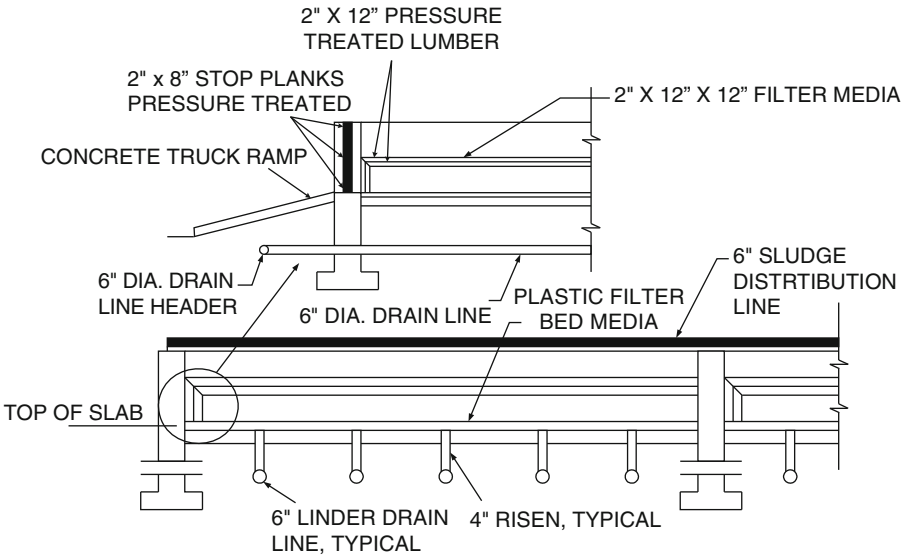


Fig. 18.7. Typical sections of a wedgewater drying bed (11). (Conversion factor: $1'' = 1$ in $= 2.54$ cm)

MGD) or less, and in moderate climates. U.S. ACERL (U.S. Army Construction Engineering Research Laboratory) has found that wedgewater systems have been used successfully in plants with flows up to 28, 387 m^3/d (7.5 MGD). A typical facility consists of the following (12–14):

- An outdoor, concrete construction with synthetic media plates
- Filtrate collection and drainage system (outlets)
- Polymer feed system
- Sludge distribution system (inlets)
- Washwater system

Figure 18.7 shows a typical section of a wedgewater drying bed.

The main structure should consist of a concrete floor with a drainage system, sidewalls, approximately 0.6 m (2 ft) high, biosolids distribution piping, supernatant decanting system, and vehicle entrance for biosolids removal. Manually removable wooden planks are to be installed at the vehicle entrance. Although most wedgewater operations are uncovered, use of a translucent roof or canopy is recommended in areas with significant precipitation. Filtrate is drained by gravity through the wedgewater media over the concrete floor. The floor should be designed with a slope of 0.5–1.0% to facilitate gravity drainage and avoid solids buildup under the media. Additional pipe drainage system may be installed. As a rule-of-thumb, there should be a drainage outlet for each 2.25 m^3 (25 ft^3) of media (11).

The media manufacturer's recommendations should be considered for design of a synthetic media dewatering system. The basis for design is the plant's average annual biosolids production rate in dry solids (i.e., kg/year or lb/year) and the number of cycles per week that can conveniently be performed. For reliability, a minimum of two beds should be constructed.

U.S. EPA states that solids loading rates of 2–5 kg/m^3/cycle (0.4–1.0 lb/ft^3/cycle) are typical (12). Adjustments, based on the expected efficiency and effectiveness of the operation, may also be considered. The number of operational cycles per year will vary. While the literature suggests that 24 h cycle times are acceptable, it is recommended that the design be based on two cycles per week. The design shall allow for downtime for cleaning of beds. Biosolids loading rates should be approximately 9.4 L/s (150 gpm). The general dimensions of each bed should be limited to approximately 7.6 m wide × 15.25 m long (25 ft by 50 ft). This will avoid problems with thermal expansion of the media and with the separation of solids before even distribution of biosolids can occur. Additional biosolids distribution inlets are also required as compared to conventional sand drying beds. Supernatant decanter devices are recommended to simultaneously remove water from the surface of the bed. High pressure washwater systems using treated effluent are recommended for tile cleaning. The supernatant and filtrate shall be routed back to the headworks, primary clarifier, or aeration basin for additional treatment (11).

Problems associated with these systems are inadequate media cleaning, front-end loader damage, and engineering errors. If the beds are properly designed, constructed, operated, and maintained, the beds will have a long life and underdrain cleaning will be required only once or twice a year. A polyurethane blade should be used on the front-end loader bucket avoiding the use of skid-steering loaders to prevent media damage. Wedgewater beds have less media clogging if high pressure hoses are used to clean the tiles (11).

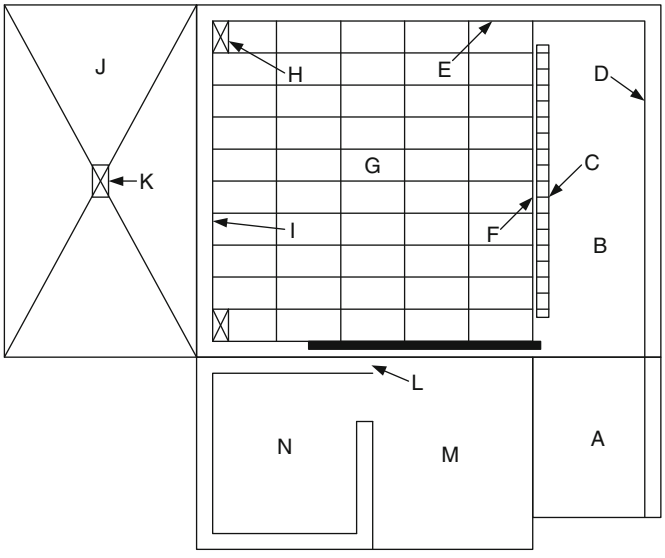
8. VACUUM ASSISTED BED

The vacuum assisted dewatering bed (VADB) uses commercially available equipment to apply a vacuum to the underside of a rigid, porous, media bed on which conditioned biosolids have been applied. The theory is that gravity, assisted by the vacuum, draws the water through the media, leaving the dry solids on top. VADB systems are capable of dewatering biosolids to final solids content of about 14% in 24 h and 18% or higher in an additional 24 h. The primary elements of a typical facility are as follows (12):

- An outdoor, concrete structure with synthetic media plates
- Filtrate collection and drainage system
- Polymer feed system
- Biosolids distribution system
- Vacuum system
- Washwater system
- Controls

Figure 18.8 shows a schematic view of vacuum assisted biosolids drying bed.

VADBs are generally used for smaller treatment plants, i.e., 7,579 m^3/d (2.0 MGD). Biosolids are seldom as dry as those removed from sand drying beds. Total solids content varies from site to site and depends on several factors including the basic type of treatment process, biosolids conditioning, biosolids feed rates, and cycle times. The bed design is similar to that of a wedgewater bed described previously. In cases of adverse weather conditions, the bed should be covered. The system equipment manufacturer's recommendations should be



A. Entrance Ramp
B. Off-Bed Level Area
C. Area Drain
D. Curbing
E. Sludge Distribution Piping
F. Bed Closure System
G. Media Filter
H. Corner Drain
I. Bed Containment Wall
J. Truck Loading Area
K. Area Drain
L. Wash Water Supply
M. Sludge Feed Inventory Tank (below grade, seldom required)
N. Control Building with
- Sludge Feed Pumps
- Polymer System
- Vacuum Pumps
- Control Panel
- Filtrate Receiver / Pumps (below grade)

Fig. 18.8. Schematic plan view of vacuum assisted sludge drying bed (11).

considered for any design of a VADB system. The basis for design is the plant's average annual biosolids production rate in dry solids (i.e., pounds or kilograms per year) and the number of cycles per week that can conveniently be performed. For reliability, a minimum of two beds should be constructed. U.S. EPA recommends that a solids loading rate of $10\,kg/m^2/cycle$ ($2\,lb/ft^2/cycle$) is acceptable. Adjustments, based on the expected efficiency and effectiveness of the operation, maybe considered. Most VADB designs are based on a 24-h cycle time. Biosolids loading rates should be approximately 9.4 L/s (150 gpm). Supernatant decanter devices should be installed to simultaneously remove water from the surface of the beds (11).

A common complaint of VADB operators is that the biosolids require long drying cycles. This problem is mainly due to inadequate drainage caused by media and/or underdrain clogging and to media destruction caused by front-end loaders or epoxy failure. Plant operators recommended a polyurethane blade be used on the front-end loader bucket to prevent damage. Skid-steering loaders are also inappropriate for this system. Tile cleaning is more difficult than

for wedgewater beds. Media blinding was reported as a major problem with a few existing VADB systems. VADB produce a faster turnover rate than sand beds. VADB systems can be operated year-round (11).

9. REED BED

An emerging and popular technique being used for biosolids dewatering in the United States for the past few years employs the use of reed bed (or sometimes referred to as wetland). Since biosolids are applied to a pre-designed stand or growth, essentially a bed of reeds, this treatment method is popularly called the "reed bed" process. The most common reed species utilized is from the genus Phragmites.

The Max-Planck Institute of West Germany originally conducted research in the late 1960s and early 1970s on the use of the reed bed system to process and dewater wastewater biosolids from small wastewater treatment plants. Although the process was originally used for wastewater treatment, it was extended to biosolids dewatering in 1980. Using the reed bed system, biosolids from wastewater treatment plants are applied to an actively growing stand of a common reed under controlled conditions. The growing reeds derive moisture and nutrients from the biosolids, and with the time, the rooted plants and the accompanying root ecosystem alter the characteristics of the biosolids, resulting in dewatering and improved biosolids characteristics. In addition to evapotranspiration, natural environmental processes, such as evaporation and drainage contribute to the moisture loss and dewatering as with conventional drying beds. Wastewater treatment plants in the northeastern United States have been using reed bed technology successfully for dewatering biosolids since the early 1980s (13).

The primary elements and characteristics of the reed bed process are as follows (12):

- Bed construction is similar to that of sand drying beds. Often retrofitted sand drying beds are used.
- Excavated trenches are lined with an impermeable material and filled with two sizes of gravel and a top layer of filter sand.
- Reed root stock or small plants are planted in the sand layer and the trenches are flooded to promote reed growth.
- A 1 m freeboard above the sand layer is provided to allow for long term biosolids storage.
- After plants are well established, stabilized, thickened biosolids (3–4% solids) are applied to the bed in 10 cm (4 in) layers at regular intervals.
- Annual harvesting of reeds and their disposal by landfilling, burning or composting is required.
- Biosolids are not removed regularly. Biosolids removal cycle time is 6–10 year.

A comparison between biosolids dewatering with conventional sand beds and the reed bed method shows that reed beds can provide adequate or marginal dewatering for both aerobically and anaerobically digested biosolids, if all the existing and drying beds are converted to reed beds. The most obvious advantage of reed beds is the elimination of labor for regular biosolids removal from sand drying beds. The process also offers many distinct advantages with respect to reduce costs, labor, and maintenance. Reed beds can also be constructed using existing biosolids drying beds. The use of greenhouses with reed bed should be made with caution. Greenhouse environments may generate severe heat and drought stress on the reeds. Higher

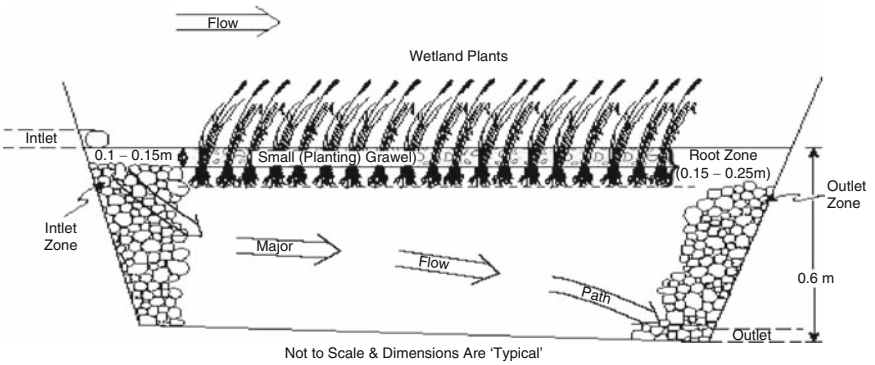


Fig. 18.9. Schematic view of reed bed (14).

volumes of aerobically digested biosolids may be dewatered than that of the anaerobically digested biosolids.

Figure 18.9 shows a typical section view of a reed bed for dewatering of biosolids.

Suggested solids loading rates are as follows (12):

- For aerobic biosolids: 9–95 kg/m^3/year (2–20 lb/ft^2/year). U.S. EPA indicates that operational systems in the northeast average loading rates of 81 kg/m^2/year (17 lb/ft^2/year). U.S. ACERL studies indicated an average loading rate of 52 kg/m^2/year (10.9 lb/ft^2/year) for systems in the U.S.
- For anaerobic biosolids: 9–57 kg/m^2/year (2–12 lb/ft^2/year). U.S. ACERL studies indicated an average loading rate of 22 kg/m^2/year (4.7 lb/ft^2/year) for systems in the U.S.

It is recommended to provide multiple beds to allow for biosolids removal and maintenance of beds (13).

U.S. EPA recommends that thickened biosolids (3–4% solids) be applied to the beds. The reeds must be harvested annually and subsequently disposed of in an acceptable manner. Operational problems include aphids and weed growth for younger reeds. Labor for weeding operations should be estimated from one to ten man-d/year. Cost estimates vary depending on size of the operation. Salinity affects the reed's height and growth. The maximum recommended salinity is 4.5%. During freezing months, biosolids application is normally stopped and the reeds are harvested (13, 14).

10. SLUDGE FREEZING BED

A sludge freezing bed is a unit operation that uses natural freeze-thaw to condition the biosolids for dewatering. It is most applicable in regions having 3 months per year or more of temperatures at or below 0°C (sub-freezing temperatures). Freezing beds can be used with conventional drying beds to provide year-round biosolids dewatering.

The design incorporates a covered, in-ground containment structure with drainage and ramp access. Drainage may be similar to conventional sand drying beds or synthetic media (wedgewire) systems. During winter months, the biosolids are added to the bed in layers. Successive layers are added as the previous layer freezes. At the end of the cold season, the bed is allowed to thaw and drain. Dewatering occurs by the removal of the melt water by the

underdrain system. Once the desired solid/liquid content is achieved, the dewatered biosolids are removed by mechanical means. The bed may be used as a conventional covered drying bed during warmer months (15, 16).

The Cold Regions Research and Engineering Laboratory (U.S. ACRREL) was involved in demonstration project at Fort Greely, Alaska and assisted in freezing bed design for projects constructed at Eielson Air Force Base, Alaska and Fort McCoy, Wisconsin.

The primary features and characteristics of the freezing bed dewatering system are as follows (15, 16):

- The facility consists of a basin with an underdrain system where biosolids are deposited in layers and allowed to freeze.
- Basins are usually covered to keep precipitation out.
- The process requires no chemical addition. i.e., polymers are not required.
- The operation requires no special skills to operate.

Freezing is dependent on natural climatic conditions at the proposed site. Any location that has 3 months per year or more of temperatures at or below 0°C may be considered. Biosolids freezing is a reliable dewatering method for most of the northern U.S. Any type of biosolids will benefit from the freeze thaw cycle. However, it is recommended that stabilized and thickened biosolids (3–7% solids content) be applied to avoid odor problems, maximize effectiveness, and reduce cost. The system is designed for worst case condition to ensure successful performance, i.e., warmest winter. A pre-engineered metal roof will be considered as part of the facility design to protect the area from snow.

The size and capacity of the freezing beds depends on the depth of biosolids that can be frozen and subsequently thawed in a season. In very cold climates, the depth of biosolids that can be frozen may be greater than the depth that can be thawed. In that case, the thawing depth will be limiting and should be used for design. The freezing depth ranges from less than 30 cm (12 in) to more than 180 cm (70 in) for most of the northern United States (15–17).

11. BIOLOGICAL FLOTATION

In a biological flotation system, fermentations take place in the presence of anaerobic bacteria, nitrates, and substrates under anaerobic environment. Anaerobic bacteria in the biosolids convert nitrate and the organic substrate as a carbon source (such as methanol) to nitrite, water, and carbon dioxide fine bubbles. Nitrite further reacts with a substrate (such as methanol) in the same biosolids, producing fine nitrogen bubbles, more fine carbon dioxide bubbles, water, and hydroxide ions. The biological biosolids, such as activated sludge can then be floated to the surface by the fine nitrogen and carbon dioxide bubbles. While the energy consumption of this process is low, its detention time is long in the range of 1 or 2 days (2, 18). Wang's chemical reactions in a biological flotation reactor for thickening of secondary activated sludge, under anaerobic conditions assuming nitrate (NO_3^-) is present in

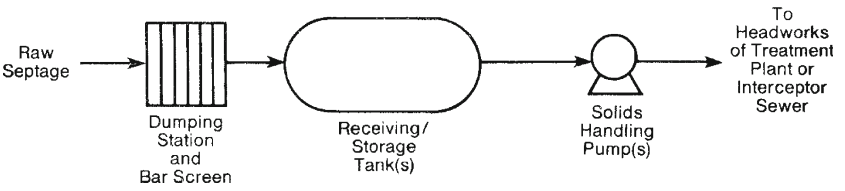


Fig. 18.10. Flow diagram of septage receiving station (23).

the biosolids and methanol (CH_3OH) is added as the substrate, are as given below:

$$6NO_3^- + 2CH_3OH \rightarrow 6NO_2^- + 2CO_2 + 4H_2O$$

$$6NO_2^- + 3CH_3OH \rightarrow 3N_2 + 3CO_2 + 3H_2O + 6OH^-$$

$$6NO_3^- + 5CH_3OH \rightarrow 5CO_2 + 3N_2 + 7H_2O + 6OH^-$$

It should be noted that the secondary activated sludge usually contains residual soluble BOD, COD, or TOC, which may avoid the necessity of adding an external organic substrate such as CH_3OH.

12. TREATMENT OF SEPTAGE AS SLUDGE BY LAND APPLICATION, LAGOON, AND COMPOSTING

12.1. Receiving Station (Dumping Station/Storage Facilities)

A septage receiving station with dumping station and storage facilities (see Fig. 18.10) provides for the transfer of septage from hauler trucks to a temporary holding tank from which it can be drawn at a controlled rate. With such a facility, septage can be discharged to an interceptor sewer or directly to the headworks of a treatment plant. The dumping station should provide for both direct hose connections (preferred) and open pit discharges. The dumping pit should be equipped with a coarse bar screen, and should be covered and preferably locked when not in use. A manual-controlled or timer-controlled pump discharge facilities feeding septage at a predetermined rate over specific periods of time in order to maximize the dilution of septage by wastewater (19–22).

Where septage is to be transferred from haulers' trucks to other vehicles (e.g., large tanker trucks for transport to centralized treatment facilities, or specialized land application equipment), the same basic facilities as described earlier could be used, with the exception that tanker trucks or trailers would replace the permanent storage tanks. Where land application is involved longer term storage may be required during adverse weather conditions, lagoon storage facilities should be considered in such cases. If septage is to be discharged to an interceptor sewer where flows are high, storage facilities might not be required. Odor control may be required depending on station location.

Grit and solids residuals which may accumulate in holding tank must be cleaned out periodically. This can be accomplished by removing the solids using vacuum truck equipment, or by flushing the solids out of the tank using high pressure water. Periodic removal of screenings will also be required.

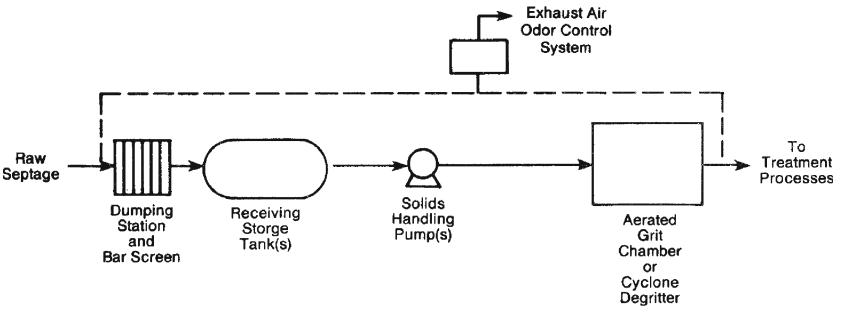


Fig. 18.11. Flow diagram of septage receiving station with pretreatment (23).

The recommended design criteria are as follows:

1. Bar Screen: $1/2 \times 11/2$ in (1.27×3.81 cm) bar stock, 1/2–3/4 in ($1.27 - 1.91$ cm) spacing
2. Hauler truck hose connection: 4 in. (10.16 cm) diameter
3. Piping and valves: 8 in (20.32 cm) diameter
4. Holding tank capacity: 1 day peak flow (not including supplemental storage requirements associated with land application systems etc.)

12.2. Receiving Station (Dumping Station, Pretreatment, Equalization)

When septage is to be ultimately treated at a wastewater treatment plant or independent septage treatment facility, a receiving station is required in order to provide preliminary treatment and equalization. This normally consists of a dumping pit with screening, grit removal, and flow equalization (see Fig. 18.11). Features which should be provided include: sloped ramp and hose-down facilities at unloading location; channel in front of bar screen for more uniform flow and to avoid direct discharge of septage onto screen; manually or mechanically cleaned bar screens; solids handling pumps; sampling/monitoring capability; ventilation system and odor control (19–24).

Grit removal can either precede storage and equalization or follow it. If a grit chamber precedes equalization, it must be designed to handle the discharge of individual or multiple truckloads of septage as they come. If storage and equalization precede grit removal the grit removal, process can be designed to handle the average flow, and can be operated according to a set schedule coinciding with subsequent treatment operations. Cyclone degritters may be substituted for aerated grit chambers if septage solids concentration is less than 2%.

Provisions must be made for removal and disposal of screenings and grit residuals, plus accumulated solids which settle out in flow equalization tanks. Landfilling is the most common method of disposal.

The recommended design criteria are as follows (23):

1. Bar screen: $1/2 \times 11/2$ in (1.27×3.81 cm) bar stock, 1/2–3/4 in. (1.27–1.91 cm) spacing
2. Hauler truck hose connection: 4 in (10.16 cm) diameter
3. Piping and valves: 8 in (20.32 cm) diameter
4. Degritting equipment: as per manufacturer's specifications for design flow

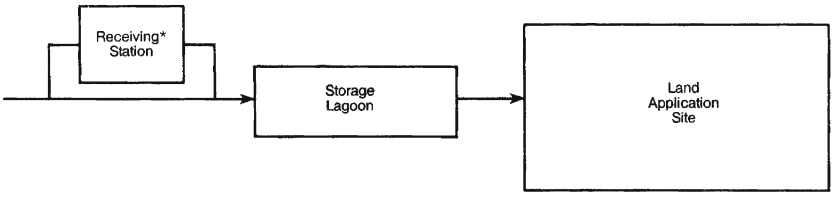


Fig. 18.12. Flow diagram of land application of septage (23).

5. Equalization tanks: multiple tanks, total capacity twice peak daily flow
6. Pumps: sized according to average design flow and operational schedule

12.3. Land Application of Septage

Raw septage and septage solids may be spread on the surface of the land or incorporated into the subsurface topsoil layers (see Fig. 18.12). Surface spreading includes spreading from septage hauler trucks or transfer vehicles such as tank wagons, spray irrigation, ridge and furrow practices, and overland flow (25, 26). Application by the hauler trucks is the most common method practiced. Spray irrigation of septage requires the use of high-pressure large nozzle systems to prevent clogging. Ridge and furrow methods involve spreading septage in the furrows and planting crops on the ridges. Overland flow methods are best suited to lands with a slope of 2–6%.

Subsurface application techniques include plow furrow cover (PFC), subsurface injection (SSI), and injection using a device such as a Terreator (a patented device). The PFC method of application applies septage in a narrow furrow created by the plow shear and is immediately covered by the plow moldboard. The SSI method of application applies septage in a narrow band behind a sweep which opens a cavity 10–15 cm (6–8 in.) deep. A Terreator or similar device opens a mole-type hole with an oscillating chisel point and injects the septage into the hole (27–31).

Federal "criteria" (40 CFR 257) specify that septage applied to the land or incorporated into the soil must be treated by a "process to significantly reduce pathogens" (PSRP) prior to application or incorporation, unless public access to the facility is restricted for at least 12 months after application has ceased, and unless grazing by animals whose products are consumed by humans is prevented for at least 1 month after application. PSRPs include aerobic digestion, air drying, anaerobic digestion, composting, lime stabilization, or other techniques which provide equivalent pathogen reduction (2).

The criteria also require septage to be treated by a "process to further reduce pathogens" (PFRP) prior to application or incorporation, if crops for direct human consumption are grown within 18 months subsequent to application or incorporation, and if contact between the septage applied and edible portion of the crop is possible. PFRPs include composting, heat drying, heat treatment, thermophilic aerobic digestion, or other techniques that provide equivalent pathogen reduction (2).

Constituents of the septage may limit the acceptable rate of application, the crop that can be grown, or the management or location of the site. Nitrogen requirements of the crop normally dictates the annual septage application rates, It is also required that soil pH be maintained at 6.5 or above to minimize the uptake of the trace elements.

The potential for contaminated runoff, soil compaction, crop damage, or trucks getting stuck preclude the application of septage during periods when soil moisture is too high. Therefore, septage application is limited only a portion of the year. For the period of the year when septage can not be applied, storage facilities must be provided. Many states regulate the total volume of septage that can be applied as a function of soil drainage characteristics.

Septage contains all the essential plant nutrients. It can be applied at rates which will supply all the nitrogen and phosphorus needed by most crops. Application rates depend on septage composition, soil characteristics, and cropping practices. Annual application rates have varied from $282\,m^3/ha$ (30, 000 gal/acre) to $1,880\,m^3/ha$ (200, 000 gal/acre). Applying septage at a rate to support the nitrogen needs of a crop avoids problems with overloading the soil (23).

There is a potential for heavy metals and pathogens to contaminate soil, water, air, vegetation, and animal life, which ultimately become hazardous to humans. Accumulations of metals in the soil may cause phytoxic effects, the degree of which varies with the tolerance level of the particular crop (32). Toxic substances such as cadmium that accumulate in plant tissues can subsequently enter the food chain, reaching human beings directly by ingestion or indirectly through animals. If available nitrogen exceeds plant requirements, it can be expected to reach groundwater in the nitrate form. Toxic materials can contaminate groundwater supplies or can be transported by runoff or erosion to surface waters if improper loading occurs. Aerosols which contain pathogenic organisms may be present in the air over a landspreading site, especially where spray irrigation is the means of septage application. Other potential impacts include public acceptance and odor.

12.4. Lagoon Disposal

The use of lagoons for the disposal of septage is a common alternative in rural areas. The design and operation of lagoons vary from simple septage pits to sealed basins with separate percolation beds. Most lagoons are operated in the unheated anaerobic or facultative phase.

A typical lagoon system consists of two earthen basins arranged in series (Fig. 18.13). The first or primary lagoon receives the raw septage via a vertical discharge chamber entering under the surface of the liquid near the lagoon bottom to minimize odors. It may be lined or unlined, depending on the geological conditions of the site. The supernatant from the primary lagoon, which has undergone some clarification and possibly anaerobic digestion, is drawn off into the second lagoon or percolation bed where it is allowed to percolate into the ground. Once the solids have accumulated in the primary lagoon until the point after which no further clarification occurs, the lagoon is drained and the solids are allowed to dry. The dried solids are then removed, sometimes further dewatered, and disposed of at a landfill or buried (33–35).

Aeration may be applied to supplement the supply of oxygen to the system and for mixing. Lagoons may be lined with various impervious materials such as rubber, plastic, or clay as required by geological conditions. Where groundwater quality is of concern, the effluent from

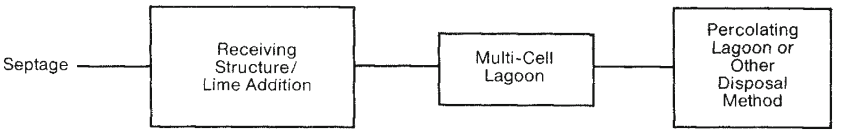


Fig. 18.13. Flow diagram of septage disposal by lagoons (23).

septage lagoons can be applied to the land or treated and discharged to surface water, rather than using percolation beds.

The pH of the lagoon must be maintained at 8.0 or greater to control odors. This may be accomplished with the use of hydrated lime added each time a truckload is discharged to the receiving chamber. Lagoon effluent can be disposed of by applying spray irrigation or overland flow. If the effluent is to be discharged to a surface water, it should be further treated using either polishing ponds or sand filters, and disinfected as required.

In very cold climates, reduced biological activity occurs and ice may form on the surface. Overloading may create potential odor problems. Potential exists for groundwater contamination with percolation beds and seepage pits or lagoons. Hence, extensive site evaluation is recommended and groundwater should be monitored near the lagoon site. Odor and vector problems are possible in immediate vicinity of lagoons.

Settled solids from primary lagoon have to be removed and properly disposed of periodically, every few months to once every 5 or 10 years depending on size of lagoon.

The recommended design criteria for lagoons are as follows (23):

1. Detention time: 20–30 days for settling alone; 1–2 year for stabilization (i.e., 80–90% removal of BOD and volatile solids)
2. Area loading rate: 20 lb VS/d/1, 000 ft^2 (facultative sludge lagoon) = 97,648 kg VSS/d/km^2
3. pH: 8.0 using lime
4. Minimum depth: 0.9 m (3 ft) (Plus additional depth for sludge storage and anaerobic zone)
5. Minimum separation distance from high groundwater level: 1.3 m (4 ft)

12.5. Composting

Composting is the stabilization of organic material through the process of aerobic, thermophylic decomposition. It is a disposal technique that offers good bactericidal action and up to 25% reduction in organic carbon. Septage is transformed into a humus-like material that can be used as a soil conditioner.

Composting is classified into three types of operations, which differ principally by the aeration mechanism they employ. They are windrow, aerated static pile, and mechanical composting (2, 35). Although all three methods may be applied for composting septage, the method that appears to offer the greatest potential as a septage treatment alternative is the aerated static pile method because it permits more uniform composting and minimizes land requirements.

Septage is usually first dewatered and then mixed with bulking agents (e.g., woodchips, sawdust, bark chips, leaves, etc.) prior to composting to decrease the moisture content of the mixture, increase the porosity of the septage, and assure aerobic conditions during

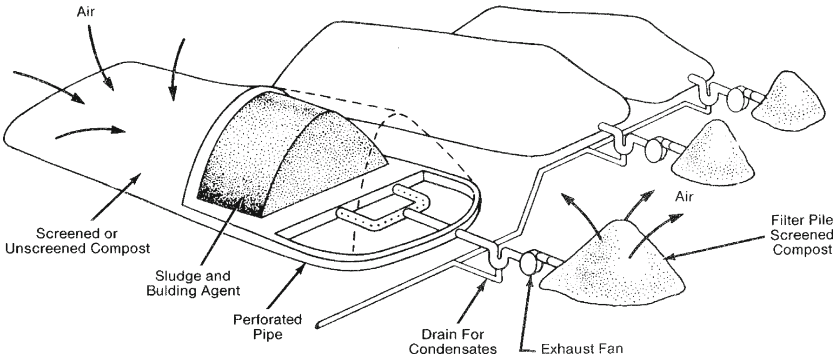


Fig. 18.14. Aerated static piles for septage composting (23).

composting. The mixture is then constructed into a pile as shown in Fig. 18.14. A blanket of finished compost completely surrounds the composting mixture in order to reduce heat loss and minimize odors.

The aerated pile undergoes decomposition by thermophylic organisms, whose activity generates a concomitant elevation in temperature to 60°C (140°F) or more. Aerated conditions in the pile are maintained by drawing air through the pile at a predetermined rate. Exhaust air is forced through a small pile of screened finished compost for odor control. The composting period normally lasts 3 weeks (36–43).

Following the composting period, the aerated piles are taken down, moved, and stored in piles for 4 or more weeks to assure no offensive odors remain and to complete stabilization. The composted material can be separated from the bulking agent, which is generally recycled for further usage. The finished compost material is then ready for utilization as a low-grade organic fertilizer, soil conditioner, or for land reclamation.

Windrow and mechanical composting are commonly used to stabilize wastewater biosolids and can be adapted to treat septage. The Lebo process which is a variation of windrow composting treats raw septage without dewatering, by first aerating the septage in a reactor and then mixing it with sawdust before composting, which takes up to 6 months. The aerated static pile method can also be used to compost raw septage; however, excessive quantities of bulking agent are required to maintain the desired moisture content.

Dewatering of septage is recommended prior to composting to minimize the amount of bulking agent required. However, if large quantities of bulking agent are available at reasonable cost, raw septage can be treated.

In areas of significant rainfall, it may be necessary to provide a cover for the pile. A drainage and collection system is generally required to control storm water runoff and leachate from the pile.

Composting represents the combined activity of succession of mixed populations of bacteria, *actinomycetes* and other fungi. The principle factors that affect the biology of composting

are moisture, temperature, pH, nutrient concentration, and availability and concentration of oxygen. A summary of pertinent design parameters follows (23, 36–42):

1. Moisture content: 40–60%
2. Oxygen: 5–15%
3. Temperature peak: 55–65°C (130°–150°F)
4. pH: 5–8
5. C/N ratio: 20:1–30:1
6. Land requirement: 0.2–0.3 acre/dry ton septage solids/d (0.09–0.13 ha/dry metric ton/d)
7. Blower size: 1/4 kW (1/3 HP)
8. Septage pile dimensions:
   - 2.7 m (9 ft) high
   - 4.6 m (15 ft) diameter
   - 0.3 m (1 ft) base
   - 0.5 m (1.5 ft) blanket
   - 0.75 m^3 (1 yd^3) filter pile

12.6. Odor Control

Soil filters provide breakdown of malodorous compounds by both chemical and biological means. This is accomplished by collection and forcing air from contained process units through networks of perforated pipes buried in the soil, or through a mixture of iron oxide and woodchips (see Fig. 18.15).

Common modifications include: use of compost rather than soil as filter media; above ground, enclosed filters for smaller volumes of gas; use of rooted vegetation to maintain loose soil and enhanced biological activity. Alternative odor control methods include exhaust gas scrubbing in aeration basins and incineration in biosolids combustion units. Chemical scrubbers and activated carbon filters have also been used with mixed success.

Odorous gases are contained and vented to the soil filter area via a piping network. Given sustained biological activity, filters may regenerate during periods when no gases are passing through. Pilot- and full-scale studies have demonstrated complete elimination of odors by use

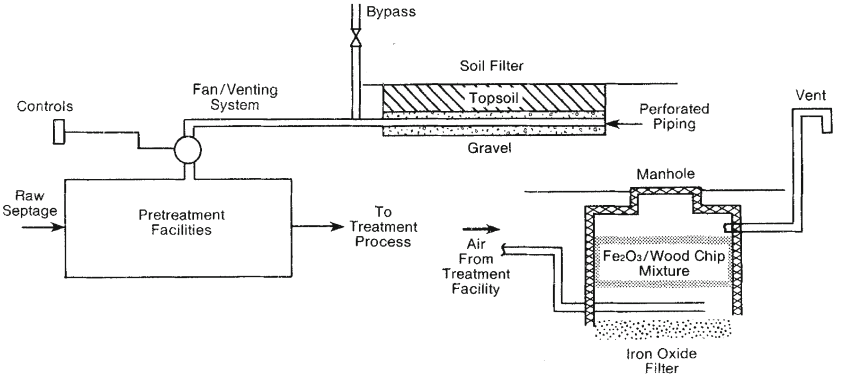


Fig. 18.15. Septage odor control (23).

of soil filters (i.e., no detectable odors in vicinity of soil filter). Gases with H_2S concentrations greater than 100 mg/L have been deodorized ($H_2S < 1$ mg/L) by this method (44–47).

The recommended design criteria for odor control are as follows (23, 44, 45):

1. Minimum soil depth: 0.5 m (20 in.)
2. Air loading rate: 60 $m^3/m^2/h$ (200 $ft^3/ft^2/h$) for soil filters
3. Detention time: not less than 30 s at peak air flow
4. Soil type: moist loam, sandy loam, compost
5. Soil temperature: above 3°C (38°F)
6. Soil Moisture: sprinkling may be required in dry summer periods; proper drainage must be provided to prevent saturation of the soil.

13. TREATMENT OF SEPTAGE AT BIOLOGICAL WASTEWATER TREATMENT PLANTS

13.1. Treating Septage as a Wastewater or as a Sludge

Biological wastewater treatment plants (BWWTPs) are one of the most frequent acceptors of septage due to their number and location, and they must be included in any comprehensive study of alternate septage treatment schemes (26, 35, 47). Septage can be disposed of in a biological wastewater treatment facility by adding it to the liquid stream or the sludge stream. In either case, a properly designed septage handling facility, including screening, degritting, and equalization, is recommended (25, 52–54).

Septage may be considered a high-strength wastewater and be dumped into an upstream sewer or placed directly into various unit processes in a BWWTP shown in Fig. 18.16 (54). At several BWWTPs, septage is considered a sludge because it is the product of an anaerobic settling/digestion septic tank, and it has approximately the same TS concentration as raw municipal sludge. The septage application points, if treated as a sludge, may include sludge stabilization, sand bed drying, or a mechanical dewatering process. The decision of where to apply the septage in a BWTP should be determined after a statistically significant sampling and analysis of a locale's septage, including: (a) solids loading; (b) oxygen demand; (c) toxic substances; (d) foaming potential; and (e) nutrient loading (N and P), where required.

The above factors, combined with a BWWTPs layout, design capacity, present loading, and other operational conditions, provide the design and operation professional with sufficient information for a reasonable septage treatment scheme for a BWWTP.

When septage is added to an upstream sewer or discharged at a BWWTP, there should be a suitable hauler truck discharge facility. It should include a hard-surfaced ramp that leads to an inlet port and is able to accept a quick-disconnect coupling directly attached to the truck's outlet. This significantly reduces odor problems. Wash-down water should also be provided for the hauler so that spills can be cleaned up (55–57).

13.2. Pretreatment of Septage at a Biological Wastewater Treatment Plant

BWWTPs handling septage have experienced better operation when septage is pretreated. Pretreatment generally includes screening, using bar screens with 3/4- to 1-in. (1.91–2.54 cm) openings; grit removal; and pre-aeration or prechlorination if the BWWTP adopts an aerobic

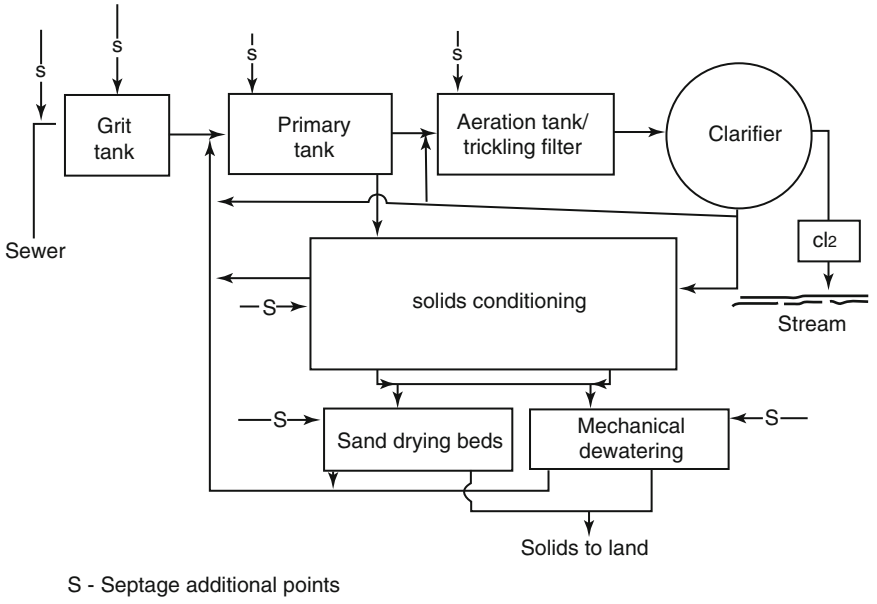


Fig. 18.16. Septage addition points in biological wastewater treatment plants (*Source:* US EPA; Ref. 54).

process. Grit removal by cyclone classifiers has been done successfully in Long Island, New York. Usually, separation of inorganic matter larger than 150 mesh is needed. Equalization/storage tanks with 2 days' average septage flow and mixing capability should also be provided. To further attenuate odors, enclosing the storage tanks and oxidation (using ozone or potassium permanganate) in tank vent lines may be considered. Pumping equipment should be used to apply a continuous dose of septage into the desired unit. Operators report slug or intermittent doses of septage are difficult to treat and may seriously upset biological treatment systems.

13.3. Primary Treatment of Septage at a Biological Wastewater Treatment Plant

In accordance with the US Environmental Protection Agency (US EPA), neither natural settling nor adding lime or polyelectrolyte resulted in consistent liquid-solids septage separation. Raw septage is relatively nonsettleable, as determined by a settleable-solids volume test, from 0 to 90% with 24.7% as the average volume. Poor settling characteristics generally may be expected from septage and that it may be divided into three types: (a) Type 1 is from septic tanks pumped before pumping is needed, settles well, and represents 25% of the samples; (b) Type 2 is from normally operating septic tank systems, shows intermediate settling characteristics, and represents 50% of the samples; and (c) Type 3 exhibits poor settling, represents 25% of the samples, and is generally from septic tanks overdue for pumping. All samples discussed in the earlier section are between 1 and 6 years old.

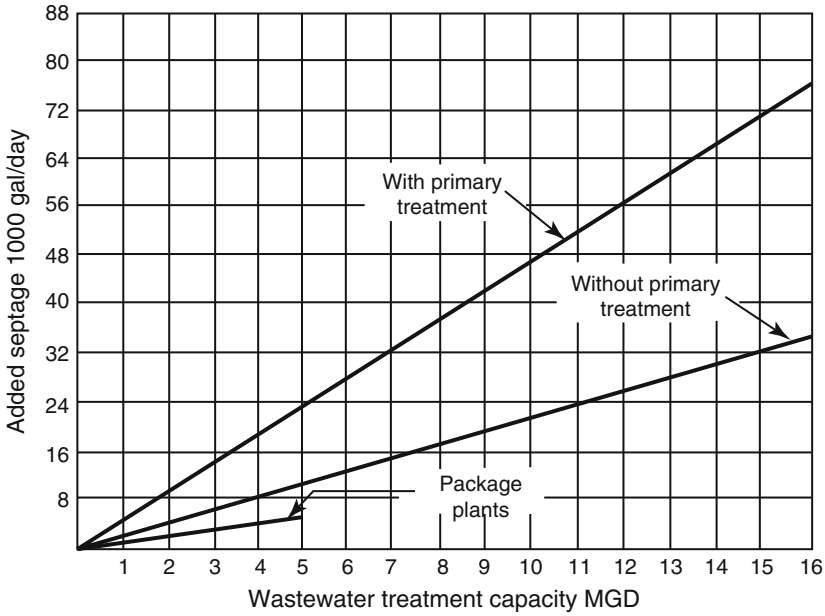


Fig. 18.17. Septage additions to activated sludge wastewater treatment plants (no equalization facilities) (*Source:* US EPA; Ref. 54). (Conversion factors: 1 gal/day = 3.785 L/day; 1 MGD = 43.8 L/s)

Elutriation, in terms of the settling of septage in a septage-sewage mixture, yields better settling results (53). It is expected that up to 75% of septage SS can settle in a BWWTPs primary sedimentation basins. A US EPA study found 55–65% SS removals in a primary clarifier, but only 15–25% BOD removals (54).

13.4. Secondary Treatment by Biological Suspended-Growth Systems

Septage may be added to a biological suspended-growth system, such as the activated sludge process, if additional aeration capacity is available, the plant is organically and hydraulically loaded below design capacity, the septage metals content can be diluted sufficiently, and foaming potential is low or controllable. Very limited quantities of septage may be added without changing the sludge-wasting rates. For instance, about 400 gal/d (1,514 L/d) slug dumps can be handled without significant upset at a 0.5 million gallons per day (21.9 L/s) activated sludge plant flowing at 40% capacity. Figures 18.17–18.18 recommend various levels of septage addition for several kinds of activated sludge plants (54).

The use of slug dumping of septage may depend on limiting the increase in mixed-liquor suspended solids (MLSS) to 10% per day to maintain a relatively stable sludge, as shown in Fig. 18.17. Higher loadings and wasting rates than the resident aquatic biomass is acclimated which may result in a poor-settling sludge. Severe temporary changes in loading beyond the 10–15% MLSS increase may cause a total loss of the system's biomass (55).

Package treatment plants should not accept septage for slug dumping if their design capacity is less than 100,000 gal/d (4.38 L/s). In a study for the U.S. Forest Service, CH2M/Hill

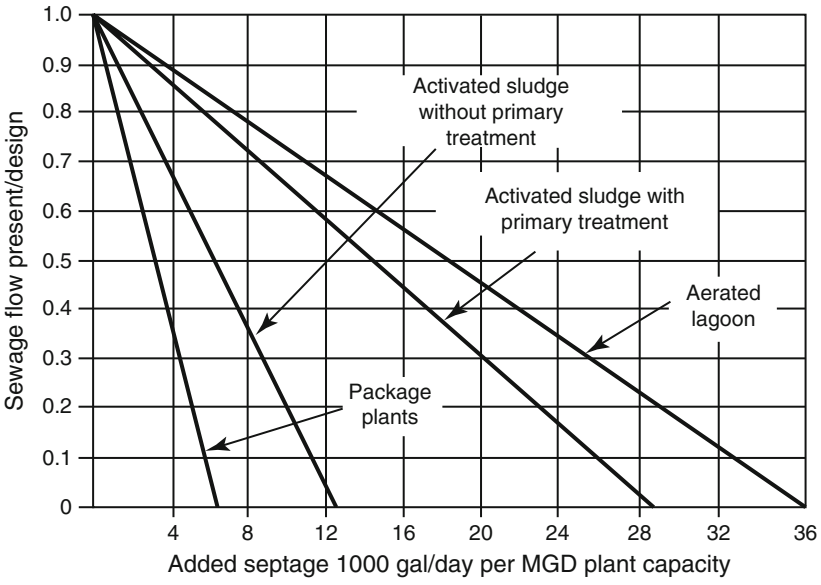


Fig. 18.18. Septage addition to biological wastewater treatment plants (with equalization facilities) (*Source:* US EPA; Ref. 54). (Conversion factors: 1 gal/day = 3.785 L/day; 1 MGD = 43.8 L/s)

determined that package treatment plants can treat septage at approximately 0.1% of the plant design capacity, whereas modified activated sludge can treat septage at twice the rate of a package plant. Conventional activated sludge plants can treat septage at about four times the rate of package plants (55).

In plants with holding and metering facilities, septage may be bled into the waste-flow stream at considerably greater rates than would be allowable if only slug-dumping procedures were available.

A research was conducted to feed septage at a controlled rate of 2–13% of the total influent flow to one of two activated sludge units (54). With a control unit food-to-microorganism (F/M) ratio of 0.4 and a septage-sewage unit F/M of 0.8, effluent BOD and SS characteristics were similar. Effluent COD of the unit receiving septage increased when septage was loaded at 10–13% of plant flow.

Figure 18.18 is based on research reported in the literature and field investigations (54). Again, it demonstrates that package plants with design capacities under 100,000 gal/d (4.38 L/s) should not accept septage. Depending on the present plant flow compared with the design plant flow, a biological treatment reserve can be estimated that will allow for a certain level of septage to be adequately treated. Under identical loading conditions, the ratio of septage addition to various kinds of treatment plants would be similar to what Table 18.5 reports.

Figure 18.18 represents continuous septage addition to an activated sludge process facility for a fully acclimated biomass. It is recommended that an initial septage feed to an unacclimated system should be substantially less than shown, that is, on the order of 10% of the graph

Table 18.5
The ratio of septage addition to various kinds of biological wastewater treatment plants under identical loading conditions. (Source: US EPA; Ref. 54)

Treatment plants	Relative volumes of septage addition
Package plants	1.00
Activated sludge (no primary treatment)	2.08
Activated sludge (conventional)	4.83
Aerated lagoons	6.00

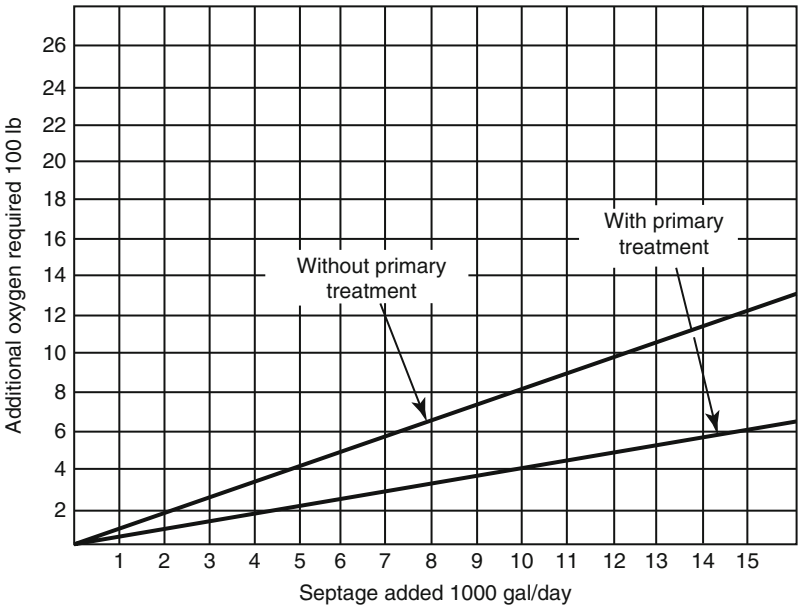


Fig. 18.19. Additional oxygen required for septage additions in activated sludge wastewater treatment plants. (*Source:* US EPA; Ref. 54). (Conversion factors: 1 lb = 0.4536 kg; 1 gal/day = 3.785 L/day)

values. Further gradual increases in daily septage loading should be made over a 2- to 3-week period up to the maximum amount shown in Fig. 18.18. Oxygen capacity must be checked continuously and gradual changes made in sludge age.

Figure 18.19 shows the additional oxygen requirements when septage is added in activated sludge treatment plants. Treatment facilities should be analyzed to determine if oxygen requirements or mixing requirements are controling factors (55).

Because septage has higher oxygen demands than raw sewage, an additional oxygen supply for activated sludge plants that accept septage having primary treatment would be 40 pounds (18.14 kg) of oxygen per 1,000 gallons (3,785 L) of septage added. For plants without primary treatment, an additional 80 pounds (36.28 kg) of oxygen per 1,000 gallons (3,785 L) of septage

added should be provided. Package treatment plants have an oxygen requirement similar to conventional activated sludge plants without primary treatment.

Higher sludge ages (such as, 10 days vs. 4 days) may result in higher percentage BOD removal and less sludge production than do lower sludge ages. Wasting must be adjusted gradually with increased loads to obtain a sludge age that produces the optimum balance between aeration tank efficiency and good settling characteristics. In general, a high sludge age produces a light sludge with poor settling ability but good substrate removal characteristics. The reverse is often true for a very young sludge.

Some odor and foaming problems may occur in aeration systems; however, the odor usually will dissipate within 6–24 h, and the degree of foaming problem depends on the surface active substances present in the septage. Commercial defoaming agents and aeration-tank spray water have been used to reduce foaming.

13.5. Secondary Treatment by Biological Attached-Growth Systems

Systems that use attached growth aerobic treatment processes, such as trickling filters and rotating biological contactors, are usually more resistant to upsets from changes in organic or hydraulic loadings and are suitable for septage treatment.

In trickling filters, additional recirculation has been shown to adequately dilute septage concentrations and diminish chances of plugging the media. At Huntington, Long Island, New York, 30,000 gal/d (113,550 L/d) septage is treated at a 1.9 million gallons per day (MGD) or 7.19 MLD facility. BOD_5 reductions of 85–90% have been observed concurrent with SS reductions of 85% (54).

Rotating biological contactors (RBC) use a long detention time and a continually rotating biological medium that is reportedly resistant to upsets. At Ridge, Long Island, New York, a BOD_5 reduction of 90%, a COD reduction of 67%, and a total suspended solids reduction of 70% were reported. Flow equalization of a low-strength septage and a surface loading of 2 $gal/d/ft^2$ (0.0816 $m^3/d/m^2$) produced these results.

13.6. Septage Treatment by Aerobic Digestion

An alternative to considering septage as a concentrated wastewater is to assume that it is the product of an unheated digester (septic tank) and, therefore, a sludge. Many researchers have reported good results in aerobic digestion of septage or septage-sludge mixtures. The time needed to produce an odor-free sludge varies up to 7 days.

Tilsworth (56) reported a high degree of septage biodegradability at a 10-day aeration time, resulting in a BOD reduction of 80% and a VSS reduction of 41%. It was reported by the US EPA that (a) treating anaerobically digested septage with an aerobic digester achieved 36% VS removal at 40 days aeration under a loading of 0.0016 pound VS per cubic foot per day (0.0256 $kg/m^3/d$); (b) Orange County, Florida, added septage to aerobic digesters at the rate of 5% of total sludge flow and obtained good reductions at a loading of 0.15 pound VS per cubic foot per day (2.4 $kg/m^3/d$); (c) Bend, Oregon obtained good removal by adding 13% septage to 87% sludge at a loading of 0.02 pound VS per cubic foot per day (0.32 $kg/m^3/d$), with 15 to 18 days aeration time (54).

Before adding septage to the aerobic digestion process, aeration capacity, toxic metal or chemical accumulations, and increased solids to be disposed of should be investigated. Investigators consistently have reported initial repulsive odors and foaming problems.

When considering septage addition to aerobic digesters, recommendations should include screening, degritting, flow equalization, and analyses of excess digestion capacity and peripheral effects on other processes such as solids handling. An initial septage addition should be limited to approximately 5% of the existing sludge flow. Further septage additions should be gradual.

13.7. Septage Treatment by Anaerobic Digestion

Septage should be screened, degritted, and equalized before it is added to single-stage digesters. Digesters should be cleaned on a regular schedule, such as every 2–3 years, or as required.

Monitoring digester performance includes long-term evaluation of volatile acid/alkalinity ratios and gas production. Mixing is vital to preventing a sour digester from developing point-source failure from a septage load containing high volatile acid concentrations.

In systems with multiple tanks, all the preceding suggestions should be followed. Spreading the septage among a number of digesters reduces septage concentrations. Recycling material from the bottom of a secondary digester or from another well-buffered primary digester at a rate of up to 50% of the raw feed per day has been found helpful. Temperature and mixing should also be adjusted for maximum performance.

A maximum addition of 2,130 gal/d (0.093 L/s) of septage to each 14,500 gal/d (0.6352 L/s) of sewage sludge per million gallons (3.785 ML) of digester capacity, with a detention time of 30 days and a loading of 0.08 pound VSS per cubic foot per day (1.28 $kg/m^3/d$) is generally recommended. Good operation of anaerobic digesters requires that toxic materials be limited.

Septage in Tallahassee, Florida, is treated in an unheated (200°C to 30°C) anaerobic digester. With an influent septage concentration of 17,700 mg/L TS, a VS reduction of 56% was reported after an 82-day retention time at a loading of 0.01 pound VSS per cubic foot per day (0.16 $kg/m^3/d$). Large quantities of grit in the septage required draining and cleaning of the open digester after only 3 years operation.

Leseman and Swanson (59) analyzed volatile acid distribution concentrations in the digester contents. The volatile acid-to-alkalinity ratio varied from 0.34 to 0.83. The 8-month volatile acid concentration averaged 703 mg/L and ranged from 408 to 1,117 mg/L at a consistent pH of 6.0. The progression of volatile acid concentrations in the digester, from two to five carbon acids, showed acetic as 276 mg/L, propionic as 294 mg/L, isobutyric as 14 mg/L, butyric as 49 mg/L, isovaleric as 28 mg/L, and valeric as 42 mg/L. The digester had an open cover, so gas production could not be monitored. Supernatant from this digester is pumped to the sewage sludge anaerobic digester.

Chuang (60) reported a 92% VS removal from a heated anaerobic digester loaded at 0.08 pound VSS per cubic foot per day (1.28 $kg/m^3/d$) with a 15-day hydraulic retention time. Incoming solids ranged from 0.3% to 8%, and TS reduction was more than 93%. BOD reductions averaged 75%, from 6,100 mg/L influent to 1,500 mg/L effluent.

13.8. Septage Treatment by Mechanical Dewatering

Long Island, New York uses a vacuum filter to dewater 100,000 gal/d (378,500 L/d) of chemically conditioned septage. A design basis of 6 pounds per hour per square foot (29.29 kg/h/m^2) of surface area is used and appears to be satisfactory. Adding lime at a rate of about 190 lb/ton (95 kg/metric ton) of dry solids and 50 gal/ton (208.6 L/metric ton) of dry solids standard concentration ferric chloride solution are added before vacuum filtering (54).

In a study at Clarkson College, Crowe (61) had successful results with vacuum filtration of mixtures of raw septage and digested sludge with up to 20% raw septage by volume. Chemical preconditioning with lime, ferric chloride, and polymers was required at doses typical of domestic sludge. He observed dewatering characteristics similar to those of mixtures without septage. The filtrate contained only 5–10% of the raw septage COD.

13.9. Septage Treatment by Sand Drying Beds

Sand drying has been used to dewater septage with varying success. Anaerobically digested septage is reported to require two to three times the drying period of digested sludge (62). After treatment in aerated lagoons and batch aerobic digesters, dewatering simulation studies yielded a septage capillary suction time (CST) in the order of 200 s vs. about 70 s for sewage treatment plant sludge. A lower CST can be correlated to a faster dewatering time. The CSTs of raw septage were found to range from 120 to 825 s; the mean was 450 s.

Adding lime to septage before sand bed dewatering has vastly improved dewatering characteristics. Feige (63) found that adding 180 pounds of lime per ton (90 kg/metric ton) dry solids, or 30 pounds per 1,000 gallons (3.6 kg/1000 L) of septage based on 40,000 mg/L TS, raised the pH to 11.5 and dried to 25% solids in 6 days and 38% solids in 19 days. An application depth of greater than 8 in. is not recommended because it slowed the drying process. The filtrate analysis showed that most heavy metals were tied up in the solids, fecal coliforms were killed effectively, fecal streptococci were more resistant than fecal coliforms, and odors were significantly reduced. Filtrate quality was generally good, but further treatment before discharge was recommended.

Perrin (64) found other chemicals worked well in modifying the ability of septage to dewater. From a mean initial CST of 450 s, septage showed a dewatering ability of 50 s after adding an average of either 1,360 mg/L ferric chloride, 1,260 mg/L alum, 1,360 mg/L Purifloc C-31, or 2,480 mg/L Purifloc C-41. The effects of freezing on dewatered samples of septage after treatment in aerated lagoons or batch aerobic digesters was also studied. Freezing lowered the CST from 225 s to 42 s, an 80% decrease in dewatering time.

If septage is to be placed on sand drying beds, treatment to a consistent CST range of 50–70 s is recommended. Further treatment of under-drainage would be required in most cases.

13.10. Costs of Septage Treatment at Biological Wastewater Treatment Plants

Of all the alternatives investigated, land disposal was reported to have the lowest operation and maintenance costs, from $3.0 to $12.00 per 1,000 gallons (2009 Cost), exclusive of the cost of the land. Lagoon treatment is reported to cost between $10.00 and $25.00 per 1,000 gallons. The cost of septage treatment in BWWTPs varies widely, but typically runs about

$35.00 per 1,000 gallons (2009 Cost). Composting process is reported to cost approximately the same as disposal in BWWTPs. Physical chemical treatments, such as the Purifax Process and chemical stabilization (52, 57, 58), range from average costs similar to those found in disposal at treatment plants to double or triple that figure. Here 1,000 gallons = 3,785 liters.

Many variables affect treatment costs, including local funding requirements; eligibility for State or Federal funds; necessity for industrial cost recovery formats; local taxes assessed in lieu of, or to offset, treatment plant expenses; level of pollutant removal capacity; climate; present loading vs. design plant capacity; and cost of land. It is easy to understand, therefore, the broad range of charges for treatment plant septage disposal.

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Environmental Control of Biotechnology Industry

Lawrence K. Wang, Nazih K. Shammas, and Ping Wang

CONTENTS

Abstract Basic principles of drug development, gene sequencing and bioinformatics, application of biotechnology information to medicine, application of biotechnology information to nonmedical markets, and the regulatory environment altogether with industrial classification of biotechnology industry's pharmaceutical manufacturing and biotechnology industry's pharmaceutical SIC subcategory under US EPA's guidelines are considered in this chapter. Manufacturing processes and waste generation in fermentation, biological product extraction, chemical synthesis, formulation/mixing/compounding, research and development, waste characterization, and options for waste disposal are described. Essential part of the chapter is information on regulations for direct and indirect discharge of wastes and strategy of waste management. Case studies on factory profile, raw materials and production process, waste generation and characteristics, and end-of-pipe treatment are described in this chapter.

Key Words Drug development • gene sequencing • bioinformatics • regulatory environment • pharmaceutical manufacturing • waste generation • fermentation • biological product extraction • chemical synthesis • waste characterization • discharge of wastes • waste management • waste generation • end-of-pipe treatment.

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1. INTRODUCTION TO BIOTECHNOLOGY

The biotechnology industry is still young, especially compared with the automotive, chemical, and steel industries. Despite its comparative youth, it is becoming an important influence on many other industry segments, as well as developing an impressive presence of its own. Its technology base continues to grow dynamically and is melding medical science with information technology in new and exciting ways. While its relationship with capital markets has sometimes been stormy, that relationship now appears to be settling into maturity as its medically oriented companies bring growing numbers of new products to market.

The growth of the biotechnology industry is a unique story and yet it rests on foundations common to other segments of industry. Years of research, both government funded and privately funded, continue to provide an ever expanding knowledge base. The capital market provides the ability to transform this knowledge into unique products and processes for markets around the world. While there is inevitable tension between the industry's desire to bring new products to market and the concerns of the industry's regulators, both sides have found new and innovative ways to work together.

Perhaps unique among industries, biotechnology is not defined by its products but by the technologies used to make those products (1). Biotechnology refers to a set of enabling technologies used by a broad array of companies in their research, development, and manufacturing activities. To date, these technologies have been used primarily by the pharmaceutical industry, but they are being used increasingly by a variety of other industries, such as agriculture, mining, and waste treatment. Various US government publications have defined biotechnology as a set of techniques that use organisms or their cellular, subcellular, or molecular components to make products or modify plants, animals, and microorganisms to carry desired traits (1). This broad definition includes methods of treating disease developed from recent research in molecular biology and other fields, as well as the centuries-old practices of animal and plant breeding and the use of microorganisms to make leavened bread and fermented beverages.

Advances in molecular biology over the past 25 years have led to the development of genetic engineering, monoclonal antibody technologies, DNA amplification, protein engineering, tissue engineering, and other methodologies with applications in the medical arena. These new techniques have enabled researchers to modify the genetic and biochemical makeup of organisms with far greater precision and speed.

In the roughly 25 years since the development of recombinant DNA technologies in research laboratories, more than 2,000 firms have been founded in the USA alone to explore and to take advantage of these new technologies (2). Approximately 30 new products have reached the medical market, and several hundred more are in human clinical trials. The market for such products has grown dramatically from $7.6 billion in 1996 to $24 billion in 2005. Similarly, the market for agricultural biotech products has increased from $295 million to $1.74 billion in the same period. Applications of the products will lead to enhanced pest resistance in food crops, improved methods of food preservation, and other advances. Table 19.1 shows the distribution of research activities and biotechnology firms in the USA.

Table 19.1
Leading biotechnology states in the USA (3)

Rank	State	Number of companies
1	California	267
2	Massachusetts	130
3	New Jersey	80
4	North Carolina	71
5	Maryland	70
6	Pennsylvania	58
7	Wisconsin	56
8	New York	55
9	Texas	50
10	Washington	40

It is clear that California and Massachusetts are the top leading biotechnology states followed by New Jersey, North Carolina, and Maryland (3, 4).

The biotechnology industry serves both medical and nonmedical markets. The medical market includes human therapeutics and human diagnostics as well as applications in veterinary medicine. Nonmedical markets encompass both agriculture and industrial applications. Agricultural applications include making plants and crops pest resistant, providing improved seed quality, modulating growth and ripening times, enhancing nutrient content of foods, and providing simple and inexpensive diagnostics for use in field testing for contaminants and toxic materials. Industrial uses of biotechnology involve many different sectors and include industrial enzymes, waste management, bioremediation, energy biomass, cosmetic formulations, and diagnostics for toxicity determinations. Tables 19.2 and 19.3 show the distribution of biotechnology firms among the various medical and nonmedical markets by primary focus and in all areas, respectively (3, 4). It is obvious that the pharmaceutical industry is by far the predominant and largest area of biotechnology.

1.1. Core Technologies

The core technique of biotechnology is elegant in its simplicity. The cell is a miniature factory, containing genetic material – DNA – that acts as a blueprint for its structure and function. Biotechnology allows researchers to isolate, copy, and rearrange this genetic blueprint at the molecular level to manipulate the quantity, structure, and function of the biomolecules that control cellular processes. As a result, researchers are expanding their abilities to identify, isolate, and modify those molecular agents.

Discoveries concerning the molecular bases of cellular processes will have a wide range of applications. For example, in the area of health, these mechanisms may lead to therapies that fight disease by regulating specific cellular processes. With the help of molecular biology, biochemistry, and biophysics, the search for molecular information is yielding an increasingly detailed guide to cell behavior and its disruption. This knowledge allows biotechnologists to develop new products, processes, and therapies of commercial interest.

Table 19.2
Participation of biotechnology companies by primary focus (3)

Market area	Number of companies	Percentage of all companies
Therapeutics	315	29.4
Diagnostics	187	17.4
Reagents	84	7.8
Plant agriculture	68	6.3
Specialty chemicals	54	5.0
Immunological products	36	3.4
Environmental testing/treatment	35	3.3
Testing/analytical services	32	3.0
Animal agriculture	29	2.7
Biotechnology equipment	26	2.4
Veterinary	26	2.4
Drug delivery systems	24	2.2
Vaccines	24	2.2

Table 19.3
Participation of biotechnology companies in all areas (3)

Market area	Number of companies	Percentage of all Companies
Therapeutics	448	41.8
Diagnostics	346	32.3
Reagents	224	20.9
Specialty chemicals	159	14.8
Immunological products	146	13.6
Cell culture products	133	12.4
Fermentation/production	116	10.8
Plant agriculture	106	9.9
Vaccines	105	9.8
Drug delivery systems	94	8.8
Environmental treatment/testing	93	8.7

1.2. Biotechnology Materials

The raw materials of biotechnology are cells and their constituent biomolecules. These materials may be used for a variety of purposes, including drug synthesis, food production, and the bioremediation of hazardous waste. Examples of biotechnology materials include (1):

- *Cytokines.* Hormone-like proteins that stimulate the growth or regulate the function of various cell types. They include such agents as erythropoeitin, which stimulates the production of red blood cells and can be used to treat severe anemia associated with renal disease; granulocyte colony-stimulating factor, which stimulates the production of white blood cells and is used to counter the loss of such cells in patients who have received anticancer therapy; and interferons, which help regulate and target the body's immune response and can be used to treat certain cancers and selected viral infections.

- *Antibodies.* Large protein molecules produced by the immune system that can bind specifically to discrete antigens, foreign substances are recognized and then attacked by the immune system.
- *Enzymes.* Protein catalysts that facilitate specific chemical or metabolic reactions necessary for cell growth and function. Enzymes can be used in such activities as food processing, the bioremediation of hazardous waste, and the synthesis of certain drugs, vitamins, and fine chemicals.
- *Restriction enzymes.* Enzymes that break DNA in specific locations, creating gaps into which new genes can be inserted. These enzymes play a vital role in genetic engineering.
- *Viral vectors.* Modified, nonpathogenic viruses that deliver useful genetic information to host cells in gene therapy and genetic engineering. In gene therapy applications, such viruses are encoded with a specific gene, which, when incorporated into a host cell, confers a clinical benefit to the patient.
- *Antisense oligonucleotides.* Strands of DNA that bind to targeted messenger RNA molecules (which tell cells what proteins to make) and block the synthesis of specific proteins. In therapeutic applications, the synthesis of disease-related proteins is inhibited. These compounds are used in drug development and in agricultural biotechnology.

1.3. Drug Development

The acceleration of the drug discovery process resulting from biotechnology research is contributing to US competitiveness in biotechnology. Many companies emerged in the past decade to become involved in this new approach to drug commercialization. Important areas of drug-related research include the following (1):

- *Rational drug design.* Scientists are using a combination of chemistry, biology, biophysics, and computer modeling to determine the structure of target proteins in molecular detail and to then design specific small-molecule drugs for those target proteins. Companies involved in rational drug design include Agouron, Arris, BioCryst, Chiron, Procept, and Vertex.
- *Natural product screening.* New methods of screening materials extracted from animals and plants offer a rich source of potentially therapeutic compounds. NPS Pharmaceuticals, Magainin, Shaman, and Xenova are among the biotech firms that literally search the air, land, and sea for new drugs.
- *Combinatorial chemistry.* This technology allows chemists to synthesize large, diverse collections of molecules quickly and efficiently and to then identify the most active compound for a given application. Because combinatorial chemistry can identify promising compounds in a fraction of the time required by traditional methods of drug discovery, it can significantly reduce the cost of commercializing new drugs. Companies using such technology include Gilead Sciences, Isis, and Pharmacopeia.

1.4. Gene Sequencing and Bioinformatics

Mutations are alterations in DNA sequence that may be associated with disease-causing genes. Such modified genes, and the proteins for which they encode, represent targets for drug therapy. Genes are sequenced by cutting pieces of DNA into small segments and cloning and copying those segments millions of times over. The order of the nucleotides (subunits of DNA) contained in those segments is then determined. A computer program is used to analyze and correlate the nucleotide sequences of the individual segments to create a map of the entire gene. The genes identified by this computer analysis are then scrutinized as possible drug targets. Rapid advances in the speed and accuracy of sequencing will revolutionize the

discovery of innovative drugs and diagnostics. Companies in the business of gene sequencing include Darwin Molecular, Human Genome Sciences, Mercator Genetics, and Sequana.

1.5. Applications of Biotechnology Information to Medicine

Biotechnology produces information that is used to alter and improve cell behavior. Many biotech companies specialize in finding ways to deliver and apply biotechnology information to cells to aid in identifying, preventing, and treating disease. Representative applications include (1):

- *Diagnostics.* Tests that use biotechnology materials to detect the presence or risk of disease or pollution of a cell or material.
- *Vaccines.* Preparations of whole or significant structural portions of viruses, microbes, plants, or other entities that are intended for active immunological prophylaxis. Companies working in this area may specialize in the route of administration as well as in the disease that the vaccine targets.
- *Gene therapy.* The process of replacing defective genes with healthy genes, either in vivo or ex vivo, to regulate cell replication or the production of proteins. Alternatively, gene function may be modulated by designing and delivering molecules to cells to inhibit or promote gene action.

1.6. Applications of Biotechnology Information to Nonmedical Markets

Biotechnology also offers significant applications in agriculture and industry. Industrial applications include specialty and fine chemicals and bioremediation. Biotechnology materials, specialized software packages, and equipment used in drug development and production are also important adjuncts to the core biotechnology markets.

In nonmedical areas, there are a number of potentially important developments under way. Genetic modification of food crops, increasing protein content or salt resistance, may help to reduce world hunger. In addition, biotechnology has the potential to shift the world's fish supply from an uncertain and threatened wild food source to an agricultural analog cultivated through mariculture and fresh water aquaculture. The exploration, study, and harvesting of marine genetic resources through biotechnology are expected to produce important commercial applications, including improved diagnostics and pharmaceuticals, increased production of ocean foods, novel energy sources, and the engineering of microorganisms to control and eliminate environmental contaminants.

1.7. The Regulatory Environment

Regulation has been and will continue to be a major factor influencing the development of the biotechnology industry and its international competitiveness, especially for products made from recombinant DNA technology. Health, safety, and environmental regulations are of critical importance, affecting the cost and time needed to get biotech products to market and the profits thereafter. At the same time, other federal regulations, such as those relating to the cleanup of waste sites and to air and water quality generally, can play an important role in the development of the markets served by the bioremediation portion of the biotech industry.

The US Environmental Protection Agency's (US EPA's) effect on the domestic industry is complex. On one hand, it has regulatory authorities that it intends to use to regulate aspects of

the industry's activities and that industry fears may result in new regulatory burdens. On the other hand, US EPA's responsibilities for overseeing the cleanup of polluted sites give it the power to create important new markets for the industry.

US EPA's broad responsibilities for the cleanup of hazardous waste sites under the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) and the Resource Conservation and Recovery Act (RCRA) give rise to important market opportunities for companies offering bioremediation technologies and services, but industry has pointed to several aspects of these activities that may discourage use of bioremediation technologies. US EPA has initiated proceedings to reexamine its approaches to its cleanup responsibilities, and many within the biotechnology industry hope this will create more opportunities for bioremediation technologies in both the RCRA and Superfund programs.

2. GENERAL INDUSTRIAL DESCRIPTION AND CLASSIFICATION

2.1. Industrial Classification of Biotechnology Industry's Pharmaceutical Manufacturing

The pharmaceutical industry is the biggest and most important biotech industry. This industry produces substances that are of value for humans and other living beings. According to the census by the U.S. Department of Commerce (U.S. DC), the industry employed about 170,000 people and produced goods which were valued at over 39 billion US dollars in 1987 (5).

The Standard Industrial Classification (SIC) has been developed and revised since the first major version in 1972, with the purpose of promoting the comparability of established data describing various facets of the US economy, such as management, budget, and data on production, sales, and cost for various industries.

While the pharmaceutical industry requires ultrapure water for their manufacturing processes (6), their process effluents contain highly toxic pollutants which must be properly treated before being discharged to a receiving water.

According to the Standard Industrial Classification Manual (7), the products of the pharmaceutical industry are segregated into four categories:

1. Medical chemicals and botanical products
2. Pharmaceutical preparations
3. In-vitro and in-vivo diagnostic substances
4. Biological products, except diagnostic substances

The pharmaceutical industry has steadily grown because of the need to market, develop, and discover a variety of drugs required throughout the world. This growth of the industry has also increased the amount of waste generation and in turn disposal problems. To control effluent discharge and to reduce the impact of waste from the pharmaceutical industry, the US EPA categorized pharmaceutical manufacturing processes according to the SIC standard, and has developed effluent discharge limitation guidelines based on the production activities and wastes from this industry (8–15).

It should be noted that the pharmaceutical SIC in the US EPA's effluent discharge limitation guidelines (8, 9, 11, 13–15) was based on the older versions rather than the 1987 SIC codes

cited above, although the 1987 SIC codes were used for the recent guidelines to pollution prevention in the pharmaceutical industry (15, 16). To follow the effluent discharge limitation guidelines established by the US EPA, the following sections present those SIC codes for the pharmaceutical manufacturing quoted by the US EPA (11–15).

2.2. *Biotechnology Industry's Pharmaceutical SIC Subcategory Under US EPA's Guidelines*

According to the US EPA's effluent discharge guidelines (11–15), pharmaceutical manufacturing includes those plants producing or utilizing the following products, processes, and activities:

1. Biological products
2. Medicinal chemicals and botanical products
3. Pharmaceutical products
4. All fermentation, biological and natural extraction, chemical synthesis, and formulation products which are considered as pharmaceutically active ingredients by the U.S. Food and Drug Administration, but which are not covered by other categories
5. Cosmetic preparations which function as a skin treatment
6. The portion of a product with multiple end uses which is attributable to pharmaceutical manufacturing either as a final pharmaceutical product, component of a pharmaceutical formulation, or pharmaceutical intermediate
7. Pharmaceutical research which includes biological, microbiological, and chemical research, product development, and clinical and pilot plant activities

The pharmaceutical manufacturing under this categorization does not include all the activities producing the substances used in medical purposes such as some medical instruments. Moreover, not all products containing pharmaceutical ingredients belong to pharmaceuticals, such as milk containing vitamin D. To clarify the confusion in the nature of pharmaceutical manufacturing, it is helpful to review the manufacturing which is similar to, but not included in, pharmaceutical manufacturing. The following lists the production or activities specifically excluded from the pharmaceutical manufacturing category (11):

1. Surgical and medical instrument and apparatus
2. Orthopedic, prosthetic, and surgical appliances and supplies
3. Dental equipment and supplies
4. Medical laboratory
5. Dental laboratory
6. Outpatient care facilities
7. Health and allied sources, not elsewhere classified
8. Diagnostic devices not covered under other categories
9. Animal feeds which include pharmaceutically active ingredients such as vitamins and antibiotics
10. Foods and beverages which are fortified with vitamins or other pharmaceutically active ingredients

Note, again, that these SIC codes are cited according to the earlier versions of the Standard Industrial Classification Manual rather than the 1987s version (11, 13).

Because each of the pharmaceutical subcategories is involved in one or more particular processes, it is difficult to make any generalization regarding various effluents discharged

from the pharmaceutical industry. The problem is even more complicated by the fact that pharmaceutical manufacturing uses both inorganic and organic raw materials. To better minimize and treat pharmaceutical wastes, the manufacturing processes must be first fully understood. This chapter will initially discuss the pharmaceutical manufacturing processes and waste generation, then discuss the waste characteristics and their environmental impact, and finally discuss waste minimization and treatment.

3. MANUFACTURING PROCESSES AND WASTE GENERATION

While the preceding section itemizes the pharmaceutical manufacturing under the SIC subcategorization, it is better to generalize the pharmaceutical manufacturing with its main processes and the waste generation, so as better to understand how to control and treat the manufacturing wastes. The five common processes used in the manufacture of pharmaceutical products are:

1. Fermentation (subcategory A)
2. Natural product extraction (subcategory B)
3. Chemical synthesis (subcategory C)
4. Formulation/mixing/compounding (subcategory D)
5. Research and development activities (subcategory E)

These five processes have been the basic pharmaceutical manufacturing processes, although the SIC subcategory codes for the pharmaceutical industry can be revised as stated in the preceding sections. The US EPA's guidelines to the point source category for pharmaceutical manufacturing (40 CFR Part 439) are established based on these five processes and their related wastes (11, 12, 14, 15). These five processes are identified by the US EPA as the subcategories of pharmaceutical manufacturing and will be used throughout this chapter, instead of using the SIC subcategories.

US EPA (13) has reported that subcategory D (formulation/mixing/compounding) is the most prevalent pharmaceutical manufacturing process, and about 80% of the plants in the industry are engaged in this activity. Furthermore, 58% of these plants conduct subcategory D operations only.

Pharmaceutical manufacturing plants generate a variety of wastes during manufacturing, maintenance, and housekeeping operations. While maintenance and housekeeping activities are similar from one plant to the next, actual processes used in pharmaceutical manufacturing vary widely. With this diversity of processes comes a similarly diverse set of waste streams. Typical waste streams include spent fermentation broths, process liquors, solvents, equipment washwaters, spilled materials, off-spec products, and used processing aids (16).

The following subsections discuss those five main manufacturing processes and their associated wastes.

3.1. Fermentation

Although only about 6% of pharmaceutical products and their wastes are generated by fermentation processes, fermentation is considered an important production process for the

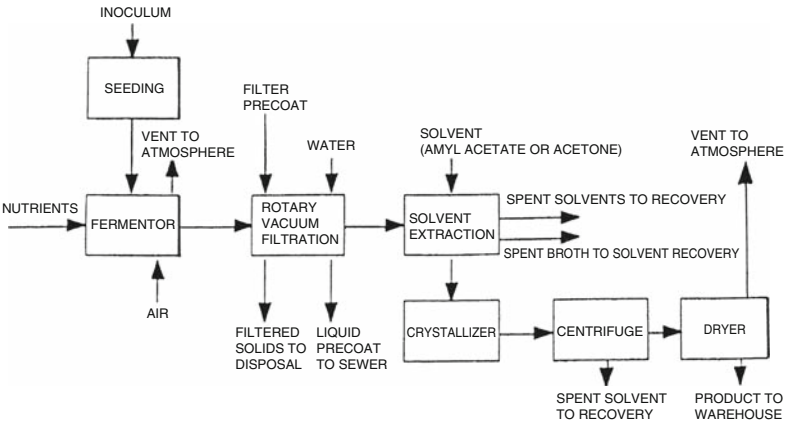

Fig. 19.1. Fermentation process flow diagram (16).

industry (14, 16). Most antibiotics (penicillin, streptomycin), steroids (such as cortisone), and vitamin B12 are produced using fermentation processes.

Fermentation processes consist of three major steps:

1. Inoculum and seed preparation
2. Fermentation
3. Product recovery and purification

Figure 19.1 shows a flow diagram for a fermentation process (16). Sterile inoculum preparation begins with a carefully maintained population of a microbial strain. A few cells from this culture are matured into a dense suspension through a series of test tubes, agar slants, and shaker flasks. The cells are then transferred to a seed tank for further propagation into a culture of sufficient quantity to function as a seed. While tailored to a specific fermentation, the volume of the final seed tank occupies from 1 to 20% of the volume used in full-scale production.

In the fermentation step, the material from the seed tank, along with selected raw materials, is introduced, through a series of sterilized lines and valves, into a sterilized fermentor (batch vessel). Once these sterilized nutrient materials are added to the vessel, fermentation commences. Dissolved oxygen content, pH, temperature, and several other parameters are carefully monitored throughout the fermentation cycle.

Following cell maturation, the fermentor broth from the batch vessel is often filtered to remove the solid residues resulting from the fermentation process; the filtrate is then processed to recover the desired product.

There are three commonly used schemes for product recovery, i.e., solvent extraction, direct precipitation or solvent evaporation, and ion exchange or adsorption (17).

In the solvent extraction process (18), an organic solvent is used to separate a pharmaceutical product from an aqueous filtrate and to form a more concentrated solution. With subsequent extractions, the product is purified, especially from contaminants. Finally, the product

is further recovered, specifically, removed from the solvent, by precipitation or crystallization, or solvent evaporation.

Normally solvents used for product recovery are recovered and reused. However, small portions left in the aqueous phase during the solvent extraction can appear in the plant's wastewater stream. Typical processing solvents used in fermentation operations are methylene chloride, benzene, chloroform, butyl acetate, l,l-dichloroethylene, and l,2-trans-dichloroethylene (11, 12, 15, 16).

In precipitation or evaporation processes, product is recovered directly from a treated broth. In an ion exchange process, a product is removed from a treated broth using ion exchange resin, then proceeded for an additional purification and a final isolation.

The waste characteristics of fermentation processes may vary depending on the production. For example, the antibiotic wastes can generally be divided into four groups (19):

Group A: spent fermentation mash
Group B: wastes containing acids, bases, and solvents (used in the purification of the product)
Group C: condensate from barometric condensers in evaporation and drying
Group D: washing water (used for cleaning equipment and floors)

The waste of Group A has a 5-day biological oxygen demand (5-day BOD or BOD_5) of 4,000–13,000 mg/L (20) if the end product is totally absent from the effluent. For example, in the production of streptomycin, the average 5-day BOD or the spent mash is approximately 2,500 mg/L, and for aureomycin, it is in the range 4,000–7,000 mg/L. When the fermentation does not proceed satisfactorily, a batch of the mash has to be discharged to waste together with the mycelium, which results in the 5-day BOD of the waste rising to 20,000 mg/L or even 30,000 mg/L, while the permanganate value increases to more than 15,000 mg/L. If the mycelium is very carefully separated from the mash, the waste liquors are fairly clear, and the combined content of organic and inorganic suspended solids in a filtered penicillin mash is about 400 mg/L. However, the waste is commonly milky-yellow in color and cannot be clarified easily. The waste directly from the fermentation tanks has a pH of 2–3 units. The pH may rise to 7.5–8.0 units when it is mixed with the effluents from Group D.

The Group B waste consists of the tailings from distillation apparatus used for the recovery of organic solvents. The concentration of these components depends on their solubility in water.

The Group C waste consists of condensates from barometric condensers are only slightly polluted. Those wastes from the manufacturer of aureomycin, however, have a 5-day BOD of 60–120 mg/L.

The Group D wastewater from washing of floor and equipment is similar to that of the waste in Group A, with 5-day BOD from 500 to 1,500 mg/L. But in penicillin production, the washing waste water contains alkaline, due to the use of basic substances for removing unwanted matter from equipment tanks and fermentators.

The fermentation process generates a large volume of waste such as the spent aqueous fermentation medium and solid cell, debris. The aqueous medium is very impure, containing unconsumed raw materials such as corn steep liquor, fish meal, and molasses. Filtration processes result in large quantities of solids in the form of spent filter cake including solid

remains of the cells, filter aid, and some residual product. After product recovery, spent filtrate is discharged as waste water (known as the "spent beers"), which contributes the most significant waste load in the fermentation process. That is, this filtrate still contains a large amount of organic material, protein, and other nutrients. Some wastewater may also come from the use of wash water and gas and dust scrubbers. While solvent extraction contributes relatively small amounts of organic solvents, direct precipitation results in increased metallic ion (particularly copper and zinc) concentration.

In general, the wastewaters from fermentation operations typically have high 5-day BOD, COD (chemical oxygen demand), and TSS (total suspended solids) levels with a pH value in the range of 4–8 units (11, 12).

Sometimes a fermentation batch can be infested with a phage, a virus that attacks microorganism (13). In such a case, very large wastewater discharges may be necessary in a short period of time, which causes a higher nutrient and 5-day BOD concentration than that of the spent broth during normal production. Some fermentation plants use heavy-metal-bearing chemicals as biocides (such as organomercury) which will introduce heavy metal contamination.

Volatile solvents used in product recovery operations may release vapors to the air. Some factories may generate acid and solvent vapors such as methanol and butyl acetate, causing air emission problems.

3.2. Biological Product Extraction

Biological product extraction is the production of pharmaceuticals from natural biological material sources such as roots, leaves, animal glands, and fungi. Such pharmaceutical, which typically exhibit unique pharmacological properties, includes allergy relief medicines, insulin, morphine, alkaloids, and papaverine (16). Despite their diversity, all extractive pharmaceuticals have a common characteristic: they are too complex to synthesize commercially.

The extraction process requires very large volumes of specialized plant or animal matter to produce very small quantities of products. In other words, these extraction techniques basically consist of methods to concentrate particular compounds from either plant or animal tissue (21).

The extraction process consists of a series of subsequent extraction operations. In almost every step, the volume of material can greatly diminish. To that end the volume on the final product may be less than one-thousandth of the initial volume. Therefore, another characteristic of natural product extraction is that the amount of finished drug product is small compared with the amount of source material used. Because of these volume reductions, conventional batch method and continuous processing method are not suitable for biological product extraction operations (11, 13). Therefore, a unique assembly-line, small-scale batch processing method has been developed. Material is transported in portable containers through the plant in batches of 75–100 gallons (283.9–378.5L). In this method, a continuous line of these containers is sent past a series of operating stations where technicians perform specific tasks on each batch, in turn.

An extraction plant may make one product for a few weeks, and then may convert to produce a different product after changing and redefining the tasks to be conducted at each station.

Due to the nature of the extraction process, the waste material generated is practically equal to the amount of raw material processed and most of the waste appears in the solid or semisolid form. Wastes from biological product extraction include spent raw materials such as leaves and roots, water-soluble solvents, solvent vapors, and wastewaters. The wastewater is mainly from the aqueous part of the spent natural materials and from the product recovery and purification processes. The wastewater also comprises organic solvents, heavy metals, and ammonia.

Organic solvents are used in product recovery to dissolve fats and oils which would contaminate the product; solvents are also used to extract the product itself. While ketones and alcohols are common extraction agents, other organic solvents, such as benzene, chloroform, and 1,2 dichloroethane, may be used to extract the alkali-treated plant alkaloids.

Common heavy metals are lead and zinc, which are used as precipitating agents. Ammonia (in solution or anhydrous forms) is often used for pH control, as are the hydroxides of various cations, and also, more importantly, as a common extraction solvent.

In general, the extraction wastewater is characterized by small flows and low pollutant concentrations. The wastewaters typically have low BOD_5, COD, and TSS levels and a pH in the range of 6–8 (13).

Similar to the fermentation process, volatile solvents used in product recovery operations may release vapors to the air.

3.3. Chemical Synthesis

Most drugs are produced by chemical synthesis. In a typical manufacturing plant, batch processing is a standard method of operation for chemical synthesis facilities, including a series of reaction, separation, and purification steps to make a desired product.

Chemicals used in chemical synthesis operations range widely and include organic and inorganic reactants and catalysts. In addition, manufacturers use a wide variety of solvents for product recovery, purification, or for process reaction, which are listed as priority pollutants (13, 15). A large number of toxic substances are used in chemical synthesis plants, and a correspondingly high incidence of toxic pollutants in the plant's wastewater has been observed.

Figure 19.2 is a process flow diagram of chemical synthesis for an anti-convulsive drug plant (16, 22). Raw materials, potassium permanganate, and water are mixed in a 3,000 gallon (11,355-L) reactor. A manganese dioxide precipitate is formed and is removed from solution by a rotary drum filter coated with Celite. The wet filter cake (manganese dioxide precipitate and Celite) is deposited into trash bins for disposal at a municipal landfill. The filtrate is neutralized with sulfuric acid and sent to a climbing film evaporator. Overhead water is collected and discharged into the sewer. The enriched product solution is then sent to an 800-gallon (3,028-L) Pfaudler vessel where a final pH adjustment is made with sulfuric acid. As the mixture is agitated and cooled, potassium sulfate is crystallized. The potassium sulfate crystals are removed from the reaction mixture by centrifugation dissolved in water and then discharged to the sewer. Butyl acetate is added to the concentrate and the mixture is azeotropically dehydrated.

In a continuous process, the overhead azeotropic mixture is condensed and sent to a decanter where the lower water layer is discharged to the sewer and butyl acetate is taken

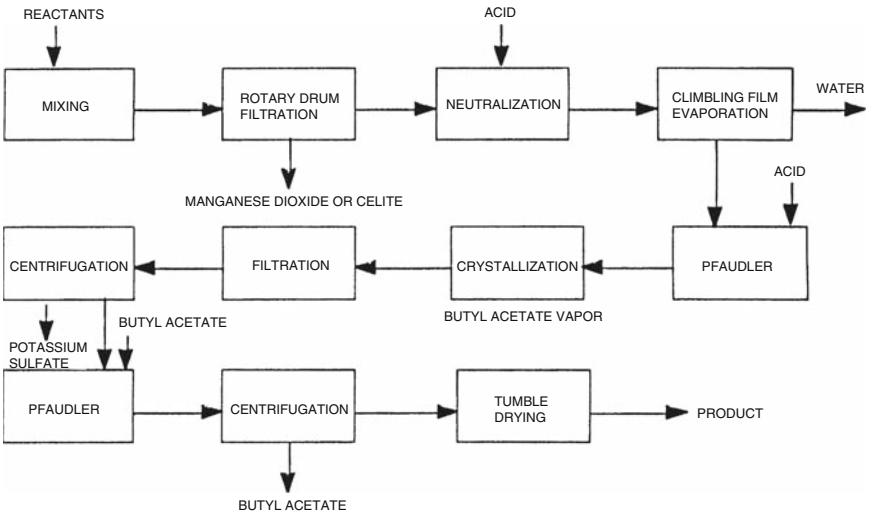

Fig. 19.2. Process flow diagram of chemical synthesis for an anti-convulsive drug plant (16).

off the top and returned to the product mixture. This process procedure is continued until all the water (which contains some butyl acetate) is removed. The butyl acetate product mixture is then filtered to remove any remaining salt. The filtered solution is then cooled, allowing product to crystallize and be separated by centrifugation. Butyl acetate is recovered and stored for reuse. The product is sent to a tumble dryer prior to packaging. Butyl acetate vapor is vented from the dryer, condensed, and recovered for reuse (16).

Solvents serve several functions in a chemical synthesis process (11, 13). They dissolve gaseous, solid, or viscous reactants to bring all reactants into close molecular proximity. They also serve to transmit heat to or from the reacting molecules. Benzene and toluene are widely used organic solvents since they are stable compounds that do not easily take part in chemical reactions.

Waste streams from chemical synthesis operations are complex due to the various operations and reactions employed. Virtually every step of an organic synthesis generates liquor that contains unconverted reactants, reaction byproducts, and residual products in the organic solvent base. Acids, bases, cyanides, and metals may also be generated. Typically, the spent solvents are recovered on-site by distillation or extraction (23), which also generate solvent recovery wastes such as still bottom tars.

Aqueous waste streams from synthesis processes may result from miscible solvents, filtrates, concentrates, equipment cleaning, wet scrubbers, and spills. Wastewaters typically have high 5-day BOD, COD, and TSS levels and have a pH value in the range of 1–11 units. Solid wastes may result from filter cakes. The use of volatile solvents can also result in air emissions.

3.4. Formulation/Mixing/Compounding

Pharmaceutical formulation is a process for preparation of dosage forms such as tablets, capsules, liquids, parenterals, and creams and ointments for consumer use.

Tablets account for over 90% of all medications taken orally (24) and are produced in three varieties: plain compressed, coated, and molded. The form of tablet depends on the desired characteristics of active ingredient, which can be slow, fast, or sustained, for example, spraying or tumbling the tablets with a coating material is one of the ways controlling the release characteristics. Tablets are produced by blending the active ingredient with fillers, such as starch or sugar, followed by compressing using either wet granulation, or direct compression, or slugging.

Capsules prepared in hard or soft form are the next most widely used oral dosage form for solid drugs. Hard capsules consist of two separate pieces which are formed by dipping pins into a solution of gelatin maintained at a specified temperature. When removed, a gelatin film is deposited on the pins. Unlike hard capsules, soft capsules are prepared by placing two continuous gelatin films between rotary die plates, then injecting in the drug.

The third type of pharmaceutical formulation is a liquid dosage form prepared for injection or oral use, which includes solutions, syrups, elixirs, suspensions, and tinctures, all of which are usually prepared by mixing the solutes with a selected solvent in a glass-lined or stainless steel vessel. Suspensions and emulsions are frequently prepared using colloid mills and homogenizers.

Parenteral dosage forms are injected into the body either intramuscularly, intravenously, or subcutaneously. Parenterals are prepared as solutions, as dry solids which are dissolved immediately before injection, as suspensions, as dry insoluble solids which are suspended before injection and as emulsions.

Ointments and creams are semisolid dosage forms prepared for topical use. Ointments are usually prepared by melting a base, which is typically the petroleum derivative petrolatum. This base is then blended with the drug and the cooled mixture is passed through a colloid or roller pill. Creams are oil-in-water or water-in-oil emulsions, rather than being petrolatum based, and are manufactured in a similar manner (16).

Most water used in the formulation process is as cooling water, which generates no contact wastewater. Wastewater is generally originated from cleanup, spills, and breakage of packaged products. Some wastewaters may come from the dust scrubbers, which are sometimes used to control dust from tablet and capsule production.

Most wastes are nontoxic, have relatively small flows, and have low 5-day BOD, COD, and TSS concentrations, and with near neutral pH (6.0–8.0).

Air emissions may result from the use of volatile solvents in the formulation processes.

3.5. Research and Development

Research and development (R&D) processes in the pharmaceutical industry involve chemical research, microbiological research, and pharmacological research to provide information for pharmaceutical production related in the above. The development of a new drug with less environmental pollution requires cooperative efforts in several fields, such as medicinal,

chemical engineering, biomedical engineering, environmental engineering, biology, biochemistry, pharmacology, and toxicology.

An example is the R&D section (16) in a plant producing a wide range of dermatological products (such as shampoos, creams, and itch soothing preparations) and ophthalmic products (such as contact lens cleaners, eye drops, and disinfecting solutions). These pharmaceutical compounds are formulated in the production section after having been thoroughly researched by the R&D section. The R&D section involved two major groups, the synthetic chemistry division and the product development division. Halogenated and nonhalogenated solvents, such as chloroform, methylene chloride, acetone, methanol, acetonitrile, acetone, ethyl ether, xylene, and hexane are commonly used for extraction and analyses. Acetonitrile and methanol are extensively used as carrier liquid in high performance liquid chromatography (HPLC). The plant consumed 400 gal (1,514 L) of acetonitrile and 990 gal (3,747 L) of methanol annually. Other chemical wastes, including photographic chemicals, radionuclides, bases, and oxidizers, can be produced from some pharmaceutical research and development sections. Sulfuric acid is the most widely used acid at an annual consumption of 450 gal (1,703 L). In addition, a large quantity of sulfuric acid is used in glassware washing at an annual acid consumption of approximately 1,080 gal (4,088 L).

The wastes from the research and development processes can be similar to those wastes generated from one or more or all of the above four processes, chemical synthesis, fermentation, biological product extraction, and formulation, and can be even more complicated, because various attempts should be made to develop a new drug or a new pharmaceutical instrument. Radioactive wastes may also be generated.

As a result of the diverse nature of pharmaceutical research and development, a wide range of chemical and biological laboratory wastes are produced. However, the quantity, quality, and the time schedule of discharging research and development wastes are usually erratic, and the problem cannot be measured entirely. The quantities of materials discharged by research and development operations are in general (25) relatively small as compared with the volumes generated by production facilities.

Pharmaceutical production can be batch, continuous, and semi-continuous operations. Batch-type production is the most common type of manufacturing technique for each of the subcategories. Table 19.1 summarizes the typical wastes and the associated process origins in pharmaceutical industry. Note that most of the process origins in the table can exist in all the five main processes but with varied qualities (i.e., having various kinds of materials and wastes) and quantities of wastes.

4. WASTE CHARACTERIZATION AND OPTIONS FOR WASTE DISPOSAL

4.1. Waste Characteristics

The preceding discussions show that numerous process wastes are generated by the pharmaceutical industry. The pharmaceutical wastes vary greatly depending upon the manufacturing processes. The very nature of the pharmaceutical industry determines the composition of each plant effluent which varies considerably from plant to plant.

There are pharmaceutical plants which discharge only solid wastes, and no waste liquors in the sense of production process. However, these plants still have to deal with certain amounts of wastewater from washing of equipment and floors, etc.

A distinguishing feature of pharmaceutical fermentation and the biological product extraction manufacturing is that a large proportion of the material input to the manufacturing process ends up as process wastes. The wastes from such a low-product-yield process may be in either solid or liquid form.

Many plants generate wastewaters with COD concentration ranging from 500 to 1,500 mg/L whereas the wastewaters from fermentation and chemical synthesis products may have COD concentrations reaching 10,000 mg/L or even higher (26).

Generally, fermentation processes and chemical synthesis processes produce large flows and have high levels of 5-day BOD and COD, and with high TSS for the fermentation processes, although they vary greatly from factory to factory; while, the biological product extraction, formulation, and research and development tend to produce low flows with low levels of 5-day BOD, COD, and TSS (13). Table 19.2 lists average waste flow and traditional pollutants from four manufacturing processes, chemical synthesis, fermentation, biological product extraction, and formulation/manufacturing.

Toxic pollutants can exist in the wastewaters. Especially, the waste from the chemical synthesis plant usually contains significant levels of a large number of toxic pollutants. Table 19.3 lists toxic organic pollutants associated with pharmaceutical industry according to the list of organic priority pollutants by the 1977 amendment to the US Clean Water Act.

Besides cyanide, many inorganic priority pollutants are commonly found in the waste streams from pharmaceutical industry, such as arsenic, beryllium, cadmium, chromium, copper, lead, mercury, nickel, selenium, silver, thallium, and zinc. Only a few of these priority pollutants are widespread in their occurrence or high in concentration. The significance of these facts affecting the regulation of these pollutants will be discussed later.

4.2. Options for Waste Disposal

There are three options of wastewater discharge for pharmaceutical manufacturing: direct discharge after treatment, indirect discharge (i.e., discharging to Publicly Owned Treatment Works, POTW), and zero discharge. Many pharmaceutical manufacturers treat their wastes and directly discharge their treated wastewaters to the navigable waters. Some of pharmaceutical plants are so located that POTW are adequate to solve their, at least a part of, waste disposal problem. Some industrial plants generate basically no wastewater, or trade out waste, or limit the treated wastewater on-site, resulting in zero discharge. The numbers of the three types of wastewaters discharge by pharmaceutical industrial plants in the USA are listed in Table 19.4.

Deep well injection (27) generates no discharge to waterways. However, most of the deep well injections that were permitted in the early times, and, at least some of them may not be allowed for such operation sooner or later especially if the injected material has a great potential threat to the environment.

Datta Gupta et al.(28) described disposal of effluent by irrigation and application of dry waste biosolids as fertilizer (29), which may generate no wastewater discharge. Lane (25)

Table 19.4
Pharmaceutical process wastes (16)

Waste description	Process origin	Composition
Process liquors	Organic syntheses	Contaminated solvents
Spent fermentation broth	Fermentation processes	Contaminated water
Spent natural product raw materials	Natural product extraction processes	Leaves, tissues
Spent aqueous solutions	Solvent extraction processes	Contaminated water
Leftover raw material containers	Unloading of materials into process equipment	Bags, drums (fiber, plastic, metal), plastic bottles
Scrubber water from pollution control equipment	Dust or hazardous vapor generating processes	Contaminated water
Volatile organic compounds	Chemical storage tanks, drums	Solvents
Off-spec or out-dated products	Manufacturing operations	Miscellaneous products
Spills	Manufacturing and lab operations	Miscellaneous chemicals
Waste water	Equipment cleaning, extraction residues	Contaminated water
Spent solvents	Solvent extraction or wash practices	Contaminated solvents
Used production materials	Manufacturing operations	Filters, tubing, diatomaceous earth
Used chemical reagents	R&D operations	Miscellaneous chemicals
Natural gas combustion products	Steam boilers	Carbon compounds, oxides of nitrogen and sulfur

described an alternative treatment and disposal of spent beer by spray irrigation. The spent beer frequently contains high amounts of nitrogen, phosphate, and other plant growth factors. However, it is also likely to contain salts, like sodium chloride and sodium sulfate, as a result of the extraction process. The presence of such salts depending on their concentration can cancel out the value of the spent beer as a fertilizer. Spray irrigation is mainly used for the purpose of disposal of the spent beer, rather than just for its value as a fertilizer. This disposal technique has a number of limitations: (a) large land areas are needed in the order of 125 acres (505,875 m^2) for 100,000 gal (378,500 L) of spent beer sprayed per day; (b) the land should be reasonably flat so that runoff from the spraying does not result in erosion or "puddling" in low spots (29). The "puddlin" will result in odors that will most likely render the entire operation a public nuisance.

Table 19.5
Characteristics of major pharmaceutical wastewater streams (13)

Process	Waste flow MGD	BOD_5 mg/L	COD mg/L	TSS mg/L	pH	Priority pollutant
Fermentation	0.622	1,668	3,452	1,023	4–8	Cu, Zn
Natural extraction	0.197	42	132	93	6–8	Pb, Zn, solvents
Chemical synthesis	0.477	2,385	4,243	414	1–11	Variety
Formulation	0.296	339	846	308	6–8	

Note: *MGD* million gallon per day ($1\text{MGD} = 3,784\,\text{m}^3/\text{day}$).

5. ENVIRONMENTAL REGULATIONS ON PHARMACEUTICAL WASTEWATER DISCHARGES

Wastes generated from pharmaceutical manufacturing could exert various impacts on the environment, such as:

1. Color and odor problems due to the spent solvent, their raw materials, and spent chemicals
2. The growth of bacteria in the biosolids from fermentation and natural extraction processes
3. Oxygen depletion due to the relatively high oxygen demand load
4. Toxic materials such as heavy metal, cyanide, and toxic organic compounds
5. Air pollution due to volatilization of volatile organic solvents.

The total pollutional load of wastewaters generated by the pharmaceutical manufacturing industry in the USA was reported by US EPA (13) as shown in Table 19.5.

5.1. Regulations for Direct Discharge

To ease the impact of waste discharge to the environment, the Clean Water Act requires a permit for any discharge into the nation's waterways. Direct discharge into surface water must have a National Pollution Discharge Elimination System (NPDES) permit and/or a State Pollution Discharge Elimination System (SPDES) permit. The NPDES permit or the SPDES permit is granted on case-by-case basis.

The US EPA (11, 12, 15) has regulated what is known as the Best Practical Control Technology Currently Available (BPT). The direct discharge limitations are presented in Table 19.6.

The regulation for cyanide is the same in the Best Available Technology Economically Achievable (BAT) and the New Source Performance Standards (NSPS). The regulations have been delineated mainly for the four subcategories: fermentation, biological extraction, chemical synthesis, and formulation. The US EPA tends to deregulate the effluent discharge from R&D, because only insignificant amount of wastes is discharged and the wastes have similarity in quality to those from the other four sections.

Note that many of the priority pollutants which may be found from pharmaceutical discharges are excluded from direct discharge regulation because either they are present at low

Table 19.6
Organic priority pollutants from pharmaceutical manufacturing

	Concentration (μg/L)	
Organic compounds	Average	Range
1. PAH (polynuclear aromatic hydrocarbons)		
Acemaphtherie	12	0–100
Naphthalene	2.8	0–14
Anthracene	1.8	0–7
Fluorine	3.5	0–41
Phenanthrene	1.8	0–7
2. Nitrogen compounds		
1,2-Diphenylhydrazine	2	0–17
N-Nitrosodiphenylamine	12	0–1400
3. Aromatic compounds		
Benzene	220	0–2100
Chlorobenzene	67	0–600
2,4-Dinitrotoluene	12	0–49
Ethylbenzene	16	0–86
Toluene	2400	0–17000
4. Halogenated hydrocarbons		
Carbon tetrachloride	460	0–6000
1,2-Dichloroethane	8.7	0–74
1,1,1-Trichloroethane	10	0–130
1,1,2-Trichloroethane	95	0–1300
l,1,2,2-Tetrachloroethane	2	0–10
Chloroform	300	0–1600
1,1-dichloroethylene	8.9	0–95
Methylene chloride	2600	0–20000
Methyl chloride	300	0–1500
Methyl bromide	3	0–15
Tetrachloroethylene	3.5	0–36
Trichloroethylene	8	0–62
5. Ethers		
Bis(2-chloroethyl) ether	19	0–170
6. Phenolic compounds		
2-Chlorophenol	2.4	0–22
2,4-Dichlorophenol	1	0–5
4-Nitrophenol	400	0–3500
Pentachlorophenol	4.4	0–62
7. Phthalates		
Bis (2-ethylhexyl)	37	0–170
Butyl benzyl phthalate	33	0–3 60
Di-n-butyl phthalate	10	0–90
Diethyl phthalate	8	0–31

level, or they are infrequent for occurrence, or their presence amount is too small to be effectively reduced by the current technology.

5.2. Regulations for Indirect Discharge

As mentioned earlier, an alternative way to discharge wastewaters from pharmaceutical plants is discharging their wastewaters to the Publicly Owned Treatment Works (POTW) for further treatment. However, the wastes and wash water from pharmaceutical plants, especially from chemical synthesis manufacturing, are not always compatible with biological waste treatment plants. The waste and wash water may be too concentrated or too toxic (such as heavy metal and cyanides) that will harm the POTW biological treatment systems. Moreover, high-acid waste can seriously destroy the material used to seal the sewer joints, and can retard biological treatment; flammable solvents may cause fire or explosion and then cause damage and interruption of sewer systems.

To assist control authorities and approval authorities for industrial discharge to POTWs, the US EPA has developed the National Categorical Pretreatment Standards for point sources. These categorical pretreatment standards are designed to prevent the discharge of pollutants which pass through, interfere with, or are otherwise incompatible with the operation of POTWs. Specifically, the Pretreatment Standards for Existing and New Sources (PSES and PSNS) were established for the indirect dischargers, to prevent the pollutants which are incompatible with or not susceptible to treatment in a POTW (15). The priority pollutants considered for pretreatment standards are listed in Table 19.7.

The PSES and PSNS regulate an indirect discharge limitation for cyanide. The limit for alternative A is the same as that in the BPT in Table 19.6; while the alternative B is $9.4 \times R$ and $33.5 \times R$, instead of $9.4 \times (0.35) \times R$ and $33.5 \times (0.18) \times R$ in the last two columns of Table 19.6, respectively.

The waste to be discharged to the POTW must meet the influent requirements and the factory must pay attention to the municipal sewer system. Pretreatment is usually required before discharging to the POTW.

5.3. Historical View on Regulations

To protect the environment, US EPA has regulated the BPT, which is basically identical to those shown in Table 19.6. As mentioned earlier the wastewaters from fermentation and

Table 19.7
Statistical data for the three types of wastewater discharges

Type of discharge	Number of plants	Wastewater flow MGD	
Direct discharger	52	24.9	11%
Indirect discharger	285	39.9	62%
Zero discharger	127	0	27%
Total plant	464	64.8	100%

Note: *MGD* million gallon per day ($1\,\text{MGD} = 3,784\,\text{m}^3/\text{day}$).

chemical synthesis of products may have COD ranging between 10,000 and 20,000 mg/L. According to the BPT, which is defined as a COD removal of 74%, the fermentation and chemical plants may be able to discharge their treated wastewater with COD concentration from 2,600 to 5,200 mg/L to meet 1976 BPT (26). In November 1982, the US EPA proposed the BAT and the NSPS to control the discharge of nonconventional pollutant, COD, as well as other pollutants from pharmaceutical manufacturing facilities (9, 10, 15). However, the industry commented that the proposed regulations could not be met based on the US EPA proposed technology. In 1983 and modified in 1998, the US EPA promulgated final Pharmaceutical Manufacturing Point Source Effluent Limitation Guidelines, Pretreatment Standards, and NSPS (11, 12, 15).

The Agency decided to return to the 1976 BPT subcategorization discharge. The 1982 proposed COD regulations are no longer valid. Therefore, the BPT limitations listed in Table 19.6 are basically the 1976 version and finalized in 1983. However, the US EPA reserved a final decision on appropriate BAT limitations and NSPS for COD which is postponed until additional information could be obtained on applicable COD removal technologies and their achievable concentrations.

On December 16, 1986, US EPA promulgated the BCT limitations for the existing pharmaceutical manufacturing facilities. The existing pharmaceutical manufacturers that are subcategorized A–D productions are covered by this regulation, which set equal to the BPT limitations in 1983. All these guidelines have been reissued in 1998 (15).

It should be pointed out that the US Pharmaceutical industry is largely an international industry in which many companies have manufacturing facilities and sales and distribution operations in countries other than the USA. In addition to US federal statutes and regulations, there are international laws, regulations, treaties, conventions, and initiatives which are drivers of the environmental programs of pharmaceutical companies. The Basel Convention, ISO 14000 standards, the environmental requirements of NAFTA, and the evolving European Union Directives and Regulations are a few examples of important international environmental standards and programs which affect this industry (14).

6. WASTE MANAGEMENT

6.1. Strategy of Waste Management

The main objectives of pharmaceutical waste management are to reduce waste generation through improved manufacturing process and enhanced solvent recovery; remove suspended matter, odor, BOD matter, and hazardous and toxic materials; and to prevent air pollution.

This section discusses three main tasks of waste management in pharmaceutical industry:

1. In-plant control
2. In-plant treatment
3. End-of-pipe treatment

The load on the end-of-pipe treatment process depends on how well the in-plant control is practiced. The in-plant control usually analogs to waste minimization. However, waste minimization is defined by the US EPA as source reduction and recycling, which covers a

somewhat different practice from the traditional in-plant control, including the interplanetary efforts to minimize wastes such as waste exchange. In general, in-plant control is a means of waste management, and an interplanetary waste exchange program in waste minimization cannot be practiced without a well oriented in-plant management. The waste exchange will be presented in the section of in-plant control.

Since wastewater treatment and pollutant removal costs are highly influenced by the pollutants and volume of water to be treated, the costs for treating a segregated stream are considerably less than that would be in treating combined wastewater. Also, chemicals other than those being treated are less likely to interfere with the treatment technology if treatment occurs before mixing (11, 13). The importance of waste separation has been recognized which is reflected by the fact that in-plant treatment deals with a segregated particular pollutant. The in-plant control is mainly a source control to reduce generation of waste while the end-of-pipe treatment mainly deals with overall waste in the plant. From the view point of treatment, in-plant treatment can be visualized as end-of-pipe treatment or a pretreatment for a particular production process; while from another point of view, it is an in-plant process to reduce waste before being discharged to an overall waste stream.

6.2. In-Plant Control

In-plant control includes water conservation, raw material substitution, chemical substitution, material recovery, extensive recycling of wastewater, and modification and improvement of processes, so that the amount of wastewater can be reduced and pollution can be minimized. The following are some examples of in-plant controls that have been demonstrated effectively in reducing pollution loads.

6.2.1. Material Substitution

Material substitution is a replacement of one or more of the raw materials used in production to reduce the toxicity or volume of wastes generated.

Material substitution has been demonstrated to be successful in pharmaceutical tablet coating operations to reduce hazardous waste generation. Wayman and Miller (30) reported a successful material substitution in tablet coating which reduced the usage of methylene chloride from 60 to 8 ton/year by converting the conventional film coating to aqueous film coating. The other example, a water-based solvent and new spray equipment for a tablet coating developed in a manufacturing plant eliminated expensive (US $180,000) air pollution control equipment, resulting in a saving of US $15,000 per year in solvent make-up cost (31).

Other material substitutions that may be suitable for pharmaceutical manufacturing include the use of aqueous-based cleaning solutions instead of solvent-based solutions and the replacement of chlorinated solvents with non-chlorinated solvents (13). Moreover, using nontoxic or less toxic biocides to substitute the heavy-metal-containing biocides in the fermentation processes can avoid the correlated heavy metal contamination.

For the pharmaceutical industry, however, product reformulation seems to be very difficult, because the reformulation must have the same therapeutic effect, stability, and purity profile as the original formulation. Moreover, it takes a considerable amount of time for the U.S. Food and Drug Administration (U.S. FDA) to approve of the reformulated drug. Another problem

that a reformulation may encounter is the possibility of customer rejection of the product due to changes of the product's aesthetic qualities such as taste, color, dosage, or form. Because of the difficulties in reformulation, waste minimization should be introduced at the research and development phase (16).

Another sort of material substitution is to substitute the toxic materials used in the waste recovery and cycling processes, such as using nontoxic chemicals to substitute for zinc and lead containing agents in a precipitation process.

6.2.2. Process Modification

Modification or modernization of the existing processes is another opportunity to reduce waste generation.

The modification can be accomplished through, for example, controlling a suitable feed rate, a proper agitating and mixing, optimizing operating temperatures, and automation control. In most cases, the product/process yield determines the product/waste ratio. Inadequate feeding rate, mixing, or temperature control in pharmaceutical manufacturing can cause a high byproduct yield. Reactor efficiency can be improved and byproduct formation can be reduced by controlling reaction parameters.

Increased automation can reduce operation errors. For example, introducing automation in material handling and transfer processes can reduce spillage.

Another process modification option is to redesign chemical transfer system to reduce physical material losses (13). For example, replacing gas pressurization with a pumped transfer eliminates the tank pressurizing step and its associated material losses (32).

Other design considerations for waste minimization include modifying tank and vessel dimensions to improve drainage, installing internal recycle systems for cooling wasters and solvents, selecting new or improved catalysts, switching from batch to continuous processes for solvent recovery, and optimizing process parameters to increase operating efficiency. Manufacturing processes have demonstrated that excessive solvent emissions from the purging of autoclaves used for the manufacture of synthetic steroids can be considerably reduced by installing rotameters with integral needle valves to control nitrogen flow into the reactor; nitrogen flow and resulting solvent vapor pickup can be reduced by a factor of six compared with the baseline situation where nitrogen flow is not controlled and operated in an on–off fashion without throttling (16).

The major obstacles of process modification to the waste minimization are: new processes must be tested and validated to ensure that the resulting product is acceptable; a considerable amount of time may be needed for the US FDA approval, if applicable, before instituting any change; extension process changes can be expensive; and downtime will occur when production is stopped for new equipment installation.

The routine cleanup in the pharmaceutical plant can be carried out most effectively by vacuum cleaning. Wash water may be a water pollutant. Special attention should be given to prevent such material from entering the sewer system. Lane (25) has shown that a central wash area with portable equipment can be usable. The portable (even large) equipment can be moved to a central washup area, providing better prevention of dumping of hazardous pollutants to the sewer system.

6.2.3. Recycling Wastewater and Recovering Materials

Recovering and recycling include directly reusing waste material, recovering used materials for a separate use, and removing impurities from waste to obtain relatively pure substances. The goal is to recover materials for reuse in the process or for reuse in a different application. The restrict quality control requirements of the pharmaceutical industry often restrict reuse opportunities. After a high degree of purification, materials recovered from manufacturing processes may be reused. Recycling can be performed either on-site or off-site. On-site can be either integral to an operation or in a separate operating area. The value of a waste depends on the type, market, purity, quantity and frequency of generation, and distance between the generator and the recycling operation.

One of the important recycling programs in the pharmaceutical industry is the recycling of solvent. Solvents are used for reaction media, extraction media, equipment cleaning, and coating media. Processes for solvent recovery from concentrated waste streams include distillation, nebulization, evaporation, liquid–liquid extraction, filtration, decantation, centrifugation, flotation, and sedimentation. The commonly used and recycled solvents are acetone, cyclohexane, methylene chloride, ethyl acetate, butyl acetate, methanol, ethanol, isopropanol, butanol, pyridine, methyl ethyl ketone, methyl isobutyl ketone, and tetrahydrofuran (33). Solvent waste recyclability can be improved through special arrangement of recycling procedure: for example, minimizing solid concentration in solvent wastes, segregating chlorinated solvent wastes from non-chlorinated solvent wastes, segregating aliphatic from aromatic solvent wastes, segregating chlorofluorocarbons from methylene chloride, and segregating water wastes from flammables.

6.2.4. Water Conservation and Reuse

It is more cost effective to treat the waste with smaller volume but higher concentration than a waste with greater volume but lower concentration. To recycle and reuse renovated wastewater is recommended. It has been estimated that about 1 to 100 tons (0.9072 to 90.72 metric tons) of water is used per ton of product. By modifying processing procedures or auxiliary equipment, water usage and wastewater generation may be significantly reduced (21). Examples are: use of surface rather than barometric condensers, reuse of noncontact water, concentration of reaction mixtures to limit waste volume, and combining several processes.

King (34) has described an oil-dehydration evaporator/pyrolysis system for energy recovery from pharmaceutical wastewater. Gas produced in the pyrolysis unit is burned to provide steam required by the evaporator for oil dehydration.

6.2.5. Segregation and Concentration of Wastes

Concentrating waste may reduce treatment cost. Concentration of wastewater may also minimize the impact of intermittent hydraulic surges, specifically in fermentation operations. Segregation of waste streams, which allows concentrating the individual waste for individual treatment, often allows more efficient removal of particular pollutants. Segregation of wastes also allows using an individual treatment method for the individual waste, such as using various evaporation or dewatering methods to treat the separated waste streams for the

fermentation wastes in an in-plant treatment program. For example, cyanide destruction, metal removal, and steam stripping to remove ammonia and organic solvents are utilized in the pharmaceutical industry for in-plant treatment. They need to be separated individually. Individual process units are now commonly designed with allowance for waste stream segregation.

For a similar reason, separation and treatment for storm runoff and sewer system may eliminate the discharge of contaminated runoff and reduce treatment cost, because the storm water from certain manufacturing areas can contain high levels of toxic pollutants, while the storm runoff from some other areas and the sewer may not. For the factories practicing in-plant treatment and direct discharge, the domestic wastewater should be separated from polluted storm runoff. The latter should be discharged directly to POTW or treated in-plant separately; while, the nonpolluted storm runoff can be separated from polluted streams and discharged directly to a river.

Sewers and pumps must be designed for peak flows to avoid flooding the mill or bypassing the treatment plant. Also a good pipe and storage system are needed for collecting the spills and the wastewater from various stages and storing wastewater and biosolids.

6.2.6. Good Operating Practices

Good operating practices, which can help reduce waste generation, material losses, and production cost, include: closer supervision, production scheduling, materials tracking, inventory control, spill prevention, material handling and storage procedures, documentation for process procedure, maintenance programs, employee training, and management incentives. As these practices all apply to the general waste minimization in all industries.

6.2.7. Reduction of Air and Dust Problems

Air pollution control in the pharmaceutical industry is mainly practiced by in-plant-control. Air and dust control technologies are fully described in *Air Pollution Control Engineering* (35) and *Advanced Air and Noise Pollution Control* (36). There are three main sources of air pollution: fermentation process gas, dust, and volatile solvents.

Most of the fermentations carried out in the pharmaceutical industry are aerobic (25). Air must be supplied to the fermentation organism. Compressed air is injected, or sparged, into the lower end of the fermentor, which is simply a large, vertical, circular tank. Supplying fresh air to the fermentation vessel on a constant basis makes it necessary to vent or discharge an equal volume of what is termed "used" air from the top of the fermentation vessel. The used air, or vent gas, has scrubbed a number of materials, including carbon dioxide and many other more complex organic materials from the fermentation as it moves up through the fermenting mass. The organic materials generate odor. These odors vary with the material being fermented and vary somewhat between different fermentors of the same material. This "used" air, or vent gas, from the fermentor is the principal air pollutant. Wet scrubbing of the vent gases may be practiced though it may not be particularly successful in many cases.

On large fermentors, the volume of gases is so great that the water needed to do a scrubbing job (if water is used alone to do the job) is so large that, consequently, generates even larger dimensions of polluted water to eliminate or even partially reduce air pollution. Activated carbon can be used to adsorb the odor of the vent gas. This method, however, may be

practical only for large fermentors, because the method requires a larger amount of carbon to accomplish a satisfactory end point.

Incinerating vent gas is a satisfactory solution. However, sometimes fuel is needed to raise the vent gas temperature from fermentation temperature (generally well below 40°C) to an incineration level. At this point, this method may be uneconomical. A possible more economical method may be: piping the vent gas from the fermentor to a boiler house and using it for combustion air in the boiler. This method was used in large scale operations such as in the fermentation plant at Abott Laboratories in North Chicago, IL and at Eli Lilly & Company in Lafayette, IN, both in the USA.

Air emission of volatile organic solvent can be a big air pollution problem, which may be reduced by employing scrubbers or condensers to reclaim the solvent vapors. Some factories may generate acid and solvent vapors such as methanol and butyl acetate which are sent to a house vacuum system for disposal. The waste mycelium, or filter cake, which results from the initial separation of solids from the fermentated beer, is a frequent source of odor. The living cell biomass is quite perishable. If housekeeping standards are not maintained at a high level, this part of the evaporation is also likely to contribute to the odor problem. Thus, good housekeeping throughout the entire plant will do much to improve an odor situation.

Dust is a secondary pollution source. Dust inside a plant may cause "cross contamination," i.e., contamination of one drug by another. Penicillin is one of the materials that are capable of causing extremely toxic reactions even when present in trace quantities (25). For example, aspirin tablet can cause a reaction of very serious proportions (might result in death) in the presence of minute amount of penicillin. Thus, penicillin dust should be absolutely isolated from the areas where other pharmaceuticals are manufactured. Besides the isolation of penicillin production in a separate area, the intake air to the areas producing other pharmaceuticals should be carefully filtered, because the intake air may contain the air out of the penicillin manufacturing area.

There are many methods used to remove dusts. A scrubber or Rotoclone can be used for removing many pollutants. However, the use of water with a scrubber or Rotoclone may result in water pollution problems. In such a case, a dry filter system may be recommended. McNeil Laboratories used an extremely large Pangborn baghouse type dust collector to exhaust all the air from most manufacturing operations. It was 33 ft (10 m) long by 17 ft (5.2 m) wide by 20 ft (6 m) high. The inlet duct was 44 in. (112 cm) in diameter. This single unit had a capacity of 36,000 scfm (1019 m^3/min). On this point, the pharmaceutical manufacturing areas in McNeil Laboratories were supplied with 100% outside air (25), thus prevented secondary pollutant from dust.

6.2.8. Waste Exchanges

Waste exchange is an alternative to recycling. It involves the transfer of waste to another company for use "as is" or for reuse after treatment. Waste exchanges are private or government-subsidized organizations that help identify the supply and demand of various wastes. Waste exchanges have been established in some areas of the USA to put waste generators in contact with potential users of the waste. US EPA (16) listed 48 state programs which offer technical and/or financial assistance for waste minimization and treatment in the USA; and 24 exchange operating offices in the USA and Canada.

There are three types of waste exchanges: information exchanges, material exchanges, and waste brokers. Metals and solvents are the most frequently recycled materials via waste exchange, because of their high recovery value. Other wastes commonly recycled through waste exchanges include acids, alkalic salts and other inorganic chemicals, organic chemicals, metal sludges, and solid residue from fermentation and natural product extraction processes. The biosolids from the treatment plant can also be beneficially reused off-site, which will be detailed in the section of end-of-pipe treatment.

6.3. In-Plant Treatment

In-plant treatment in the pharmaceutical industry is mainly for treating priority pollutants, such as solvents, metals, and cyanide, before combining the factory overall waste stream. Although all three pollutants may be removed by the end-of-pipe treatment, they can be removed more effectively by the in-plant treatment when they are concentrated in the segregated stream. Therefore, the in-plant treatment can also be regarded as a pretreatment to biological waste treatment.

6.3.1. Cyanide Destruction Technologies

Chemical oxidation and high pressure and temperature hydrolysis are two treatment processes which are effective in treating cyanide-bearing waste streams in the pharmaceutical industry.

Chemical oxidation is a reaction in which one or more electrons are transferred from the chemical being oxidized, here the cyanide waste, to the chemical initiating the transfer, the oxidizing agent (37–39).

6.3.1.1. CHLORINATION

Cyanide can be destructed by oxidation either with chlorine gas under alkaline conditions or with sodium hypochlorite. The oxidation of cyanide by chlorine under alkaline condition can be described by the following two-step reactions:

$$Cl_2 + NaCN + 2NaOH = NaOCN + 2NaC1 + H_2O \quad (1)$$

$$3C1_2 + 6NaOH + 2NaOCN = 2NaHCO_3 + N_2 + 6NaC1 + 2H_2O$$

Cyanide is oxidized to cyanate at a pH of about 9.5–10.0. Usually 30 min are required to complete the reaction, which markedly reduces the volatility and toxicity (thousandfold reduction) of the waste. Figure 19.3 sketches a chlorination process for a cyanide destruction system.

Since cyanate may revert to cyanide under some conditions, additional chlorine is provided to oxidize cyanate to carbon dioxide and bicarbonate. The complete oxidation of cyanate requires several hours at pH about 9.5–10.0, but only 1 h at a pH between 8.0 and 8.5. Also, excess chlorine must be provided to break down cyanogen chloride, a highly toxic intermediate compound formed during the oxidation of cyanate. Although stoichiometric oxidation of a part of cyanide to cyanate requires only 2.73 parts of chlorine and complete oxidation of a part of cyanide to carbon dioxide and nitrogen gas requires 6.82 parts of

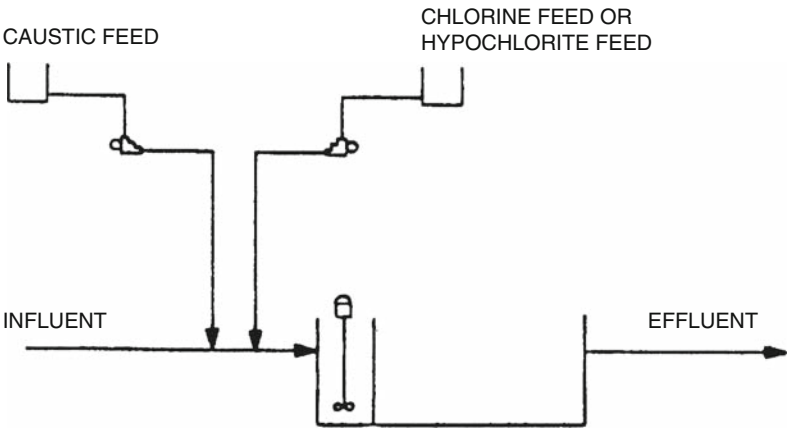

Fig. 19.3. Chlorination process for a cyanide destruction system (13).

chlorine, nearly 3 to 4 parts of chlorine is needed for oxidizing 1 part cyanide to cyanate, and 8 parts of chlorine is needed for oxidizing 1 part of cyanide to gases in practice.

Iron interferes seriously with the alkaline chlorination of cyanide wastes. However, it has been reported that ferrocyanides are treatable by alkaline chlorination at a temperature of 71°C and at a pH of about 12.0.

Ammonia also interferes with the chlorine oxidation process by the formation of chloramines, resulting in an increase of chlorine demand.

Cyanide levels around 0.040 mg/L are achievable by in-plant chlorination processes in electroplating industry, if reaction interferences are not present (13). It was reported that in inorganic chemical industries the free cyanide level after chemical oxidation treatment is generally below 0.1 mg/L.

Chlorination process is a relatively low cost system and does not require complicated equipment, and has received widespread application in the chemical industry. It also fits well into the flow scheme of a wastewater treatment facility.

There are limitations and disadvantages for the chlorination process. For example, toxic, volatile intermediate-reaction products can be formed. Thus, it is essential to control properly the pH to ensure that all reactions are carried to their end point. Also, for waste streams containing other oxidizable matter, the chlorine may be consumed in oxidizing these materials and this may interfere with the treatment of the cyanide. A potential hazardous situation may exist in storage and handling when gaseous chlorine is used.

6.3.1.2. OZONATION

Ozonation is an alternative oxidation treatment for cyanide destruction (13). In fact, ozone oxidizes many cyanide complexes (e.g., iron and nickel complexes) that are not broken down by chlorine.

The oxidation of cyanide by ozone to cyanate occurs in about 15 min at a pH of 9.0–10.0, but the reaction is almost instantaneous in the presence of traces of copper or manganese as catalysts. The pH of the cyanide waste is often raised to 12.0 to obtain complete oxidation.

Oxidation of cyanate to the final end products, nitrogen and bicarbonate, is a much slower and more difficult process unless catalysts are present. Since ozonation will not readily affect further oxidation of cyanate, it is often coupled with such independent processes as dialysis or biological oxidation.

The disadvantages of ozonation include:

1. Higher capital and operating costs than chlorination
2. Toxicity problems similar to chlorination
3. Ozone demand is increased when other oxidizable matter is present in the waste stream
4. The cyanide is not effectively oxidized beyond the cyanate level in most cases

6.3.1.3. Alkaline Hydrolysis

Alkaline hydrolysis is a process based on the application of heat and pressure (13). In this process, a caustic solution is added to the cyanide-bearing wastewaters to raise the pH to between 9.0 and 12.0. Then, the wastewater is transferred to a continuous flow reactor at temperatures in the range of 165–185°C and pressures of 90–110 psi (625–763 kPa). The breakdown of cyanide in the reactor is generally accomplished within a residence time of about 1.5 h.

It has been reported (13) that an average effluent level of 5.25 mg/L is achievable for cyanide destruction. Alkaline hydrolysis is an economic process, and has much less storage and handling problems than chlorination. It is more likely suitable for wastewaters with high concentrations of cyanide.

6.3.2. Metal Removal

Although US EPA does not promulgate effluent guidelines limitations for metals in the pharmaceutical industry, it is useful to improve metal removal to release the impact of heavy metals on the environment. In fact, some factories are practicing removal of heavy metals in the waste stream (13). The methods usually used for metal removal are precipitation through adjustment to the optimum pH, sulfide precipitation, and chemical reduction.

6.3.2.1. Alkaline Precipitation

The solubility of metal hydroxides, in most cases, is a function of pH. Therefore, adjustment to the optimal pH for precipitation of the metal hydroxide will result in an effective removal of the metal. The alkaline precipitation for metal removal system is schematically shown in Fig. 19.4. It should be noted that the solids contact clarifier shown in Fig. 19.4 can be either a settling or a dissolved air flotation clarifier (40).

The solid metal hydroxides are coagulated (using coagulating agents) in clarifier and deposited as sludge.

Lime is the commonly used chemical. In wastewaters containing substantial sulfate compounds, insoluble calcium sulfate precipitates will form when using lime. In such instances, sodium hydroxide may be used.

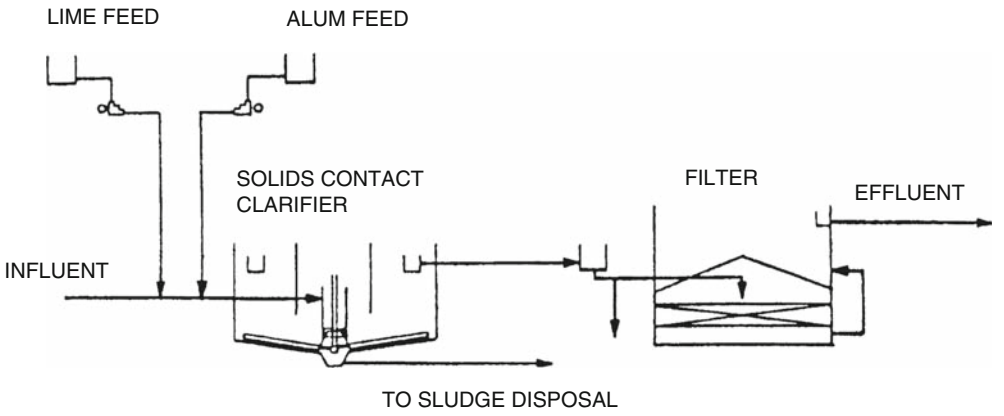

Fig. 19.4. Alkaline precipitation for a metal removal system (13).

The alkaline precipitation method is a well demonstrated wastewater treatment technology. It is easy to operate, and has lower cost than other methods. Its limitations and disadvantages are that: (1) alkaline precipitation is subject to interference when mixed wastes are treated; and (2) relatively high quantities of residue can be generated.

6.3.2.2. Sulfide Precipitation

For many heavy metals (such as copper, nickel, and zinc), their sulfides have much lower K_{sp} than their hydroxides (see Table 19.8). Hence, the sulfide precipitation method is applicable to the removal of all heavy metals by precipitating them as metal sulfides. In the process, sulfide is supplied by the addition of a slightly soluble metal sulfide that has solubility somewhat greater than that of the sulfide of the metal to be removed. Normally ferrous sulfide is used (40).

Heavy metal sulfide sludges are less subject to leaching than hydroxide sludges. However, sulfide precipitation produces sludge in greater volumes than does alkaline precipitation. Separation of heavy metal sulfides by dissolved air flotation is also a viable alternative (41).

6.3.2.3. Chemical Reduction

Some heavy metals (e.g., chromium which is a common metal contaminant in pharmaceutical wastewater) have higher solubility in their higher valency (e.g., hexavalent chromium) than those in their lower valency (e.g., trivalent chromium). The general procedure is first to reduce the valency of chromium from +6 to +3, then second to precipitate the product, chromium sulfate at a suitable pH range by either alkaline precipitation or sulfide precipitation, forming insoluble chromium precipitates (either chromium hydroxide or chromium sulfide depending on the process method used). Sulfur dioxide, sodium bisulfite, sodium metabisulfite, and ferrous sulfate are strong reducing agents in aqueous solution, and are used for chromium reduction. The chromium precipitates can be removed by filtration, sedimentation clarification, or flotation clarification (41, 42).

Some heavy metals are bonded in organic compounds making their removal to be more complicated. A typical example is from Merck one of the largest pharmaceutical companies. The company used an organo-mercury compound (thinerosal, RSHgEt) as a slow killing

Table 19.8
Annual mass loadings from direct and indirect pharmaceutical wastewater discharges

	Mass loadings for direct dischargers (1,000 lb/year)				Mass loadings for indirect dischargers (1,000 lb/year)+			
	Subcategories A,B, & C		Subcategory D		Subcategories A,B, & C		Subcategory D	
Pollutants	Raw waste water	Final effluent	Raw waste water	Final effluent	Raw waste water	Discharge to POTW	Raw waste water	Discharge to POTW
Conventional pollutants								
BOD_5	83,000	5,900	4,100	300	169,000	169,000	5,600	5,600
TSS	45,000	4,600	1,200	290	64,500	64,500	3,000	3,000
Priority pollutants								
Volatile organics	2,000	77	240	6	2,400	2,000	18	18
Semivolatile organics	120	2	17	0.2	390	330	16	16
Pesticides	–	–	–	–	0.02	0.02	–	–
Metals	60	22	1.2	0.7	51	45	2	2
Cyanide	22	7	0.3	0.2	4.3	4.1	0.3	0.3
Nonconventional pollutants								
COD	192,000	44,000	7,500	800	411,000	411,000	24,000	24,000
Volatile organics	5,100	*	1,000	*	7,700	*	2,200	*
Semivolatile organics	59	*	10	*	87	*	25	*
Pesticides/Herbicides	63	*	11	*	92	*	26	*
Industry characteristics								
Number of facilities	30		21		130		155	
Wastewater flow, MGD	21.38		3.54		31.1		8.8	

– Negligible.
*Insufficient data available.
+1 lb = 0.4536 kg.

biocide in the fermentation process (43). They developed an at-source treatment technology to remove and recover mercury from the spent fermentation wastewater. The removal and reclamation of mercury from wastewater is accomplished by the following four steps:

1. Using aluminum (at pH = 11.5) to reduce the sulfur-hydrogen of thinerosal to release mercury at cationic state in water with the reaction:

$$Al \rightarrow Al^{3+} + 3e$$

and one of the following reactions:

$$2e^- + RSHgEt + 2H_2O \rightarrow RH + HSHgEt + 2OH^- \quad (4)$$

$$2e^- + RSHgEt + H_2O \rightarrow RH + HOHHgEt + S^{2-} \quad (5)$$

$$4e^- + RSHgEt + 2H_2O \rightarrow RH + \left[HHgEt\right] + S^{2-} + 2OH^- \quad (6)$$

(Note: since most of the biocides are associated with cell mass, caustic hydrolysis is used to release organomercury compound from cell paste before treatment.)

2. Using sodium borohydride to reduce mercury ions to the element state:

$$4Hg^{2+} + NaBH_4 + 8OH^- \rightarrow 4Hg + NaBO_2 + 6H_2O \quad (7)$$

This process is at the ambient temperature and at pH = 10; the pH should be maintained at 10 for about 10 min to complete the reaction. It should be noted that at low pH borohydride is unstable. For example, at pH = 7 the following reaction will occur:

$$BH_4^- + 4H_2O \rightarrow B(OH)_3 + OH^- + 4H_2$$

3. Applying ultrafiltration: the treated water is stirred for 1 h and the colloid mercury is separated by ultrafiltration; 99.7% removal can be reached (the Hg concentration in the effluent will be 110 ppb from an initial Hg concentration of 56 ppm).
4. Using granular activated carbon adsorption, the mercury concentration can be reduced from the 110 to 10 ppb. The overall mercury removal can be reduced by as much as 99.99% with the GAC filtration/polishing process (from an initial Hg concentration of 56 ppm to 10 ppb in the effluent). Mercury can be reclaimed from the filter cake of the ultrafiltration process.

6.3.3. Solvent Recovery and Removal

Solvents are used extensively in pharmaceutical manufacturing. Because solvents are expensive, most factories try to recover and purify them for reuse whenever possible. Solvent recovery and recycling is one of the in-plant source control operations, and is also an in-plant treatment process. Typical techniques used for solvent recovery are decantation, evaporation, distillation, extraction (13), and nebulization (44). Stripping has also been proved to be an effective method to recover solvents from pharmaceutical manufacturing processes.

6.3.3.1. Steam Stripping

Steam stripping transfers the volatile constituents of a wastewater to a vapor phase when steam is passed through preheated wastewater. The basic theory of steam stripping is associated with the partitioning of the organic compound in the vapor phase and in the wastewater phase. The partitioning coefficient (K_i), also called the vapor–liquid equilibrium constant, of compound i is expressed as follows:

$$K_i = V_i / W_i$$

where K_i is the partitioning coefficient, also called the vapor–liquid equilibrium constant, V_i is the mole fraction of organic compound i in the vapor phase, and W_i is the mole fraction of organic compound i in the wastewater phase.

K_i can be calculated, for low pressures, from

$$K_i = r_i (P_i / P)$$

where r_i is the activity coefficient of organic compound i in the wastewater at a certain temperature, P_i is the vapor pressure of the pure substance at the operating temperature, P is the total pressure.

Equations (9) and (10), show that the extent of separation is a function of the physical properties of the volatile compounds and the temperature and pressure in the stripper. The separation is also governed by the arrangement and type of equipment.

The process is performed in a steam stripper which has various types, such as packed tower, tray column, and steam flash tank. Flash tanks, which provide essentially one stage of liquid–vapor contact, are used to strip extremely volatile compounds. For the more difficult separations, columns filled with packing materials, which provide large surface areas for liquid–vapor contact, can be used.

Figure 19.5 shows the processes and flow directions in a typical column stripper. The solvent-containing wastewater is preheated allowing the components of the wastewater to separate by partial vaporization, then is introduced at the top or near the middle of the column and flows by gravity through the stripper. Steam is injected through a sparger and rises countercurrent to the flow of the water. When contacted with steam, the volatile organic compounds in a wastewater are driven into the vapor phase.

Solvent-containing wastewater and condensed overhead vapors from the stripper are allowed to accumulate in a gravity phase separation tank. Because the condensate mixes with fed wastewater accumulated in the tank, the solvent concentration increases to the point at which it is saturated with solvent, when a two phase mixture is formed. The difference between the specific gravities of water and solvents creates two immiscible liquid layers. One layer contains the immiscible solvents; the other layer is an aqueous solution which is saturated with solvents.

The solvent layer is pumped to storage. The solvent can be recovered by decanting the immiscible liquid layers, or by recycling the condensed vapors directly to the gravity phase separation tank. While the aqueous phase from the gravity phase separation tank is pumped through a preheater where the temperature is raised by heat exchange with the stripper effluent. After preheating, the solvent-saturated water is introduced with the feed wastewater at the top or near the middle of the column and flows by gravity through the stripper.

The hot effluent, which is discharged at the bottom of the stripper, is used as a heating medium in the feed preheater. The temperatures of the feed, overhead, and bottom are controlled at about boiling point. For example, the temperatures for a methylene chloride removal in packed column steam stripper are at about 85–100°C and with the highest for the bottom temperature and the lowest for the feed temperature (Table 19.9). The table indicates a poorer removal occurred under an upset condition when the overhead temperature is too low (< 85°C). The pressure is usually under atmospheric pressure.

This practice is particularly advantageous in cases where the wastewater to be stripped contains low concentration of the recovering solvents. The most economical operation of a wastewater steam stripper occurs when the feed is saturated with the solvent to be recovered. The composition of the recovered solvent and economic factors determines whether the solvent is reused within the plant, disposed of, used as incinerator fuel, sold to solvent reclamation facility, or sold for other users. Solvents recovered by steam stripping are normally not used directly in pharmaceutical synthesis because of the US FDA purity requirements.

If the feed contains high concentrations of suspended solids, a filter may be installed prior to the preheater to prevent fouling in the preheater and the column.

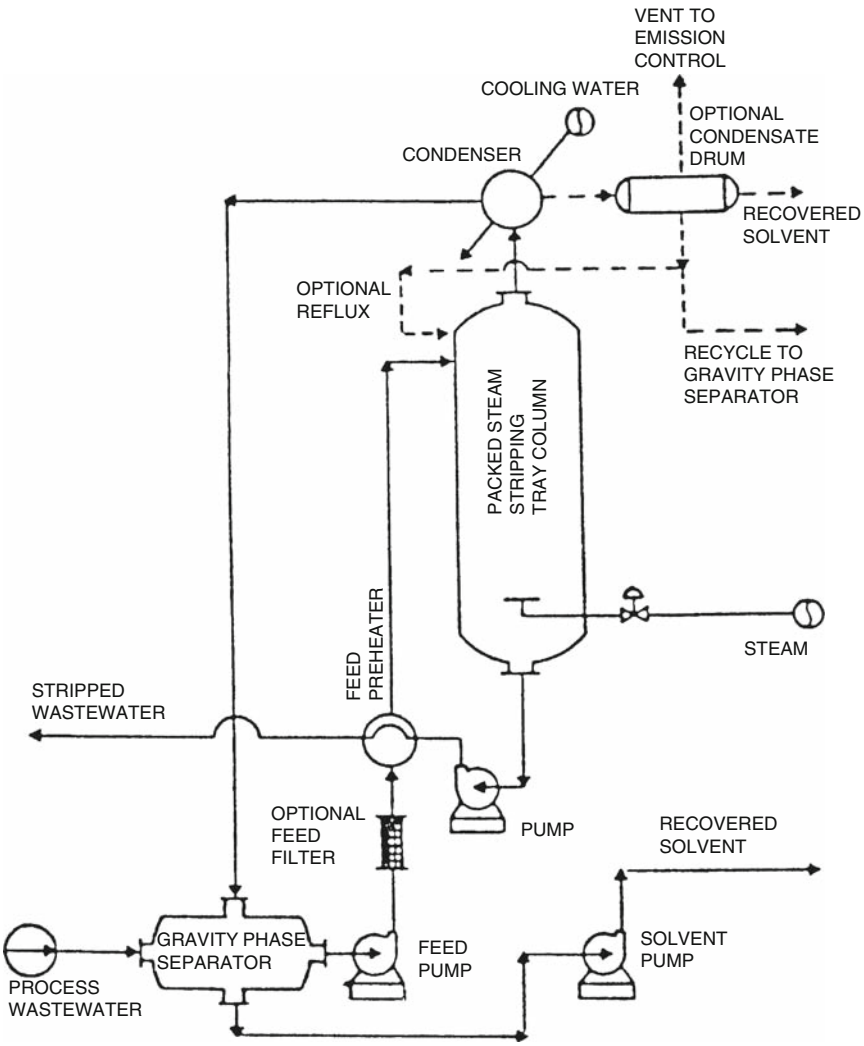

Fig. 19.5. Equipment for stream stripping solvents from wastewater (13).

Steam stripping usually is a pretreatment method. It can effectively remove solvent from wastewater. Steam stripping has been successfully used to remove methylene chloride, toluene, chloroform, and benzene.

Many factories have reported that steam stripping enables the plants to meet a POTW requirement that the concentration of explosive vapors in the plant sewer pipes not exceed 40% of the lower explosion limit (LEL). Moreover, it has been reported (13) that greater than 99% removal and an effluent with less than 10 mg/L concentration have been achieved for a toluene wastewater. The stripped wastewater is combined with other process wastewater

Table 19.9
Summary of BPT Regulation (11,12)

Parameter	Maximum 30-day average	Daily maximum
BOD_5 (mg/L)	Reduction 90% from raw waste	
COD (mg/L)	Reduction 74% from raw waste	
pH (unit)	6.0–9.0	
TSS (mg/L)	1.7 times BOD concentration limitation	–
Cyanide (mg/L)		
Alternative A[a]	9.4	33.5
Alternative B[b]	9.4 (0.35) R	33.5 (0.18) R

[a] Alternative A: Measure at effluent from cyanide destruction unit. Applies only when all cyanide-bearing wastes are diverted to a cyanide destruction unit and subsequently are discharged to a biological treatment system.

[b] Alternative B: Measure at final effluent discharge point. R: equals the dilution ratio of the cyanide contaminated waste streams to the total process wastewater discharge flow.

in another pretreatment system for further end-of-pipe treatment, or further combined with sanitary wastewater then discharged to the POTW.

6.3.3.2. Air Stripping

Air stripping is also used to recover volatile organic compounds, such as benzene, chloroform, 1,1,1-trichloroethane, 1, 2-dichloroethane, ethylbenzene, methyl chloridem tetrachloroethylene, trichloroethylene, and toluene in pharmaceutical plants. The air stripping process is similar to steam stripping. The basic theory of air stripping is associated with the partitioning of the organic compound between air and wastewater.

6.3.3.3. Other Methods of Solvent Removal

Carbon adsorption can also be used to remove organic solvents from a segregated waste stream, especially in small quantities. Carbon adsorption method is widely used in tertiary treatment.

The feasibility and extent of recovery and purification are governed largely by the quantities involved and by the complexity of the solvent mixtures to be separated. If recovery is not economically practicable, the used solvents may have to be disposed of by means of incineration, landfilling, or contract disposal. It is expected that some solvents can still be present in the wastewater even after an effort for recovery. Further removal of solvents can be accomplished in the end-of-pipe treatment in the combined overall waste stream.

6.4. End-of-Pipe Treatment

End-of-pipe treatment is mainly designed to treat a number of pollutants in a plant's overall waste stream before it is discharged directly to a body of surface water, although it is sometimes used for pretreating the waste stream when a wastewater is designed for indirect discharge, i.e., discharging to the POTW for further treatment. The pretreatment for pharmaceutical waste is mainly for reducing the toxicity of the wastewater in order not to be

harmful for the biological treatment system. Pretreatment is mainly accomplished by the so called in-plant treatment as stated previously. This section discusses the end-of-pipe treatment for direct discharge.

Generally, a secondary treatment facility is needed for an end-of-pipe treatment for pharmaceutical wastes (13). The treatment schemes involve primary treatment (screening, equalization, neutralization) followed by either a secondary biological treatment or a secondary physicochemical treatment. Additional tertiary treatments may also be needed.

6.4.1. Primary Treatment

The common primary treatment methods in the pharmaceutical industry are: (a) coarse solid removal by screening; (b) primary sedimentation, applying gravity separation to remove grit and settleable solids and using a skimmer to remove floating oil and grease; (c) primary chemical flocculation/clarification; and (d) dissolved air flotation.

6.4.1.1. Equalization and Neutralization

Flows are usually required to be equalized, especially if the waste from the production plant is not equally distributed (either in flow rate or in waste characteristics) around the clock. In this case, an equalization tank is needed to minimize or control fluctuations in wastewater characteristics to provide optimum conditions for the subsequent treatment processes. The main benefits of equalization are:

1. Providing continuous feed to biological systems over periods when the manufacturing plant is not operating
2. Providing adequate dampening of organic fluctuations to prevent shock loading to biological systems
3. Preventing high concentrations of toxic materials from entering the biological systems
4. Minimizing chemical requirements necessary for neutralization

Also, neutralization and nutrients addition can be accomplished in the equalization step. A pH between 6.5 and 8.5 should be maintained in a biological system to ensure optimum biological activities. Neutralization is important for chemical synthesis plants as shown in Table 19.2.

Neutralization is performed by adding basic or acidic substances depending on the pH of the waste stream. An economical option is by adding a proportional combination of acid and basic wastewater streams.

The raw materials used in fermentation and biological product extraction manufacturing are mainly from natural plants and animals. Nutrients (such as nitrogen and phosphorous) may not be needed. However, for some other wastes, nutrients addition may be necessary prior to biological waste treatment. Mixing is usually provided to ensure adequate equalization and to prevent settleable solids from depositing in the basin (45).

6.4.1.2. Screening and Sedimentation

All waste flows should be passed through screens to remove large suspended matter, and through sedimentation tanks to remove suspended solids. Rectangular gravity clarifiers are usually used for primary sedimentation, although circular gravity tanks are equally efficient.

Chemical coagulation and flocculation can also be combined with primary treatment to increase TSS removals.

Primary treatment is important for the efficiency of subsequent secondary treatment, which may remove 20–50% of 5-day BOD.

6.4.1.3. Primary Flotation Clarification

When conventional sedimentation cannot effectively remove suspended solids or oil and grease, primary flotation may be used instead before secondary treatment (46, 47).

In dissolved air flotation, wastewater is pressurized to 50–90 psi (347–624 kPa) in the presence of sufficient air to approach saturation (40, 45, 48, 49). When the pressure in the air–liquid mixture is released to atmospheric pressure in the flotation unit, micro air bubbles are released from solution. The suspended solids or oil globules are floated by these micro air bubbles, rising to the surface where they are skimmed off.

6.4.2. Secondary Biological Treatment

6.4.2.1. Activated Sludge

Activated sludge is the most widely used secondary biological process for treating pharmaceutical wastewater (50–57). It is mainly used for medium and large wastewater flows.

A typical activated sludge treatment system consists of an aeration tank for aerobic biological treatment, a secondary clarifier for solid separation and an activated sludge return system for sludge recycle (58). The aeration tanks are loaded with the equalized, neutralized, and pretreated wastewater. In the aerobic biological degradation, the soluble biodegradable wastes are transferred to insoluble microbial biomass.

The secondary sedimentation clarifiers settle the biosolids from the biologically treated wastewater, resulting in a clear effluent which meets the standards (mainly the BOD and TSS) for direct discharge. The major part of the settled biosolids is further treated before disposal or reuse. A part of the settled biomass is returned to the aeration tank as the return activated sludge.

The return activated sludge is fed to the aeration tank to ensure a sufficient amount of microbial population for the degradation of the organic waste is present. The biomass is measured by the Mixed Liquor Volatile Suspended Solids (MLVSS).

Complete mixing and adequate aeration are essential in the aeration tanks. Sufficient oxygen should be furnished to maintain dissolved oxygen throughout the aeration volume.

There are various types of modes for operating the activated sludge system, such as conventional, extended aeration, step aeration, contact stabilization, and completely mixed. Figure 19.6 shows the flow diagrams of a few selected activated sludge processes. The treatment mode is selected according to the characterization of the wastes and the goal of treatment (59–61).

Once maximum and normal raw waste loads and flows have been determined, the design criteria for the biological treatment plant can be established. In addition to the removal 5-day BOD and suspended solids, some toxic organic matters are slightly reduced during the process. Activated sludge treatment systems can be designed for the purpose of nitrogen removal by operating the system to accomplish nitrification and denitrification (62, 63).

Some activated sludge treatment systems experience severe filamentous microorganisms buildup accompanied with very poor settling. A pilot-scale experiment was conducted to

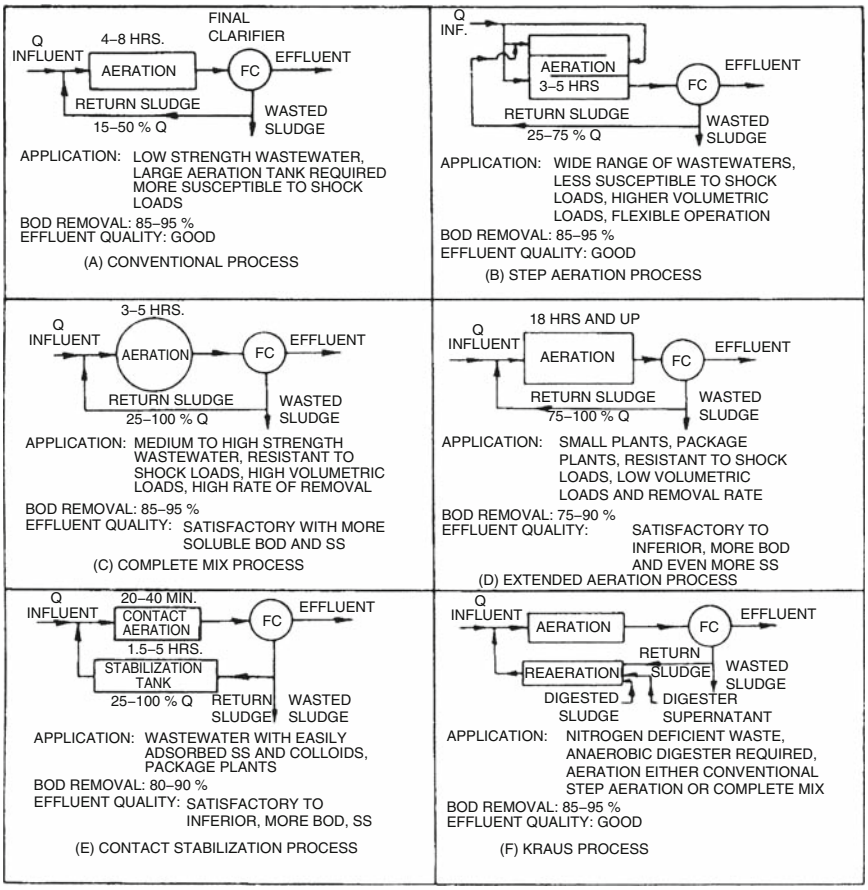

Fig. 19.6. Flow diagrams and applications of major activated sludge processes.

improve sludge settling for a nitrifying activated sludge system, treating 1.2 MGD (4.54 MLD), equivalent to 10,000–15,000 kg 5-day BOD per day, of pharmaceutical wastewater from both synthetic and fermentation processes. The concentration of filamentous organisms and the mixed liquor sludge volume index (SVI) can be reduced by changing the aeration pattern from three aeration basins in parallel flow to three completely mixed compartments in series. Such process change results in reducing the filamentous population and improving settling characteristics.

Alternatively, a secondary flotation clarifier can be adopted to replace a secondary sedimentation clarifier to solve the problems of sludge bulking and rising (40, 58, 64).

According to Mayabhate et al. (65), an oxidation ditch activated sludge system was capable of providing acceptable treatment for pharmaceutical wastes.

Datta Gupta et al. (28) described a complete treatment system for antibiotic production wastewater including lime neutralization, clarification, activated sludge treatment, post-aeration, and chlorination. The effluent was disposed of by irrigation while the biosolids were dried and utilized as fertilizer.

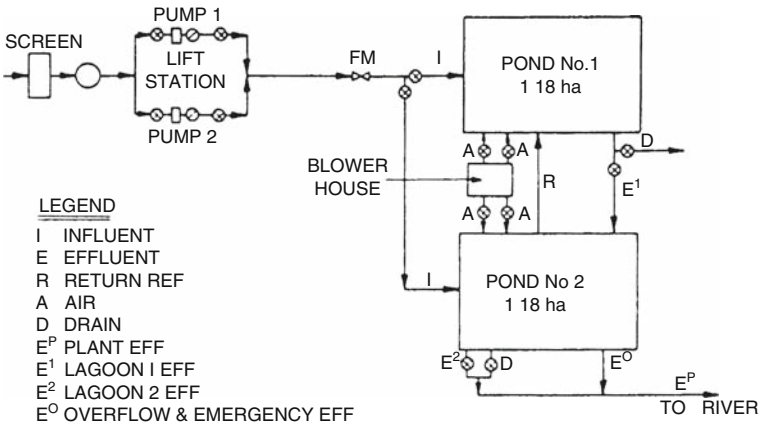

Fig. 19.7. Aerated lagoon system.

Schumann (66) described a treatment system for high strength pharmaceutical wastewater which included neutralization and aerobic activated sludge treatment with aerobic sludge stabilization (29).

6.4.2.2. Aerated Lagoon

Aerated lagoons are usually rectangular in shape, with a length-to-width ratio of 2 to 1. The depth of lagoons is usually about 8–12 ft (2.44–3.66 m). The lagoon bottom and sides are lined and have a freeboard of at least 3 ft. About 1–2 months of retention time is required for treatment by an aerated lagoon. The detention time and waste loading determine the required lagoon volume which in turn determines the surface area of the lagoon (67).

Complete mixing and adequate aeration are essential. Sufficient oxygen should be furnished to maintain dissolved oxygen throughout the entire 8–12-ft depth (Fig. 19.7). Aerators should be spaced to provide uniform blending for dispersion of dissolved oxygen and suspension of microbial mass. The oxygen provided for aerated lagoons is commonly provided by mechanical aeration, diffused aeration, or by induced surface aeration. The mechanical aeration units can be either floating or platform-mounted.

The aerated lagoon is the second widely used biological treatment method for treating pharmaceutical wastewater. It is mainly used for relatively small plants and can achieve 85–95% reduction of 5-day BOD.

6.4.2.3. Trickling Filter

Trickling filters are fixed film reactors using a biological process for wastewater treatment (68). It is widely used in pharmaceutical waste treatment for plants medium to large in size. The filter medium consists of a bed of coarse material such as broken stones, plastic rings, corrugated plastic sheets, or plastic tubes over which wastewater is distributed. The plastic media are predominant for high rate filters such as for strong industrial wastewaters with high loading rates. Nitrification–denitrification can be accomplished by using low loading rates and multistage trickling filtration.

Wastewater is applied to trickling filters by a rotary distributing system. The wastewater then trickles downward through the media, on which a zoogleal slime layer is formed (Fig. 19.8). Dissolved organic material in the wastewater is transported into the slime layer where biological oxidation takes place. The effluent liquid is then collected by an underdrain system. Organic removal occurs by adsorption and assimilation of the soluble and suspended waste materials by microorganisms attached to the media. Oxygen for the process is supplied from air circulating through the interstices between the filter media which increases dissolved oxygen in wastewater.

The quantity of biological slime produced is controlled by available food. Growth will increase as the organic load increases until a maximum effective thickness is reached. This maximum growth is controlled by physical factors including hydraulic dosage rate, type of media, type of organic matter, amount of essential nutrients present, temperature, and the nature of the particular biological growth. During trickling filter operations, biological slime is sloughed off, either periodically or continuously. The sloughed biomass is removed in the subsequent clarification process. Recirculation of trickling filter effluent is practiced in high rate trickling filters which improve the filter efficiency.

The overall performance of trickling filters is related to the hydraulic and organic loading. The performance can be correlated to either hydraulic loading or organic loading when the BOD concentration in wastewater and the depth of the filter remain constant (68–70). Other factors that affect the performance of trickling filter plants include the specific surface area of media, flow distribution and dosing frequencies, wastewater temperature, recirculation rate, underdrain and ventilation system, filter staging, and secondary clarification (68, 71, 72).

It is important to note that either sedimentation clarifiers or dissolved air flotation clarifiers can be used as the secondary clarification units for separating the biomass from the effluent of trickling filters (64).

6.4.2.4. Anaerobic Treatment

Anaerobic treatment involves the breakdown of organic wastes to gas (mainly methane and carbon dioxide) in the absence of oxygen. This process involves two steps: the breakdown of organics by facultative and anaerobic organisms to organic acids, and the subsequent breakdown of these acids to methane and carbon dioxide (51, 73).

Since the anaerobic process has less cell synthesis than that in the aerobic system, the nutrient requirements are correspondingly less. The conversion of organic acids to methane gas yields little energy. The rate of growth is slow, and the yield of organisms by synthesis is low. Therefore, the kinetic rate of removal and the sludge yield are considerable less than those in the activated sludge process or the trickling filter process. Figure 19.9 illustrates several anaerobic processes that have been used in the treatment of pharmaceutical wastewater (74–77).

The conventional anaerobic treatment process provides a continuous or intermittent feeding without solids separation. The detention time is usually 10–30 days and the minimum time is 3–5 days.

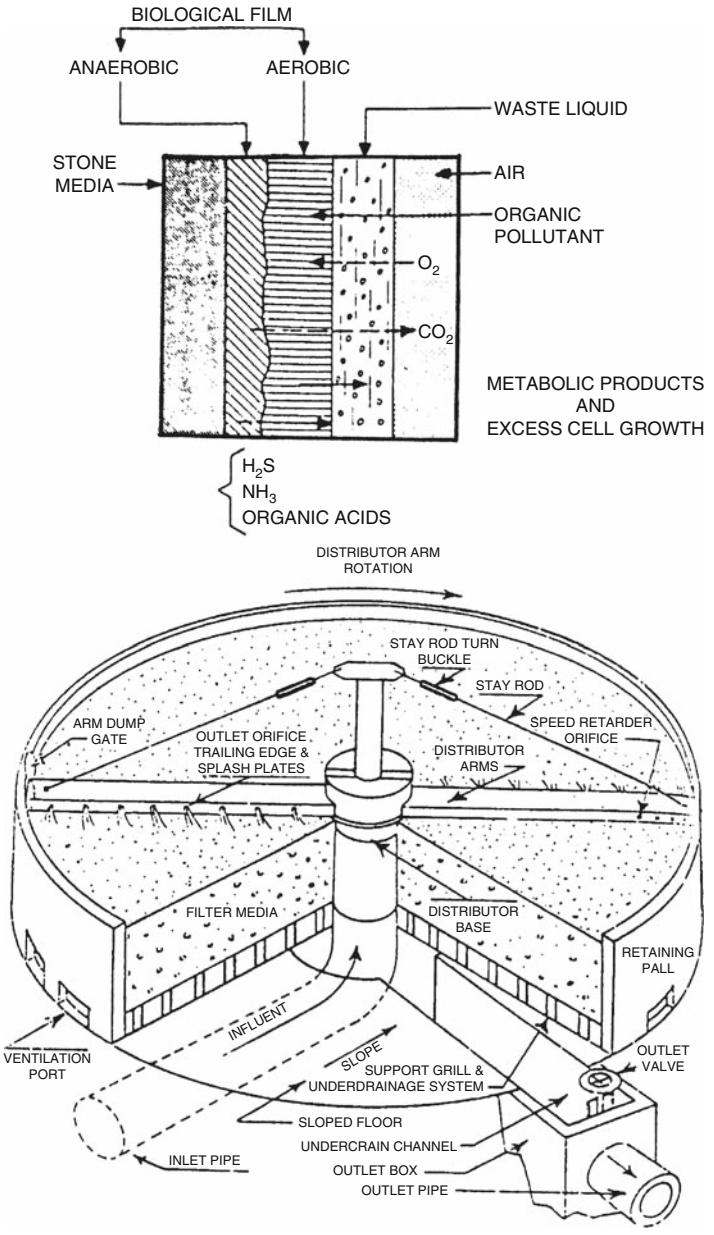

Fig. 19.8. Trickling filter.

An anaerobic-contact process provides for separation and recirculation of seed organisms, therefore allowing process operation at detention periods of 6–12 h. A 90% removal of COD was reported for wastewater at a loading of 2.5 kg COD/m^3/day (78).

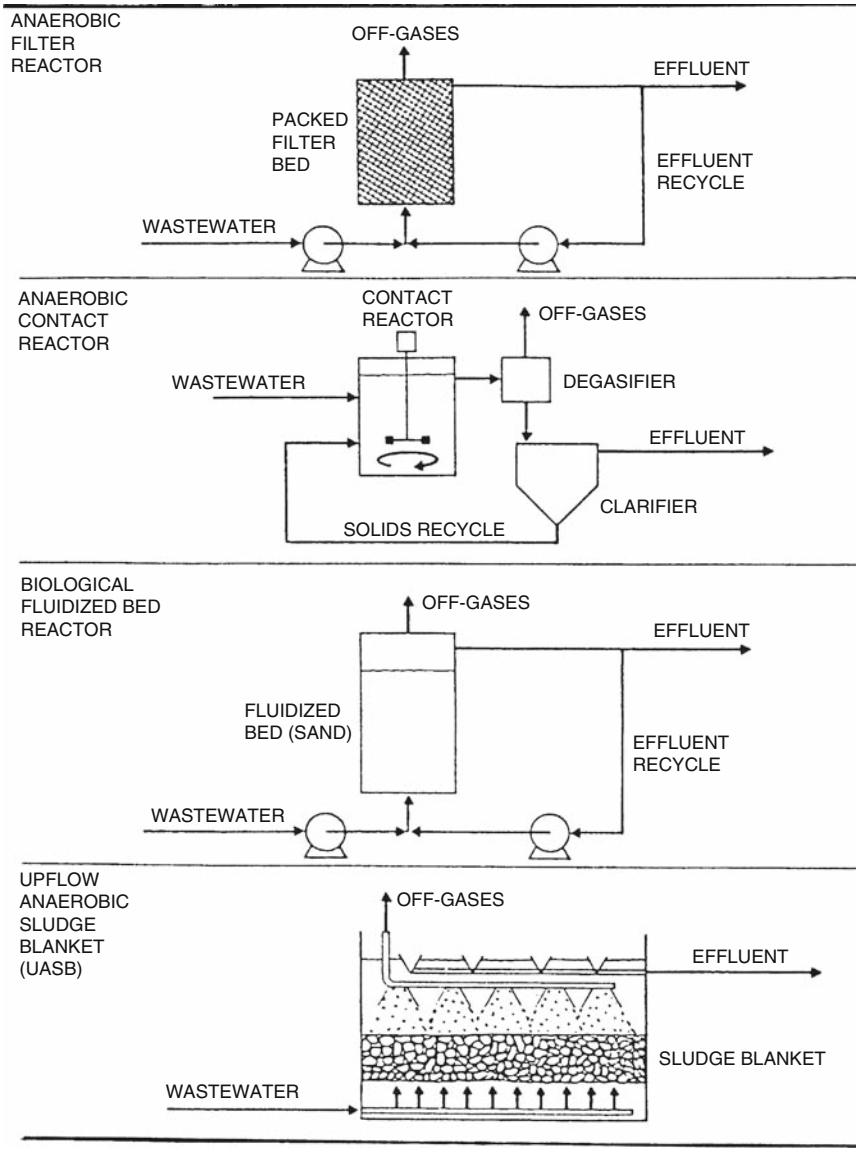

Fig. 19.9. Anaerobic wastewater treatment processes.

In an anaerobic filter, the growth of the anaerobic microorganisms occurs on the surface of packed media. The filter is operated either in the upflow or downflow mode and part of the effluent is recirculated. The packed filter media also provide for the separation of solids and the gas generated in the anaerobic process. Jennet and Dennis (79) treated pharmaceutical wastewater and achieved a 97% removal of COD at a loading of 3.5 kgCOD/m^3/day at 37°C. Sachs et al. (80) used an anaerobic filter to treat biological or chemically synthesized pharmaceutical

wastewater. With a loading of 0.56 kg COD/m^3/day at 35°C and 36 h hydraulic retention time, achieved 80% COD removal.

In a fluidized bed reactor, the wastewater is pumped upward through a sand bed. Part of the effluent is recycled. Stronach et al. (81) utilized anaerobic fluidized beds to treat two types of wastes. The first waste, a propanol-containing waste, was nutrient limited and caused inhibition of methanogenesis; whereas the second waste, a methylformamide-containing waste, appeared to contain a non-biodegradable and toxic fraction which did not inhibit methanogenesis but caused a reduction in COD removal and erratic volatile acids production. The feed flow had a COD concentration of 2,500 mg/L was applied at an organic loading rate of 4.5 kg COD/m^3/day and with a hydraulic retention time of 0.53 day. Final COD removal was 54 and 45% for the first and second wastes, respectively.

In an upflow anaerobic sludge blanket process reactor, wastewater is directed to the bottom of the reactor where it is distributed uniformly. Methane and carbon dioxide rise upward and are captured in a gas dome. The flow passes into the settling portion of the reactor where solid–liquid separation takes place.

An anaerobic degradation of pharmaceutical antibiotic fermentation wastewater was studied at a pilot scale (82) and then was applied to a full-scale treatment plant. The waste contained a high proportion of suspended solids representing about 40% of the COD as well as residual amounts of antibiotics, extraction solvents, grain flours, sugars, protein, and nutrients. Four treatment configurations were piloted: a downflow anaerobic filter, a downflow/upflow anaerobic filter, an upflow anaerobic sludge blanket, and a low rate anaerobic reactor. The high rate systems were ultimately incapable of assimilating the feed pollutants, resulting in excessive loss of biomass and, therefore, low soluble COD removals. The low rate system adequately hydrolyzed the feed pollutants and yielded 70% COD and 80–90% TSS removals. The presence of antibiotic residuals did not affect the system.

Shafai and Oleszkiewicz (83) investigated the anaerobic ammonification of wastewater from an estrogen extracting pharmaceutical plant. Both flow-through and batch anaerobic reactors were used to treat a waste with high loading of total dissolved solids (TDS), TKN nitrogen, and total organic carbon (TOC). It was found that TDS concentrations over 17 g/L in the flow-through reactors and in excess of 10 g/L in the batch reactors to be inhibitory to both ammonification and methanogenesis.

Anaerobic treatment has also been used as an additional treatment to supplement the main treatment system. One example is at the Abbott Laboratories in North Chicago, Illinois. The health care products manufacturer operates a large fermentation and chemical synthesis plant. The total wastewater flow from the factory is 0.92 MGD (3.48 MLD); the COD, BOD, and TSS loads are 25,000, 11,500, and 3,500 lb/day, respectively (11340, 5216, and 1588 kg/day, respectively). About 70–85% of the waste is from the fermentation process. The wastewater flow was treated in an extended aeration activated sludge plant. To accommodate the growth and expanding load from the fermentation process, a low-rate anaerobic reactor was added as a pretreatment step for the high strength fermentation wastewater prior to aerobic treatment. The anaerobic reactor was also used for the digestion of the raw waste solids from fermentation and for the wasted sludge from the aerobic system. The flow diagram of the treatment plant is shown in Fig. 19.10. The low-rate anaerobic reactor performance operating at a temperature

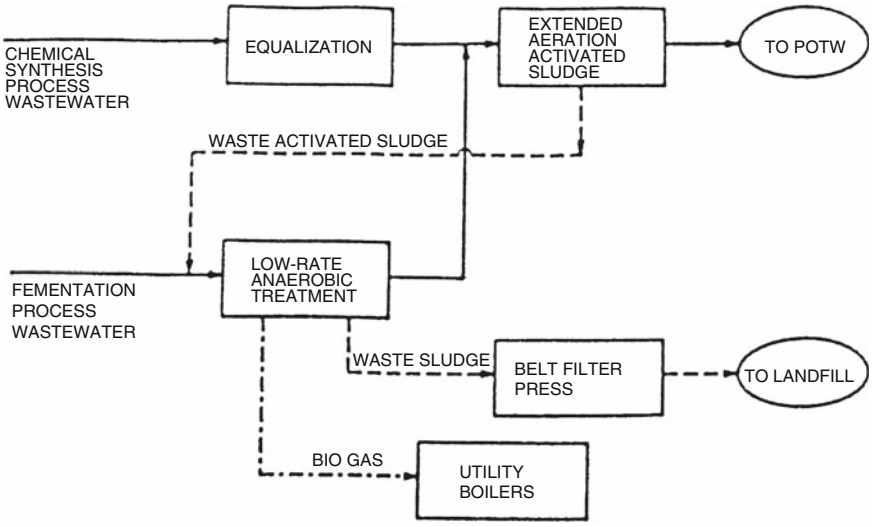

Fig. 19.10. Aerobic–anaerobic treatment of chemical synthesis and fermentation wastewater effluents.

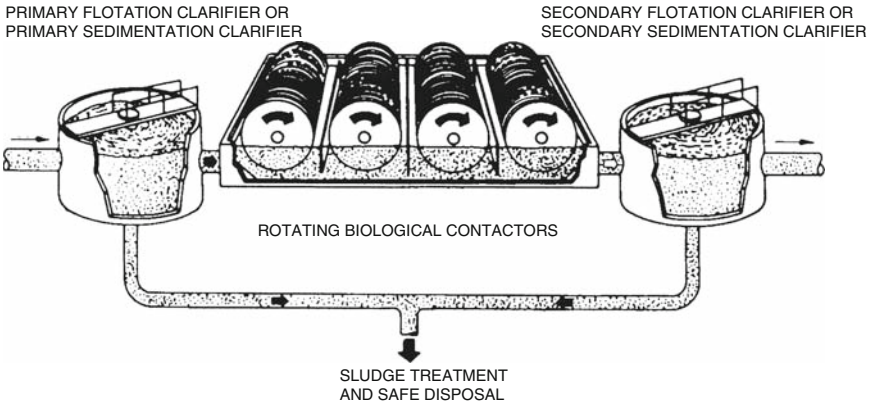

Fig. 19.11. Schematic diagram of rotating biological contactors.

of 28.5–32.5°C and with a hydraulic retention time of 9.5–10.0 days was as follows: 79% removal of COD, 86% removal of 5-day BOD, and 83% removal of TSS

6.4.2.5. Other Biological Treatment Methods

Other biological treatment methods utilized in pharmaceutical wastewater are waste stabilization ponds (67), rotating biological contactors (84–86) (see Fig. 19.11, polishing ponds, sequencing batch reactors (87), and sequencing batch biofilters (88). For detailed description of these processes the readers are referred to the books: *Biological Treatment Processes* (51) and *Advanced Biological Processes* (73).

6.4.3. Tertiary Treatment

Tertiary treatment using physicochemical processes is usually applied for further improving the quality of the secondary effluent following biological treatment. Examples of these additional treatment methods are the polishing pond, coagulation/flocculation/clarification, secondary neutralization, chlorination, ion exchange, and filtration (multimedia, sand, and granular activated carbon) (40, 89).

6.4.3.1. Filtration and Carbon Adsorption

Filtration is widely used for polishing wastewater. The most common filter type is a multimedia of activated carbon and sand. The filter needs a periodical backwash and is used mainly for removal of relatively coarse particles. Granular activated carbon is more versatile in dealing with various kinds of small suspended solid particles, colloidal, and dissolved pollutants.

Carbon adsorption uses activated carbon which has a great specific surface area (surface area per unit volume) to effectively adsorb pollutants (40, 89). Granular activated carbon is an effective and economical adsorbent because besides its higher specific surface area it has a high hardness which lends itself to reactivation and repeated use.

The granular activated carbon adsorption process is usually preceded by preliminary filtration or clarification to remove insoluble particles. Once the carbon is depleted, it can be reactivated by heating to a temperature between 1,600 and 1, 800°F (871–982°C) to volatilize and oxidize the adsorbed contaminates. Oxygen in the furnace is normally controlled at less than 1% to avoid loss of carbon by combustion (13).

The application of carbon adsorption in pharmaceutical industry is limited. Most of the priority pollutants (heavy metals, volatile organics, and cyanide) are generally reduced more effectively and with less cost by other technologies. This method is particularly applicable in situations where pollutants in low concentrations not amenable to treatment by other technologies must be removed from waste streams. Holler and Schinner (90) arrived at the same conclusion and stated that for economic reasons carbon adsorption should be mainly used as a tertiary treatment for final polishing of secondary ef1luents. Bauer et al. (91) used activated carbon filtration in an activated sludge system to remove toxic compounds. More details on the removal of organics and toxic material from pharmaceutical wastewater effluents can be found in (92–100).

Besides the usage of granular activated carbon as a filtration media, powdered activated carbon (PAC) has been used as an additive in an activated sludge system (26). One of the experiments showed that the MLSS concentration increased from 5,850 to 8,830 mg/L as the PAC dosage to the influent was increased from 208 to 1,520 mg/L. The 0.7 mg/mg PAC dosage resulted in 50% additional removal of COD.

6.4.3.2. Coagulation/Flocculation/Clarification

Coagulation is a process used for the removal of colloidal and fine suspended particles (101, 102). Kharlamova et al. (103) used alum, lime, and bentonite clay as coagulants to treat pharmaceutical waste effluents. The treated effluents had lighter coloring and increased transparency. The reduction in BOD and COD, however, was limited. On the other hand,

Table 19.10
Pretreatment pollutants standards(13)

Pollutant	No. of occurrences in wastewaters	Max. wastewater concentration level (μg/L)
Cyanide	5	590
Acrolein	2	100
Acrylonitrile	1	100
Benzene	6	580
Carbon tetrachloride	1	300
Chlorobenzene	2	11
1,2-dichloroethane	2	290
1,1,1-trichloroethane	4	360,000
1,1-dichloroethane	3	27
Chloroform	6	1,350
l,l-dichloroethylene	2	10
1,2-trans-dichloroethylene	1	550
Ethylbenzene	3	21
Methylene chloride	9	890,000
Bromoform	1	12
Tetrachloroethylene	1	2
Toluene	6	1,050
Trichloroethylene	1	7

the researchers were successful in destroying synthetic surfactants used in the production of antibiotics using hydrogen peroxide as an oxidant and iron and aluminum ions as catalysts. However, flocculation and coagulation may not be effective or cost efficient for pharmaceutical wastewater treatment although it is able to reduce COD concentrations (65).

PAC can also be applied to a coagulation/flocculation/clarification system for removal of toxic substances (64). Clarification can be either a sedimentation clarification or a flotation clarification.

6.4.3.3. CHLORINATION

Chlorination as a means of disinfection is needed before the discharge of effluent after biological treatment. For example, post-aeration and chlorination are used in addition to activated sludge treatment for wastewater treatment at a penicillin production facility (28).

Table 19.10 shows a summary of end-of-pipe treatment methods used for wastewater treatment in the pharmaceutical industry. It is estimated (13) that the activated sludge process is the most widely used biological treatment method, at about 60% of the biological treatment plants. Physicochemical treatment methods have been used in only 20% of the plants out of which, thermal oxidation is the most widely used.

6.4.4. Residue Treatment and Waste Disposal

A large proportion of the material input to the manufacturing process ends up as process waste. Fermentation and biological extraction, as well as the formulation processes, are typical

examples. Besides excess sludges generated during production processes, sludge can also be generated in the processes of pretreatment, primary treatment, secondary treatment, and tertiary treatment.

Fat and oil may also occur during biological extraction manufacturing procedures, which are skimmed-off in flotation or settling tanks. The sludges generated in the pretreatment stages usually contain contaminants such as traces of solvents and heavy metals. Organic contaminants in the sludge are either (a) traces of solvents used in the fermentation, chemical synthesis and biological extraction manufacturing steps; or (b) reactants or by-products of the chemical synthesis steps. Biological sludges, also known as biosolids, need to be thickened, dewatered, conditioned, and stabilized before disposal. Disposal methods of sludge include incineration, landfill, and reuse. In the latter two cases, sludge stabilization and disinfection will be needed (29, 66).

Recovered solvents may be used as fuel for incineration or other kinds of beneficial uses. Fats and oil may be incinerated or landfilled along with sludge or may also be transferred to other industry such as soap manufacturing to be used as raw materials. Such a beneficial usage of residue is one of the waste exchange programs that should be encouraged.

Sludge may be spread on land for agricultural purposes (104) or sold as an animal feed supplement. However, the wasted biological sludges are generally contaminated with varying degrees of potentially toxic materials, which may exclude the above two types of beneficial usage.

Wickramanyake (105, 106) discussed the treatment of sludge generated at a DNA processing facility. The sludge consisted mainly of biological solids (i.e., biosolids) such as cells and cell debris. The solid levels in the sludge samples can vary depending on the process used to concentrate solid materials. The solids content and physical properties of biosolids significantly affect decontamination processes including incineration, thermal (dry-heat and steam) treatment, gamma and electron radiation, microwave radiation, and chemical decontamination (29). Each of these microbial inactivation techniques can be effective in the treatment of the DNA biosolids. Since verification of the extent of decontamination is difficult with biosolids, high safety factors should be incorporated into the design of treatment units and good maintenance and operating procedures should be employed.

Incineration may not be legally practiced in some areas, such as New York City. The New York City Department of Environmental Protection has developed comprehensive plans to handle sludge problems (107). The plan includes heat drying, composting, chemical stabilizing of dewatered biosolids, landfilling (mainly for toxic-containing biosolids), and, more importantly, beneficial usage. The beneficial applications include the spreading of biosolids on or just below the surface of land to benefit soil and plants and as a substitute for soils imported by the city for daily cover at active landfills or as capping material for closed landfills.

7. CASE STUDY

This section uses a factory producing antibiotics by fermentation as an example of waste generation and end-of-pipe treatment in the fermentation pharmaceutical industry.

7.1. Factory Profiles

Ansa, a plant at Izmit, Turkey, produces antibiotics pharmaceutical products by fermentation. It has the capacity to produce 120 metric ton/year of tetracycline and oxytetracycline derivatives and 1.5–2.0 metric ton ton/year of gentamicin sulfate. The following description covers the period when the production rate of the factory was 50–60% of full capacity. The production was carried out year round, 7 days a week and 24 h a day with three shifts. The maximum daily production capacity was 400 kg/day for tetracycline and oxytetracycline, and 20 kg per 3 days (intermittent production) for gentamicin (108).

7.2. Raw Materials and Production Process

The production used different raw materials from agricultural sources and used various chemicals (Table 19.11).

Figure 19.12 shows the production mode. A bacterial-based mycelium was first produced in the microbiology laboratory.

The fermentation involved two phases: solubilization of antibiotics by acidification and filtration. The whole process was carried out on a batch basis.

The processes following the filtration of fermentation product were slightly different between tetracycline and oxytetracycline production and gentamicin production. For tetracycline and oxytetracycline production, the fermentor filtrates were treated by extraction, pH adjustment, filtration, precipitation, centrifugation, complex formation and crystallization, and purification, before yielding the final product. For gentamicin production, the filtrates were treated by extraction, chromatographic resin adsorption, evaporation, filtration, crystallization, or spray drying to yield the final product.

7.3. Waste Generation and Characteristics

The production generated 33 sources of wastewater discharges. They can be grouped into seven main processes:

1. Wastewaters from fermentation processes (strong)
2. Wastewaters from extraction and purification processes (strong)
3. Wastewaters from recovery process (strong)
4. Floor and equipment washings (dilute)

Table 19.11
Solubility Products (K_{sp}) for Insoluble Metal Salts (13)

Compound	K_{sp}	Metal Ion Conc. (μg/L)
CuS	6×10^{-36}	1×10^{-10}
NiS	2×10^{-25}	8×10^{-6}
ZnS	1.6×10^{-25}	2×10^{-5}
$Cu(OH)_2$	3.5×10^{-19}	25
$Ni(OH)_2$	1.5×10^{-15}	400
$Zn(OH)_2$	1.8×10^{-14}	1×10^{-3}

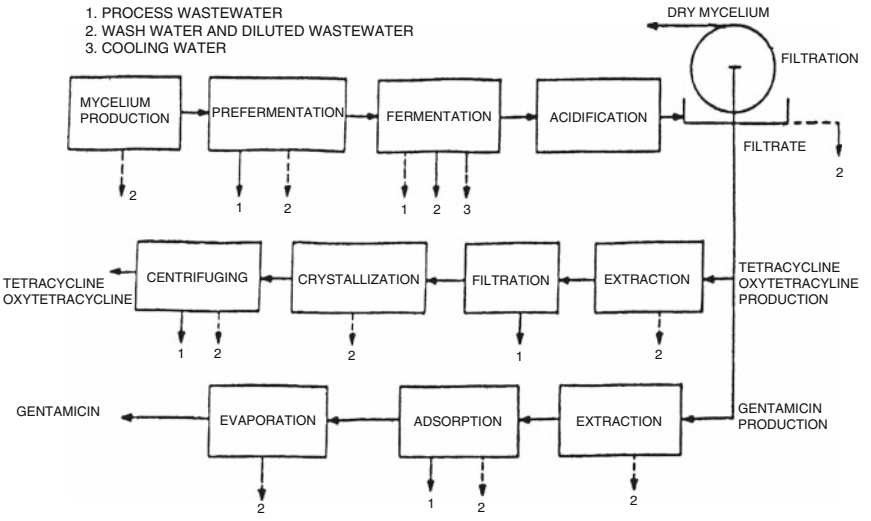

Fig. 19.12. Antibiotic production process system (108).

5. Laboratory wastes, miscellaneous wastes (varied)
6. Sanitary wastes
7. Waste cooling water (uncontaminated)

These waste streams can be further grouped into three groups: the strong process wastes, the diluted wastes, and cooling water. The strong process wastes from fermentation process, extraction and purification processes, and recovery process. The diluted wastes from the floor and equipment washings, laboratory wastes, and miscellaneous wastes (varied). The cooling water was confined, without contacting with processing water, which, in fact, was uncontaminated and generated no waste.

The flow rates for the three main streams were:

1. Strong process wastes: $Q = 120\,\mathrm{m}^3/\mathrm{day}$
2. Diluted wastes: $Q = 160\,\mathrm{m}^3/\mathrm{day}$
3. Cooling water: $Q = 1,000\,\mathrm{m}^3/\mathrm{day}$

Table 19.12 lists the flow and concentrations of some major traditional wastes for the above first two major types of wastewater. The process wastes were very strong in organic content, having a 5-day BOD of 13,500 mg/L, a COD of 34,000 mg/L, and a BOD/COD ratio of 1:2. The total loads were 1,680 kg/day of 5-day BOD and 4,180 kg/day of COD. The diluted wastes had 400 mg/L of 5-day BOD and 600 mg/L of COD.

In fact, full segregation of the strong and dilute waste streams was not possible due to the complexity of existing piping system. The process wastes and dilute wastes were actually diluted with the wasted cooling water down to a 5-day BOD of 8,400 and 50 mg/L, respectively, and the flow rates at 200 and 800 $\mathrm{m}^3/\mathrm{day}$, respectively, instead of as shown in

Table 19.12
Methylene chloride removal in packed column steam stripper (13)

Sample number	Feed temp. (°C)	Overhead temp. (°C)	Bottoms temp. (°C)	Feed rate (gpm)	Steam rate (L/h)	Methylene chloride (mg/L)	
						Influent	Effluent
1	87	97	104	9.6	160	NA^a	0.926
2	86	98	102	8.9	160	NA	5.10
3	86	94	101	9.0	150	NA	4.94
4	86	89	102	9.0	150	NA	3.00
5	85	89	102	9.0	150	NA	1.99
6	85	86	102	9.0	150	NA	5.70
7	85	84	102	9.0	155	NA	22.80^b
8	84	84	101	9.0	155	NA	38.05^b
Composite of Influent samples						260	NA
Average of all effluent datum points							10.31
Average of effluent datum points obtained under normal operating conditions							3.61

[a]NA means not analyzed. 1 gpm = 3.785 LPM = 3.785 L/min.
[b]Efffluent concentrations under upset conditions, overhead temperature < 85°.

Table 19.12. Combining the waste streams yielded a total flow of $1,000\,m^3$/day and 5-day BOD of 1,720 mg/L.

The strong process waste didn't maintain a uniform composition which was drastically affected when tetracycline and oxytetracycline were alternately produced together with gentamicin. Moreover, the strong waste had strong sulfate level and frequent changes in the products and wastewater properties. An adequate dilution of process waste could avoid the toxicity and BOD shock load when otherwise treating a smaller flow and stronger waste where a high concentration of sulfate and more variable discharge were encountered. These factors all affected the treatability properties of the wastes.

7.4. End-of-Pipe Treatment

Table 19.13 presents a summary of all end-of-pipe treatment processes (11, 12). However, aerobic treatment scheme was selected for end-of-pipe waste treatment, as an engineering project. Anaerobic treatment was not chosen because (a) a total of $360,000\,m^3$/day of air, with oxygen content, was regularly discharged from the plant, favoring an aerobic process as an economic treatment system, and (b) the inhibition problems possibly due to high sulfate levels, frequent changes in products and fluctuation in wastewater characteristics.

An activated sludge treatment system shown in Fig. 19.13 was selected and designed for the pharmaceutical plant (108). Table 19.14 and Table 19.15 introduce the raw material consumption and the wastewater characteristics, respectively, of the antibiotic production plant (108). It basically involved a separate equalization of waste streams, pH adjustment, aeration, activated sludge system, secondary clarification, and biosolids treatment.

Table 19.13
Summary of end-of-pipe treatment processes (11,12)

End-of-pipe technology	Number of plants
Equalization	62
Neutralization	80
Primary treatment	61
Coarse settleable solids removal	41
Primary sedimentation	37
Primary chemical flocculation/clarification	12
Dissolved air flotation	3
Biological treatment	76
Activated sludge	52
Pure oxygen	1
Powdered activated carbon	2
Trickling filter	9
Aerated lagoon	23
Waste stabilization pond	9
Rotating biological contactor	1
Other biological treatnent	2
Physical/chemical treatment	17
Thermal oxidation	3
Evaporation	6
Additional treatment	40
Polishing ponds	10
Filtration	17
Multimedia	7
Activated carbon	4
Sand	5
Other polishing	17
Secondary chemical flocculation/clarification	5
Secondary neutralization	5
Chlorination	11

Note: Subtotals may not add to totals because: (a) some plants employ more than one treatment process; (b) minor treatment processes were not listed separately; (c) details for some treatment processes were not available.

The strong and diluted wastes (flow rates of 200 and 800 m^3/day and with 5-day BOD at 8,400 and 50 mg/L, respectively) were equalized in separate tanks, because they had quite different waste discharge rates and continuous variation in waste characters around the clock. The two equalized waste streams were then combined for the next treatment step: pH adjustment. The combined waste had a 5-day BOD of 1,720 mg/L and a flow rate of 1, 000 m^3/day.

Table 19.14
Raw materials consumption for antibiotic production – case study (108)

Raw materials	Usage (tone per year)
Carbohydrate sources: Starch, dextrin, sugars, vegetable oils	1500
Protein sources: Soy meal, soy flour, corn, steep liquor gluten	300–400
Minerals: ammonium sulfate, ferrous sulfate, manganese sulfate, cobalt chloride, calcium chloride, sodium ferrocyanide, sodium hydrogen sulfide, phosphates	25
Ammonia, 23%	100–200
Acids, Bases: NaOH, HC1,H_2SO_4, oxalic acid	600–700
Quarternary ammonium salts	100–125
Antifoams	30
Solvents (all regenerated): acetone, methanol, oxitol, n-butanol	500
Urea	150–200

Note: 1 ton/year = 907.2 kg/year

Table 19.15
Characteristics of wastewater streams – case study (108)

Parameters	Process wastes	Other diluted wastes
Flow, m^3/day	120	160
pH	6.5–8.5	7.0–8.0
Alkalinity, mg/L	2000	–
BOD_5, mg/L	13500	400
COD, mg/L	34000	600
SS, mg/L	1500	300
TKN-N, mg/L	1500	40
Total P, mg/L	70	10
Sulfates, mg/L	3000	
Temperature, °C	Ambient	Ambient

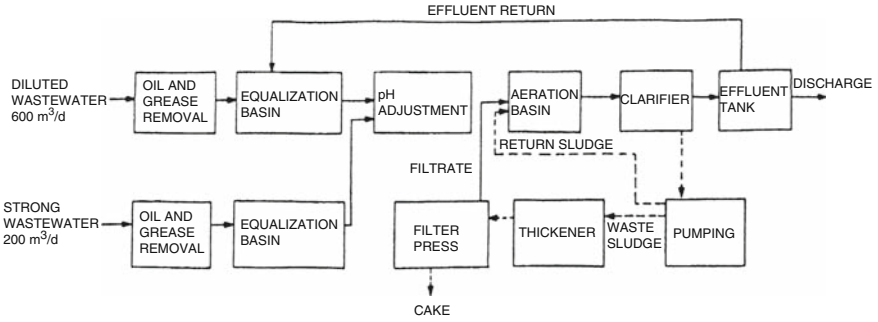

Fig. 19.13. Wastewater treatment system – a case study (108)

The waste stream was then sent to a single-stage activated sludge unit. The aeration tank had four aeration compartments in series and was designed for a hydraulic detention time of 24 h.

The two alternating process wastes (i.e., tetracycline and oxytetracycline were alternately produced together with gentamicin) showed substantially different properties affecting the mode of treatment. The yield value was much lower for oxytetracycline waste. Oxytetracycline had also a very high maximum substrate utilization rate (k), but it took a significantly large range of substrate concentration to reach this level as attested by a high half saturation constant (K_s). The tetracycline waste appeared to be biodegradable at a much slower rate ($k = 0.5$/day) but it had an inherent instability as far as substrate removal rates to be employed in the treatment, since its half saturation constant was comparatively too low. The operation showed that, under the hydraulic detention time of 1 day, the activated sludge system could yield an effluent 5-day BOD of 120 mg/L with a substrate removal rate of 0.31/day and an MLVSS concentration of 4,200 mg/L. The designed treatment plant was capable to achieve 90% removal for 5-day BOD and 80% removal for COD.

NOMENCLATURE

k = maximum substrate utilization rate
K_i = the partitioning coefficient, also called the vapor–liquid equilibrium constant
K_s = half saturation constant
P = total pressure
P_i = vapor pressure of the pure substance at the operating temperature
r_i = activity coefficient of organic compound i in the wastewater at a certain temperature
V_i = mole fraction of organic compound i in the vapor phase, and
W_i = mole fraction of organic compound i in the wastewater phase

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Appendix: Conversion Factors for Environmental Engineers

Lawrence K. Wang

CONTENTS

Abstract With the current trend toward metrication, the question of using a consistent system of units has been a problem. Wherever possible, the authors of this *Handbook of Environmental Engineering* series have used the British system (fps) along with the metric equivalent (mks, cgs, or SIU) or vice versa. For the convenience of the readers around the world, this book provides a detailed Conversion Factors for Environmental Engineers. In addition, the basic and supplementary units, the derived units and quantities, important physical constants, the properties of water, and the Periodic Table of the elements, are also presented in this document.

Key Words Conversion factors • British units • metric units • physical constants • water properties • periodic table of the elements • environmental engineers • Lenox Institute of Water Technology • mks (meter-kilogram-second) • cgs (centimeter-gram-second) • SIU (Système international d'unités; International System of Units) • fps (foot-pound-second).

1. CONSTANTS AND CONVERSION FACTORS

Multiply	*by*	*to obtain*
abamperes	10	amperes
abamperes	2.99796×10^{10}	statamperes
abampere-turns	12.566	gilberts
abcoulombs	10	coulombs (abs)
abcoulombs	2.99796×10^{10}	statcoulombs
abcoulombs/kg	30,577	statcoulombs/dyne
abfarads	1×10^{9}	farads (abs)
abfarads	8.98776×10^{20}	statfarads
abhenries	1×10^{-9}	henries (abs)
abhenries	1.11263×10^{-21}	stathenries
abohms	1×10^{-9}	ohms (abs)
abohms	1.11263×10^{-21}	statohms
abvolts	3.33560×10^{-11}	statvolts
abvolts	1×10^{-8}	volts (abs)
abvolts/centimeters	2.540005×10^{-8}	volts (abs)/inch
acres	0.4046	ha
acres	43,560	square feet
acres	4047	square meters
acres	1.562×10^{-3}	square miles
acres	4840	square yards
acre-feet	43,560	cubic feet
acre-feet	1233.5	cubic meters
acre-feet	325,850	gallons (U.S.)
amperes (abs)	0.1	abamperes
amperes (abs)	1.036×10^{-5}	faradays/second
amperes (abs)	2.9980×10^{9}	statamperes
ampere-hours (abs)	3600	coulombs (abs)
ampere-hours	0.03731	faradays
amperes/sq cm	6.452	amps/sq in
amperes/sq cm	10^{4}	amps/sq meter
amperes/sq in	0.1550	amps/sq cm
amperes/sq in	1550.0	amps/sq meter
amperes/sq meter	10^{-4}	amps/sq cm
amperes/sq meter	6.452×10^{-4}	amps/sq in
ampere-turns	1.257	gilberts
ampere-turns/cm	2.540	amp-turns/in
ampere-turns/cm	100.0	amp-turns/meter
ampere-turns/cm	1.257	gilberts/cm
ampere-turns/in	0.3937	amp-turns/cm
ampere-turns/in	39.37	amp-turns/meter
ampere-turns/in	0.4950	gilberts/cm

Multiply	*by*	*to obtain*
ampere-turns/meter	0.01	amp-turns/cm
ampere-turns/meter	0.0254	amp-turns/in
ampere-turns/meter	0.01257	gilberts/cm
angstrom units	1×10^{-8}	centimeters
angstrom units	3.937×10^{-9}	inches
angstrom unit	1×10^{-10}	meter
angstrom unit	1×10^{-4}	micron or μm
ares	0.02471	acre (U.S.)
ares	1076	square feet
ares	100	square meters
ares	119.60	sq yards
assay tons	29.17	grams
astronomical unit	1.495×10^{8}	kilometers
atmospheres (atm)	0.007348	tons/sq inch
atmospheres	76.0	cms of mercury
atmospheres	1.01325×10^{6}	dynes/square centimeter
atmospheres	33.90	ft of water (at 4°C)
atmospheres	29.92	inches of mercury (at 0°C)
atmospheres	1.033228	kg/sq cm
atmospheres	10,332	kg/sq meter
atmospheres	760.0	millimeters of mercury
atmospheres	14.696	pounds/square inch
atmospheres	1.058	tons/sq foot
avograms	1.66036×10^{-24}	grams
bags, cement	94	pounds of cement
barleycorns (British)	1/3	inches
barleycorns (British)	8.467×10^{-3}	meters
barrels (British, dry)	5.780	cubic feet
barrels (British, dry)	0.1637	cubic meters
barrels (British, dry)	36	gallons (British)
barrels, cement	170.6	kilograms
barrels, cement	376	pounds of cement
barrels, cranberry	3.371	cubic feet
barrels, cranberry	0.09547	cubic meters
barrels, oil	5.615	cubic feet
barrels, oil	0.1590	cubic meters
barrels, oil	42	gallons (U.S.)
barrels, (U.S., dry)	4.083	cubic feet
barrels (U.S., dry)	7056	cubic inches
barrels (U.S., dry)	0.11562	cubic meters
barrels (U.S., dry)	105.0	quarts (dry)
barrels (U.S., liquid)	4.211	cubic feet
barrels (U.S., liquid)	0.1192	cubic meters
barrels (U.S., liquid)	31.5	gallons (U.S.)

Multiply	*by*	*to obtain*
bars	0.98692	atmospheres
bars	10^6	dynes/sq cm
bars	1.0197×10^4	kg/sq meter
bars	1000	millibar
bars	750.06	mm of Hg (0°C)
bars	2089	pounds/sq ft
bars	14.504	pounds/sq in
barye	1.000	dynes/sq cm
board feet	1/12	cubic feet
board feet	144 sq.in. × 1 in.	cubic inches
boiler horsepower	33,475	BTU (mean)/hour
boiler horsepower	34.5	pounds of water evaporated from and at 212°F (per hour)
bolts (U.S., cloth)	120	linear feet
bolts (U.S., cloth)	36.576	meters
bougie decimales	1	candles (int)
BTU (mean)	251.98	calories, gram (g. cal)
BTU (mean)	0.55556	centigrade heat units (chu)
BTU (mean)	1.0548×10^{10}	ergs
BTU (mean)	777.98	foot-pounds
BTU (mean)	3.931×10^{-4}	horsepower-hrs (hp-hr)
BTU (mean)	1055	joules (abs)
BTU (mean)	0.25198	kilograms, cal (kg cal)
BTU (mean)	107.565	kilogram-meters
BTU (mean)	2.928×10^{-4}	kilowatt-hr (Kwh)
BTU (mean)	10.409	liter-atm
BTU (mean)	6.876×10^{-5}	pounds of carbon to CO_2
BTU (mean)	0.29305	watt-hours
BTU (mean)/cu ft	37.30	joule/liter
BTU/hour	0.2162	foot-pound/sec
BTU/hour	0.0700	gram-cal/sec
BTU/hour	3.929×10^{-4}	horsepower-hours (hp-hr)
BTU/hour	0.2930711	watt (w)
BTU/hour (feet)°F	1.730735	joule/sec (m)°k
BTU/hour (feet^2)	3.15459	joule/m^2-sec
BTU (mean)/hour(feet^2)°F	1.3562×10^{-4}	gram-calorie/second (cm^2)°C
BTU (mean)/hour(feet^2)°F	3.94×10^{-4}	horsepower/(ft^2)°F
BTU (mean)/hour(feet^2)°F	5.678264	joule/sec (m^2)°k
BTU (mean)/hour(feet^2)°F	4.882	kilogram-calorie/hr (m^2)°C
BTU (mean)/hour(feet^2)°F	5.682×10^{-4}	watts/(cm^2)°C
BTU (mean)/hour(feet^2)°F	2.035×10^{-3}	watts/(in^2)°C
BTU (mean)/(hour)(feet^2) (°F/inch)	3.4448×10^{-4}	calories, gram (15°C)/sec (cm^2) (°C/cm)
BTU (mean)/(hour)(feet^2) (°F/in.)	1	chu/(hr)(ft^2)(°C/in)

Multiply	*by*	*to obtain*
BTU (mean)/(hour)(feet^2) (°F/inch)	1.442×10^{-3}	joules (abs)/(sec)(cm^2) (°C/cm)
BTU (mean)/(hour)(feet^2) (°F/inch)	1.442×10^{-3}	watts/(cm^2) (°C/cm)
BTU/min	12.96	ft lb/sec
BTU/min	0.02356	hp
BTU/min	0.01757	kw
BTU/min	17.57	watts
BTU/min/ft^2	0.1221	watts/sq inch
BTU/pound	0.5556	calories-gram(mean)/gram
BTU/pound	0.555	kg-cal/kg
BTU/pound/°F	1	calories, gram/gram/°C
BTU/pound/°F	4186.8	joule/kg/°k
BTU/second	1054.350	watt (W)
buckets (British, dry)	1.818×10^4	cubic cm
buckets (British, dry)	4	gallons (British)
bushels (British)	1.03205	bushels (U.S.)
bushels (British)	1.2843	cubic feet
bushels (British)	0.03637	cubic meters
bushels (U.S.)	1.2444	cubic feet
bushels (U.S.)	2150.4	cubic inch
bushels (U.S.)	0.035239	cubic meters
bushels (U.S.)	35.24	liters (L)
bushels (U.S.)	4	pecks (U.S.)
bushels (U.S.)	64	pints (dry)
bushels (U.S.)	32	quarts (dry)
butts (British)	20.2285	cubic feet
butts (British)	126	gallons (British)
cable lengths	720	feet
cable lengths	219.46	meters
calories (thermochemical)	0.999346	calories (Int. Steam Tables)
calories, gram (g. cal or simply cal.)	3.9685×10^{-3}	BTU (mean)
calories, gram (mean)	0.001459	cubic feet atmospheres
calories, gram (mean)	4.186×10^7	ergs
calories, gram (mean)	3.0874	foot-pounds
calories, gram (mean)	4.186	joules (abs)
calories, gram (mean)	0.001	kg cal (calories, kilogram)
calories, gram (mean)	0.42685	kilograms-meters
calories, gram (mean)	0.0011628	watt-hours
calories, gram (mean)/gram	1.8	BTU (mean)/pound
cal/gram-°C	4186.8	joule/kg-°k
candle power (spherical)	12.566	lumens
candles (int)	0.104	carcel units
candles (int)	1.11	hefner units
candles (int)	1	lumens (int)/steradian
candles (int)/square centimeter	2919	foot-lamberts

Multiply	*by*	*to obtain*
candles (int)/square centimeter	3.1416	lamberts
candles (int)/square foot	3.1416	foot-lamberts
candles (int)/square foot	3.382×10^{-3}	lamberts
candles (int)/square inch	452.4	foot-lamberts
candles (int)/square inch	0.4870	lamberts
candles (int)/square inch	0.155	stilb
carats (metric)	3.0865	grains
carats (metric)	0.2	grams
centals	100	pounds
centares (centiares)	1.0	sq meters
centigrade heat units (chu)	1.8	BTU
centigrade heat units (chu)	453.6	calories, gram (15°C)
centigrade heat units (chu)	1897.8	joules (abs)
centigrams	0.01	grams
centiliters	0.01	liters
centimeters	0.0328083	feet (U.S.)
centimeters	0.3937	inches (U.S.)
centimeters	0.01	meters
centimeters	6.214×10^{-6}	miles
centimeters	10	millimeters
centimeters	393.7	mils
centimeters	0.01094	yards
cm of mercury	0.01316	atm
cm of mercury	0.4461	ft of water
cm of mercury	136.0	kg/square meter
cm of mercury	1333.22	newton/meter^2 (N/m^2)
cm of mercury	27.85	psf
cm of mercury	0.1934	psi
cm of water (4°C)	98.0638	newton/meter^2 (N/m^2)
centimeters-dynes	1.020×10^{-3}	centimeter-grams
centimeter-dynes	1.020×10^{-8}	meter-kilograms
centimeter-dynes	7.376×10^{-8}	pound-feet
centimeter-grams	980.7	centimeter-dynes
centimeter-grams	10^{-5}	meter-kilograms
centimeter-grams	7.233×10^{-5}	pound-feet
centimeters/second	1.969	fpm (ft/min)
centimeters/second	0.0328	fps (ft/sec)
centimeters/second	0.036	kilometers/hour
centimeters/second	0.1943	knots
centimeters/second	0.6	m/min
centimeters/second	0.02237	miles/hour
centimeters/second	3.728×10^{-4}	miles/minute
cms/sec./sec.	0.03281	feet/sec/sec
cms/sec./sec.	0.036	kms/hour/sec

Multiply	*by*	*to obtain*
cms/sec./sec.	0.02237	miles/hour/sec
centipoises	3.60	kilograms/meter hour
centipoises	10^{-3}	kilograms/meter second
centipoises	0.001	newton-sec/m^2
centipoises	2.089×10^{-5}	pound force second/square foot
centipoises	2.42	pounds/foot hour
centipoises	6.72×10^{-4}	pounds/foot second
centistoke	1.0×10^{-6}	$meter^2$/sec
chains (engineers' or Ramden's)	100	feet
chains (engineers' or Ramden's)	30.48	meters
chains (surveyors' or Gunter's)	66	feet
chains (surveyors' or Gunter's)	20.12	meters
chaldrons (British)	32	bushels (British)
chaldrons (U.S.)	36	bushels (U.S.)
cheval-vapours	0.9863	horsepower
cheval-vapours	735.5	watts (abs)
cheval-vapours heures	2.648×10^6	joules (abs)
chu/(hr)(ft^2)(°C/in.)	1	BTU/(hr)(ft^2)(°F/in.)
circular inches	0.7854	square inches
circular millimeters	7.854×10^{-7}	square meters
circular mils	5.067×10^{-6}	square centimeters
circular mils	7.854×10^{-7}	square inches
circular mils	0.7854	square mils
circumferences	360	degrees
circumferences	400	grades
circumferences	6.283	radians
cloves	8	pounds
coombs (British)	4	bushels (British)
cords	8	cord feet
cords	$8' \times 4' \times 4'$	cubic feet
cords	128	cubic feet
cords	3.625	cubic meters
cord-feet	$4' \times 4' \times 1'$	cubic feet
coulombs (abs)	0.1	abcoulombs
coulombs (abs)	6.281×10^{18}	electronic charges
coulombs (abs)	2.998×10^9	statcoulombs
coulombs (abs)	1.036×10^{-5}	faradays
coulombs/sq cm	64.52	coulombs/sq in
coulombs/sq cm	10^4	coulombs/sq meter
coulombs/sq in	0.1550	coulombs/sq cm
coulombs/sq in	1550	coulombs/sq meter
coulombs/sq meter	10^{-4}	coulombs/sq cm
coulombs/sq meter	6.452×10^{-4}	coulombs/sq in
cubic centimeters	3.531445×10^{-5}	cubic feet (U.S.)

Multiply	*by*	*to obtain*
cubic centimeters	6.102×10^{-2}	cubic inches
cubic centimeters	10^{-6}	cubic meters
cubic centimeters	1.308×10^{-6}	cubic yards
cubic centimeters	2.6417×10^{-4}	gallons (U.S.)
cubic centimeters	0.001	liters
cubic centimeters	0.033814	ounces (U.S., fluid)
cubic centimeters	2.113×10^{-3}	pints (liq.)
cubic centimeters	1.057×10^{-3}	quarts (liq.)
cubic feet (British)	0.9999916	cubic feet (U.S.)
cubic feet (U.S.)	0.8036	bushels (dry)
cubic feet (U.S.)	28317.016	cubic centimeters
cubic feet (U.S.)	1728	cubic inches
cubic feet (U.S.)	0.02832	cubic meters
cubic feet (U.S.)	0.0370	cubic yard
cubic feet (U.S.)	7.48052	gallons (U.S.)
cubic feet (U.S.)	28.31625	liters
cubic feet (U.S.)	59.84	pints (liq.)
cubic feet (U.S.)	29.92	quarts (liq.)
cubic feet of common brick	120	pounds
cubic feet of water (60°F)	62.37	pounds
cubic foot-atmospheres	2.7203	BTU (mean)
cubic foot-atmospheres	680.74	calories, gram (mean)
cubic foot-atmospheres	2116	foot-pounds
cubic foot-atmospheres	2869	joules (abs)
cubic foot-atmospheres	292.6	kilogram-meters
cubic foot-atmospheres	7.968×10^{-4}	kilowatt-hours
cubic feet/hr	0.02832	m^3/hr
cubic feet/minute	472.0	cubic cm/sec
cubic feet/minute	1.6992	cu m/hr
cubic feet/minute	0.0283	cu m/min
cubic feet/minute	0.1247	gallons/sec
cubic feet/minute	0.472	liter/sec
cubic feet/minute	62.4	lbs of water/min
cubic feet/min/1000 cu ft	0.01667	liter/sec/cu m
cubic feet/second	1.9834	acre-feet/day
cubic feet/second	1.7	cu m/min
cubic feet/second	0.02832	m^3/sec
cubic feet/second	448.83	gallons/minute
cubic feet/second	1699	liter/min
cubic feet/second	28.32	liters/sec
cubic feet/second (cfs)	0.64632	million gallons/day (MGD)
cfs/acre	0.07	m^3/sec-ha
cfs/acre	4.2	cu m/min/ha
cfs/sq mile	0.657	cu m/min/sq km

Multiply	*by*	*to obtain*
cubic inches (U.S.)	16.387162	cubic centimeters
cubic inches (U.S.)	5.787×10^{-4}	cubic feet
cubic inches (U.S.)	1.0000084	cubic inches (British)
cubic inches (U.S.)	1.639×10^{-5}	cubic meters
cubic inches (U.S.)	2.143×10^{-5}	cubic yards
cubic inches (U.S.)	4.329×10^{-3}	gallons (U.S.)
cubic inches (U.S.)	1.639×10^{-2}	liters
cubic inches (U.S.)	16.39	mL
cubic inches (U.S.)	0.55411	ounces (U.S., fluid)
cubic inches (U.S.)	0.03463	pints (liq.)
cubic inches (U.S.)	0.01732	quarts (liq.)
cubic meters	8.1074×10^{-4}	acre-feet
cubic meters	8.387	barrels (U.S., liquid)
cubic meters	28.38	bushels (dry)
cubic meters	10^6	cubic centimeters
cubic meters	35.314	cubic feet (U.S.)
cubic meters	61,023	cubic inches (U.S.)
cubic meters	1.308	cubic yards (U.S.)
cubic meters	264.17	gallons (U.S.)
cubic meters	1000	liters
cubic meters	2113	pints (liq.)
cubic meters (m^3)	1057	quarts (liq.)
cubic meters/day	0.183	gallons/min
cubic meters/ha	106.9	gallons/acre
cubic meters/hour	0.2272	gallons/minute
cubic meters/meter-day	80.53	gpd/ft
cubic meters/minute	35.314	cubic ft/minute
cubic meters/second	35.314	cubic ft/sec
cubic meters/second	22.82	MGD
cubic meters/sec-ha	14.29	cu ft/sec-acre
cubic meters/$meters^2$-day	24.54	gpd/ft^2
cubic yards (British)	0.9999916	cubic yards (U.S.)
cubic yards (British)	0.76455	cubic meters
cubic yards (U.S.)	7.646×10^5	cubic centimeters
cubic yards (U.S.)	27	cubic feet (U.S.)
cubic yards (U.S.)	46,656	cubic inches
cubic yards (U.S.)	0.76456	cubic meters
cubic yards (U.S.)	202.0	gallons (U.S.)
cubic yards (U.S.)	764.6	liters
cubic yards (U.S.)	1616	pints (liq.)
cubic yards (U.S.)	807.9	quarts (liq.)
cubic yards of sand	2700	pounds
cubic yards/minute	0.45	cubic feet/second
cubic yards/minute	3.367	gallons/second

Multiply	*by*	*to obtain*
cubic yards/minute	12.74	liters/second
cubits	45.720	centimeters
cubits	1.5	feet
dalton	1.65×10^{-24}	gram
days	1440	minutes
days	86,400	seconds
days (sidereal)	86164	seconds (mean solar)
debye units (dipole moment)	10^{18}	electrostatic units
decigrams	0.1	grams
deciliters	0.1	liters
decimeters	0.1	meters
degrees (angle)	60	minutes
degrees (angle)	0.01111	quadrants
degrees (angle)	0.01745	radians
degrees (angle)	3600	seconds
degrees/second	0.01745	radians/seconds
degrees/second	0.1667	revolutions/min
degrees/second	0.002778	revoltuions/sec
degree Celsius	$^{\circ}\mathrm{F} = (^{\circ}\mathrm{C} \times 9/5) + 32$	Fahrenheit
degree Celsius	$^{\circ}\mathrm{K} = {}^{\circ}\mathrm{C} + 273.15$	Kelvin
degree Fahrenheit	$^{\circ}\mathrm{C} = (^{\circ}\mathrm{F} - 32) \times 5/9$	Celsius
degree Fahrenheit	$^{\circ}\mathrm{K} = (^{\circ}\mathrm{F} + 459.67)/1.8$	Kelvin
degree Rankine	$^{\circ}\mathrm{K} = {}^{\circ}\mathrm{R}/1.8$	Kelvin
dekagrams	10	grams
dekaliters	10	liters
dekameters	10	meters
drachms (British, fluid)	3.5516×10^{-6}	cubic meters
drachms (British, fluid)	0.125	ounces (British, fluid)
drams (apothecaries' or troy)	0.1371429	ounces (avoirdupois)
drams (apothecaries' or troy)	0.125	ounces (troy)
drams (U.S., fluid or apoth.)	3.6967	cubic cm
drams (avoirdupois)	1.771845	grams
drams (avoirdupois)	27.3437	grains
drams (avoirdupois)	0.0625	ounces
drams (avoirdupois)	0.00390625	pounds (avoirdupois)
drams (troy)	2.1943	drams (avoirdupois)
drams (troy)	60	grains
drams (troy)	3.8879351	grams
drams (troy)	0.125	ounces (troy)
drams (U.S., fluid)	3.6967×10^{-6}	cubic meters
drams (U.S., fluid)	0.125	ounces (fluid)
dynes	0.00101972	grams

Multiply	*by*	*to obtain*
dynes	10^{-7}	joules/cm
dynes	10^{-5}	joules/meter (newtons)
dynes	1.020×10^{-6}	kilograms
dynes	1×10^{-5}	newton (N)
dynes	7.233×10^{-5}	poundals
dynes	2.24809×10^{-6}	pounds
dyne-centimeters (torque)	7.3756×10^{-8}	pound-feet
dynes/centimeter	1	ergs/square centimeter
dynes/centimeter	0.01	ergs/square millimeter
dynes/square centimeter	9.8692×10^{-7}	atmospheres
dynes/square centimeter	10^{-6}	bars
dynes/square centimeter	2.953×10^{-5}	inch of mercury at 0°C
dynes/square centimeter	4.015×10^{-4}	inch of water at 4°C
dynes/square centimeter	0.01020	kilograms/square meter
dynes/square centimeter	0.1	newtons/square meter
dynes/square centimeter	1.450×10^{-5}	pounds/square inch
electromagnetic fps units of magnetic permeability	0.0010764	electromagnetic cgs units of magnetic permeability
electromagnetic fps units of magnetic permeability	1.03382×10^{-18}	electrostatic cgs units of magnetic permeability
electromagnetic cgs units, of magnetic permeability	1.1128×10^{-21}	electrostatic cgs units of magnetic permeability
electromagnetic cgs units of mass resistance	9.9948×10^{-6}	ohms (int)-meter-gram
electronic charges	1.5921×10^{-19}	coulombs (abs)
electron-volts	1.6020×10^{-12}	ergs
electron-volts	1.0737×10^{-9}	mass units
electron-volts	0.07386	rydberg units of energy
electronstatic cgs units of Hall effect	2.6962×10^{31}	electromagnetic cgs units of Hall effect
electrostatic fps units of charge	1.1952×10^{-6}	coulombs (abs)
electrostatic fps units of magnetic permeability	929.03	electrostatic cgs units of magnetic permeability
ells	114.30	centimeters
ells	45	inches
ems, pica (printing)	0.42333	centimeters
ems, pica (printing)	1/6	inches
ergs	9.4805×10^{-11}	BTU (mean)
ergs	2.3889×10^{-8}	calories, gram (mean)
ergs	1	dyne-centimeters
ergs	7.3756×10^{-8}	foot-pounds
ergs	0.2389×10^{-7}	gram-calories
ergs	1.020×10^{-3}	gram-centimeters

Multiply	*by*	*to obtain*
ergs	3.7250×10^{-14}	horsepower-hrs
ergs	10^{-7}	joules (abs)
ergs	2.390×10^{-11}	kilogram-calories (kg cal)
ergs	1.01972×10^{-8}	kilogram-meters
ergs	0.2778×10^{-13}	kilowatt-hrs
ergs	0.2778×10^{-10}	watt-hours
ergs/second	5.692×10^{-9}	BTU/min
ergs/second	4.426×10^{-6}	foot-pounds/min
ergs/second	7.376×10^{-8}	foot-pounds/sec
ergs/second	1.341×10^{-10}	horsepower
ergs/second	1.434×10^{-9}	kg-calories/min
ergs/second	10^{-10}	kilowatts
farad (international of 1948)	0.9995	farad (F)
faradays	26.80	ampere-hours
faradays	96,500	coulombs (abs)
faradays/second	96,500	amperes (abs)
farads (abs)	10^{-9}	abfarads
farads (abs)	10^{6}	microfarads
farads (abs)	8.9877×10^{11}	statfarads
fathoms	6	feet
fathom	1.829	meter
feet (U.S.)	1.0000028	feet (British)
feet (U.S.)	30.4801	centimeters
feet (U.S.)	12	inches
feet (U.S.)	3.048×10^{-4}	kilometers
feet (U.S.)	0.30480	meters
feet (U.S.)	1.645×10^{-4}	miles (naut.)
feet (U.S.)	1.893939×10^{-4}	miles (statute)
feet (U.S.)	304.8	millimeters
feet (U.S.)	1.2×10^{4}	mils
feet (U.S.)	1/3	yards
feet of air (1 atmosphere, 60°F)	5.30×10^{-4}	pounds/square inch
feet of water	0.02950	atm
feet of water	0.8826	inches of mercury
feet of water at 39.2°F	0.030479	kilograms/square centimeter
feet of water at 39.2°F	2988.98	newton/meter^2 (N/m^2)
feet of water at 39.2°F	304.79	kilograms/square meter
feet of water	62.43	pounds/square feet (psf)
feet of water at 39.2°F	0.43352	pounds/square inch (psi)
feet/hour	0.08467	mm/sec
feet/min	0.5080	cms/sec
feet/min	0.01667	feet/sec
feet/min	0.01829	km/hr
feet/min	0.3048	meters/min

Multiply	*by*	*to obtain*
feet/min	0.01136	miles/hr
feet/sec	30.48	cm/sec
feet/sec	1.097	km/hr
feet/sec	0.5921	knots
feet/sec	18.29	meters/min
feet/sec	0.6818	miles/hr
feet/sec	0.01136	miles/min
feet/sec/sec	30.48	cm/sec/sec
feet/sec/sec	1.097	km/hr/sec
feet/sec/sec	0.3048	meters/sec/sec
feet/sec/sec	0.6818	miles/hr/sec
feet/100 feet	1.0	percent grade
firkins (British)	9	gallons (British)
firkins (U.S.)	9	gallons (U.S.)
foot-candle (ft-c)	10.764	lumen/sq m
foot-poundals	3.9951×10^{-5}	BTU (mean)
foot-poundals	0.0421420	joules (abs)
foot-pounds	0.0012854	BTU (mean)
foot-pounds	0.32389	calories, gram (mean)
foot-pounds	1.13558×10^{7}	ergs
foot-pounds	32.174	foot-poundals
foot-pounds	5.050×10^{-7}	hp-hr
foot-pounds	1.35582	joules (abs)
foot-pounds	3.241×10^{-4}	kilogram-calories
foot-pounds	0.138255	kilogram-meters
foot-pounds	3.766×10^{-7}	kwh
foot-pounds	0.013381	liter-atmospheres
foot-pounds	3.7662×10^{-4}	watt-hours (abs)
foot-pounds/minute	1.286×10^{-3}	BTU/minute
foot-pounds/minute	0.01667	foot-pounds/sec
foot-pounds/minute	3.030×10^{-5}	hp
foot-pounds/minute	3.241×10^{-4}	kg-calories/min
foot-pounds/minute	2.260×10^{-5}	kw
foot-pounds/second	4.6275	BTU (mean)/hour
foot-pounds/second	0.07717	BTU/minute
foot-pounds/second	0.0018182	horsepower
foot-pounds/second	0.01945	kg-calories/min
foot-pounds/second	0.001356	kilowatts
foot-pounds/second	1.35582	watts (abs)
furlongs	660.0	feet
furlongs	201.17	meters
furlongs	0.125	miles (U.S.)
furlongs	40.0	rods
gallons (Br.)	3.8125×10^{-2}	barrels (U.S.)

Multiply	*by*	*to obtain*
gallons (Br.)	4516.086	cubic centimeters
gallons (Br.)	0.16053	cu ft
gallons (Br.)	277.4	cu inches
gallons (Br.)	1230	drams (U.S. fluid)
gallons (Br.)	4.54596	liters
gallons (Br.)	7.9620×10^4	minims (Br.)
gallons (Br.)	7.3783×10^4	minims (U.S.)
gallons (Br.)	4545.96	mL
gallons (Br.)	1.20094	gallons (U.S.)
gallons (Br.)	160	ounces (Br., fl.)
gallons (Br.)	153.72	ounces (U.S., fl.)
gallons (Br.)	10	pounds (avoirdupois) of water at 62°F
gallons (U.S.)	3.068×10^{-4}	acre-ft
gallons (U.S.)	0.031746	barrels (U.S.)
gallons (U.S.)	3785.434	cubic centimeters
gallons (U.S.)	0.13368	cubic feet (U.S.)
gallons (U.S.)	231	cubic inches
gallons (U.S.)	3.785×10^{-3}	cubic meters
gallons (U.S.)	4.951×10^{-3}	cubic yards
gallons (U.S.)	1024	drams (U.S., fluid)
gallons (U.S.)	0.83268	gallons (Br.)
gallons (U.S.)	0.83267	imperial gal
gallons (U.S.)	3.78533	liters
gallons (U.S.)	6.3950×10^4	minims (Br.)
gallons (U.S.)	6.1440×10^4	minims (U.S.)
gallons (U.S.)	3785	mL
gallons (U.S.)	133.23	ounces (Br., fluid)
gallons (U.S.)	128	ounces (U.S., fluid)
gallons	8	pints (liq.)
gallons	4	quarts (liq.)
gal water (U.S.)	8.345	lb of water
gallons/acre	0.00935	cu m/ha
gallons/day	4.381×10^{-5}	liters/sec
gpd/acre	0.00935	cu m/day/ha
gpd/acre	9.353	liter/day/ha
gallons/capita/day	3.785	liters/capita/day
gpd/cu yd	5.0	L/day/cu m
gpd/ft	0.01242	cu m/day/m
gpd/sq ft	0.0408	cu m/day/sq m
gpd/sq ft	1.698×10^{-5}	cubic meters/hour/sq meter
gpd/sq ft	0.283	cu meter/minute/ha
gpm (gal/min)	8.0208	cfh (cu ft/hr)
gpm	2.228×10^{-3}	cfs (cu ft/sec)

Multiply	*by*	*to obtain*
gpm	4.4021	cubic meters/hr
gpm	0.00144	MGD
gpm	0.0631	liters/sec
gpm/sq ft	2.445	cu meters/hour/sq meter
gpm/sq ft	40.7	L/min/sq meter
gpm/sq ft	0.679	liter/sec/sq meter
gallons/sq ft	40.743	liters/sq meter
gausses (abs)	3.3358×10^{-4}	electrostatic cgs units of magnetic flux density
gausses (abs)	0.99966	gausses (int)
gausses (abs)	1	lines/square centimeter
gausses (abs)	6.452	lines/sq in
gausses (abs)	1	maxwells (abs)/square centimeters
gausses (abs)	6.4516	maxwells (abs)/square inch
gausses (abs)	10^{-8}	webers/sq cm
gausses (abs)	6.452×10^{-8}	webers/sq in
gausses (abs)	10^{-4}	webers/sq meter
gilberts (abs)	0.07958	abampere turns
gilberts (abs)	0.7958	ampere turns
gilberts (abs)	2.998×10^{10}	electrostatic cgs units of magneto motive force
gilberts/cm	0.7958	amp-turns/cm
gilberts/cm	2.021	amp-turns/in
gilberts/cm	79.58	amp-turns/meter
gills (Br.)	142.07	cubic cm
gills (Br.)	5	ounces (British, fluid)
gills (U.S.)	32	drams (fluid)
gills	0.1183	liters
gills	0.25	pints (liq.)
grade	0.01571	radian
grains	0.036571	drams (avoirdupois)
grains	0.01667	drams (troy)
grains (troy)	1.216	grains (avdp)
grains (troy)	0.06480	grams
grains (troy)	6.480×10^{-5}	kilograms
grains (troy)	64.799	milligrams
grains (troy)	2.286×10^{-3}	ounces (avdp)
grains (troy)	2.0833×10^{-3}	ounces (troy)
grains (troy)	0.04167	pennyweights (troy)
grains	1/7000	pounds (avoirdupois)
grains	1.736×10^{-4}	pounds (troy)
grains	6.377×10^{-8}	tons (long)
grains	7.142×10^{-8}	tons (short)
grains/imp gal	14.254	mg/L

Multiply	*by*	*to obtain*
grains/imp. gal	14.254	parts/million (ppm)
grains/U.S. gal	17.118	mg/L
grains/U.S. gal	17.118	parts/million (ppm)
grains/U.S. gal	142.86	lb/mil gal
grams	0.5611	drams (avdp)
grams	0.25721	drams (troy)
grams	980.7	dynes
grams	15.43	grains
grams	9.807×10^{-5}	joules/cm
grams	9.807×10^{-3}	joules/meter (newtons)
grams	10^{-3}	kilograms
grams	10^{3}	milligrams
grams	0.0353	ounces (avdp)
grams	0.03215	ounces (troy)
grams	0.07093	poundals
grams	2.205×10^{-3}	pounds
grams	2.679×10^{-3}	pounds (troy)
grams	9.842×10^{-7}	tons (long)
grams	1.102×10^{-6}	tons (short)
grams-calories	4.1868×10^{7}	ergs
gram-calories	3.0880	foot-pounds
gram-calories	1.5597×10^{-6}	horsepower-hr
gram-calories	1.1630×10^{-6}	kilowatt-hr
gram-calories	1.1630×10^{-3}	watt-hr
gram-calories	3.968×10^{-3}	British Thermal Units (BTU)
gram-calories/sec	14.286	BTU/hr
gram-centimeters	9.2967×10^{-8}	BTU (mean)
gram-centimeters	2.3427×10^{-5}	calories, gram (mean)
gram-centimeters	980.7	ergs
gram-centimeters	7.2330×10^{-5}	foot-pounds
gram-centimeters	9.8067×10^{-5}	joules (abs)
gram-centimeters	2.344×10^{-8}	kilogram-calories
gram-centimeters	10^{-5}	kilogram-meters
gram-centimeters	2.7241×10^{-8}	watt-hours
grams-centimeters^2 (moment of inertia)	2.37305×10^{-6}	pounds-feet^2
grams-centimeters^2 (moment of inertia)	3.4172×10^{-4}	pounds-inch^2
gram-centimeters/second	1.3151×10^{-7}	hp
gram-centimeters/second	9.8067×10^{-8}	kilowatts
gram-centimeters/second	0.065552	lumens
gram-centimeters/second	9.80665×10^{-5}	watt (abs)
grams/cm	5.600×10^{-3}	pounds/inch
grams/cu cm	62.428	pounds/cubic foot
grams/cu cm	0.03613	pounds/cubic inch

Multiply	*by*	*to obtain*
grams/cu cm	8.3454	pounds/gallon (U.S.)
grams/cu cm	3.405×10^{-7}	pounds/mil-foot
grams/cu ft	35.314	grams/cu meter
grams/cu ft	10^6	micrograms/cu ft
grams/cu ft	35.314×10^6	micrograms/cu meter
grams/cu ft	35.3145×10^3	milligrams/cu meter
grams/cu ft	2.2046	pounds/1000 cu ft
grams/cu m	0.43700	grains/cubic foot
grams/cu m	0.02832	grams/cu ft
grams/cu m	28.317×10^3	micrograms/cu ft
grams/cu m	0.06243	pounds/cu ft
grams/liter	58.417	grains/gallon (U.S.)
grams/liter	9.99973×10^{-4}	grams/cubic centimeter
grams/liter	1000	mg/L
grams/liter	1000	parts per million (ppm)
grams/liter	0.06243	pounds/cubic foot
grams/liter	8.345	lb/1000 gal
grams/sq centimeter	2.0481	pounds/sq ft
grams/sq centimeter	0.0142234	pounds/square inch
grams/sq ft	10.764	grams/sq meter
grams/sq ft	10.764×10^3	kilograms/sq km
grams/sq ft	1.0764	milligrams/sq cm
grams/sq ft	10.764×10^3	milligrams/sq meter
grams/sq ft	96.154	pounds/acre
grams/sq ft	2.204	pounds/1000 sq ft
grams/sq ft	30.73	tons/sq mile
grams/sq meter	0.0929	grams/sq ft
grams/sq meter	1000	kilograms/sq km
grams/sq meter	0.1	milligrams/square cm
grams/sq meter	1000	milligrams/sq meter
grams/sq meter	8.921	pounds/acre
grams/sq meter	0.2048	pounds/1000 sq ft
grams/sq meter	2.855	tons/sq mile
g (gravity)	9.80665	meters/sec^2
g (gravity)	32.174	ft/sec^2
hand	10.16	cm
hands	4	inches
hectare (ha)	2.471	acre
hectares	1.076×10^5	sq feet
hectograms	100	grams
hectoliters	100	liters
hectometers	100	meters
hectowatts	100	watts
hemispheres	0.5	spheres

Multiply	*by*	*to obtain*
hemispheres	4	spherical right angles
hemispheres	6.2832	steradians
henries (abs)	10^9	abhenries
henries	1000.0	millihenries
henries (abs)	1.1126×10^{-12}	stathenries
hogsheads (British)	63	gallons (British)
hogsheads (British)	10.114	cubic feet
hogsheads (U.S.)	8.422	cubic feet
hogsheads (U.S.)	0.2385	cubic meters
hogsheads (U.S.)	63	gallons (U.S.)
horsepower	2545.08	BTU (mean)/hour
horsepower	42.44	BTU/min
horsepower	7.457×10^9	erg/sec
horsepower	33,000	ft lb/min
horsepower	550	foot-pounds/second
horsepower	7.6042×10^6	g cm/sec
horsepower, electrical	1.0004	horsepower
horsepower	10.70	kg.-calories/min
horsepower	0.74570	kilowatts (g = 980.665)
horsepower	498129	lumens
horsepower, continental	736	watts (abs)
horsepower, electrical	746	watts (abs)
horsepower (boiler)	9.803	kw
horsepower (boiler)	33.479	BTU/hr
horsepower-hours	2545	BTU (mean)
horsepower-hours	2.6845×10^{13}	ergs
horsepower-hours	6.3705×10^7	ft poundals
horsepower-hours	1.98×10^6	foot-pounds
horsepower-hours	641,190	gram-calories
horsepower-hours	2.684×10^6	joules
horsepower-hours	641.7	kilogram-calories
horsepower-hours	2.737×10^5	kilogram-meters
horsepower-hours	0.7457	kilowatt-hours (abs)
horsepower-hours	26,494	liter atmospheres (normal)
horsepower-hours	745.7	watt-hours
hours	4.167×10^{-2}	days
hours	60	minutes
hours	3600	seconds
hours	5.952×10^{-3}	weeks
hundredweights (long)	112	pounds
hundredweights (long)	0.05	tons (long)
hundredweights (short)	1600	ounces (avoirdupois)
hundredweights (short)	100	pounds
hundredweights (short)	0.0453592	tons (metric)

Multiply	*by*	*to obtain*
hundredweights (short)	0.0446429	tons (long)
inches (British)	2.540	centimeters
inches (U.S.)	2.54000508	centimeters
inches (British)	0.9999972	inches (U.S.)
inches	2.540×10^{-2}	meters
inches	1.578×10^{-5}	miles
inches	25.40	millimeters
inches	10^3	mils
inches	2.778×10^{-2}	yards
inches^2	6.4516×10^{-4}	meter^2
inches^3	1.6387×10^{-5}	meter^3
in. of mercury	0.0334	atm
in. of mercury	1.133	ft of water
in. of mercury (0°C)	13.609	inches of water (60°F)
in. of mercury	0.0345	kgs/square cm
in. of mercury at 32°F	345.31	kilograms/square meter
in. of mercury	33.35	millibars
in. of mercury	25.40	millimeters of mercury
in. of mercury (60°F)	3376.85	newton/meter^2
in. of mercury	70.73	pounds/square ft
in. of mercury at 32°F	0.4912	pounds/square inch
in. of water	0.002458	atmospheres
in. of water	0.0736	in. of mercury
in. of water (at 4°C)	2.540×10^{-3}	kgs/sq cm
in. of water	25.40	kgs/square meter
in. of water (60°F)	1.8663	millimeters of mercury (0°C)
in. of water (60°F)	248.84	newton/meter^2
in. of water	0.5781	ounces/square in
in. of water	5.204	pounds/square ft
in. of water	0.0361	psi
inches/hour	2.54	cm/hr
international ampere	.9998	ampere (absolute)
international volt	1.0003	volts (absolute)
international volt	1.593×10^{-19}	joules (absolute)
international volt	9.654×10^4	joules
joules	9.480×10^{-4}	BTU
joules (abs)	10^7	ergs
joules	23.730	foot poundals
joules (abs)	0.73756	foot-pounds
joules	3.7251×10^{-7}	horsepower hours
joules	2.389×10^{-4}	kg-calories
joules (abs)	0.101972	kilogram-meters
joules	9.8689×10^{-3}	liter atmospheres (normal)
joules	2.778×10^{-4}	watt-hrs

Multiply	*by*	*to obtain*
joules-sec	1.5258×10^{33}	quanta
joules/cm	1.020×10^{4}	grams
joules/cm	10^{7}	dynes
joules/cm	100.0	joules/meter (newtons)
joules/cm	723.3	poundals
joules/cm	22.48	pounds
joules/liter	0.02681	BTU/cu ft
joules/m^2-sec	0.3167	BTU/ft^2-hr
joules/sec	3.41304	BTU/hr
joules/sec	0.056884	BTU/min
joules/sec	1×10^{7}	erg/sec
joules/sec	44.254	ft lb/min
joules/sec	0.73756	ft lb/sec
joules/sec	1.0197×10^{4}	g cm/sec
joules/sec	1.341×10^{-3}	hp
joules/sec	0.01433	kg cal/min
joules/sec	0.001	kilowatts
joules/sec	668	lumens
joules/sec	1	watts
kilograms	564.38	drams (avdp)
kilograms	257.21	drams (troy)
kilograms	980,665	dynes
kilograms	15,432	grains
kilograms	1000	grams
kilograms	0.09807	joules/cm
kilograms	9.807	joules/meter (newtons)
kilograms	1×10^{6}	milligrams
kilograms	35.274	ounces (avdp)
kilograms	32.151	ounces (troy)
kilograms	70.93	poundals
kilograms	2.20462	pounds (avdp)
kilograms	2.6792	pounds (troy)
kilograms	9.84207×10^{-4}	tons (long)
kilograms	0.001	tons (metric)
kilograms	0.0011023	tons (short)
kilogram-calories	3.968	British Thermal Units (BTU)
kilogram-calories	3086	foot-pounds
kilogram-calories	1.558×10^{-3}	horsepower-hours
kilogram-calories	4186	joules
kilogram-calories	426.6	kilogram-meters
kilogram-calories	4.186	kilojoules
kilogram-calories	1.162×10^{-3}	kilowatt-hours
kg-cal/min	238.11	BTU/hr
kg-cal/min	3.9685	BTU/min

Multiply	*by*	*to obtain*
kg-cal/min	6.9770×10^{8}	erg/sec
kg-cal/min	3087.4	ft-lb/min
kg-cal/min	51.457	ft-lb/sec
kg-cal/min	7.1146×10^{5}	g cm/sec
kg-cal/min	0.0936	hp
kg-cal/min	69.769	joules/sec
kg-cal/min	0.0698	kw
kg-cal/min	46636	lumens
kg-cal/min	69.767	watts
kgs-cms. squared	2.373×10^{-3}	pounds-feet squared
kgs-cms. squared	0.3417	pounds-inches squared
kilogram-force (kgf)	9.80665	newton
kilogram-meters	0.0092967	BTU (mean)
kilogram-meters	2.3427	calories, gram (mean)
kilogram-meters	9.80665×10^{7}	ergs
kilogram-meters	232.71	ft poundals
kilogram-meters	7.2330	foot-pounds
kilogram-meters	3.6529×10^{-6}	horsepower-hours
kilogram-meters	9.80665	joules (abs)
kilogram-meters	2.344×10^{-3}	kilogram-calories
kilogram-meters	2.52407×10^{-6}	kilowatt-hours (abs)
kilogram-meters	2.7241×10^{-6}	kilowatt-hours
kilogram-meters	0.096781	liter atmospheres (normal)
kilogram-meters	6.392×10^{-7}	pounds carbon to CO_2
kilogram-meters	9.579×10^{-6}	pounds water evap. at 212°F
kilograms/cubic meter	10^{-3}	grams/cubic cm
kilograms/cubic meter	0.06243	pounds/cubic foot
kilograms/cubic meter	3.613×10^{-5}	pounds/cubic inch
kilograms/cubic meter	3.405×10^{-10}	pounds/mil. foot
kilograms/m^3-day	0.0624	lb/cu ft-day
kilograms/cu meter-day	62.43	pounds/1000 cu ft-day
kilograms/ha	0.8921	pounds/acre
kilograms/meter	0.6720	pounds/foot
kilograms/sq cm	980,665	dynes
kilograms/sq cm	0.96784	atmosphere
kilograms/sq cm	32.81	feet of water
kilograms/sq cm	28.96	inches of mercury
kilograms/sq cm	735.56	mm of mercury
kilograms/sq cm	2048	pounds/sq ft
kilograms/sq cm	14.22	pounds/square inch
kilograms/sq km	92.9×10^{-6}	grams/sq ft
kilograms/sq km	0.001	grams/sq meter
kilograms/sq km	0.0001	milligrams/sq cm
kilograms/sq km	1.0	milligrams/sq meter

Multiply	*by*	*to obtain*
kilograms/sq km	8.921×10^{-3}	pounds/acre
kilograms/sq km	204.8×10^{-6}	pounds/1000 sq ft
kilograms/sq km	2.855×10^{-3}	tons/sq mile
kilograms/sq meter	9.6784×10^{-5}	atmospheres
kilograms/sq meter	98.07×10^{-6}	bars
kilograms/sq meter	98.0665	dynes/sq centimeters
kilograms/sq meter	3.281×10^{-3}	feet of water at 39.2°F
kilograms/sq meter	0.1	grams/sq centimeters
kilograms/sq meter	2.896×10^{-3}	inches of mercury at 32°F
kilograms/sq meter	0.07356	mm of mercury at 0°C
kilograms/sq meter	0.2048	pounds/square foot
kilograms/sq meter	0.00142234	pounds/square inch
kilograms/sq mm.	10^6	kg/square meter
kilojoule	0.947	BTU
kilojoules/kilogram	0.4295	BTU/pound
kilolines	1000.0	maxwells
kiloliters	10^3	liters
kilometers	10^5	centimeters
kilometers	3281	feet
kilometers	3.937×10^4	inches
kilometers	10^3	meters
kilometers	0.53961	miles (nautical)
kilometers	0.6214	miles (statute)
kilometers	10^6	millimeters
kilometers	1093.6	yards
kilometers/hr	27.78	cm/sec
kilometers/hr	54.68	feet/minute
kilometers/hr	0.9113	ft/sec
kilometers/hr	0.5396	knot
kilometers/hr	16.67	meters/minute
kilometers/hr	0.2778	meters/sec
kilometers/hr	0.6214	miles/hour
kilometers/hour/sec	27.78	cms/sec/sec
kilometers/hour/sec	0.9113	ft/sec/sec
kilometers/hour/sec	0.2778	meters/sec/sec
kilometers/hour/sec	0.6214	miles/hr/sec
kilometers/min	60	kilometers/hour
kilonewtons/sq m	0.145	psi
kilowatts	56.88	BTU/min
kilowatts	4.425×10^4	foot-pounds/min
kilowatts	737.6	ft-lb/sec
kilowatts	1.341	horsepower
kilowatts	14.34	kg-cal/min
kilowatts	10^3	watts

Multiply	*by*	*to obtain*
kilowatt-hrs	3413	BTU (mean)
kilowatt-hrs	3.600×10^{13}	ergs
kilowatt-hrs	2.6552×10^{6}	foot-pounds
kilowatt-hrs	859,850	gram-calories
kilowatt-hrs	1.341	horsepower hours
kilowatt-hrs	3.6×10^{6}	joules
kilowatt-hrs	860.5	kg-calories
kilowatt-hrs	3.6709×10^{5}	kilogram-meters
kilowatt-hrs	3.53	pounds of water evaporated from from and at 212°F
kilowatt-hrs	22.75	pounds of water raised from 62° to 212°F
knots	6080	feet/hr
knots	1.689	feet/sec
knots	1.8532	kilometers/hr
knots	0.5144	meters/sec
knots	1.0	miles (nautical)/hour
knots	1.151	miles (statute)/hour
knots	2,027	yards/hr
lambert	2.054	candle/in^2
lambert	929	footlambert
lambert	0.3183	stilb
langley	1	15° gram-calorie/cm^2
langley	3.6855	BTU/ft^2
langley	0.011624	Int. kw-hr/m^2
langley	4.1855	joules (abs)/cm^2
leagues (nautical)	3	miles (nautical)
leagues (statute)	3	miles (statute)
light years	63,274	astronomical units
light years	9.4599×10^{12}	kilometers
light years	5.8781×10^{12}	miles
lignes (Paris lines)	1/12	ponces (Paris inches)
lines/sq cm	1.0	gausses
lines/sq in	0.1550	gausses
lines/sq in	1.550×10^{-9}	webers/sq cm
lines/sq in	10^{-8}	webers/sq in
lines/sq in	1.550×10^{-5}	webers/sq meter
links (engineer's)	12.0	inches
links (Gunter's)	0.01	chains (Gunter's)
links (Gunter's)	0.66	feet
links (Ramden's)	0.01	chains (Ramden's)
links (Ramden's)	1	feet
links (surveyor's)	7.92	inches
liters	8.387×10^{-3}	barrels (U.S.)

Multiply	*by*	*to obtain*
liters	0.02838	bushels (U.S. dry)
liters	1000.028	cubic centimeters
liters	0.035316	cubic feet
liters	61.025	cu inches
liters	10^{-3}	cubic meters
liters	1.308×10^{-3}	cubic yards
liters	270.5179	drams (U.S. fl)
liters	0.21998	gallons (Br.)
liters	0.26417762	gallons (U.S.)
liters	16,894	minims (Br.)
liters	16,231	minims (U.S.)
liters	35.196	ounces (Br. fl)
liters	33.8147	ounces (U.S. fl)
liters	2.113	pints (liq.)
liters	1.0566828	quarts (U.S. liq.)
liter-atmospheres (normal)	0.096064	BTU (mean)
liter-atmospheres (normal)	24.206	calories, gram (mean)
liter-atmospheres (normal)	1.0133×10^{9}	ergs
liter-atmospheres (normal)	74.735	foot-pounds
liter-atmospheres (normal)	3.7745×10^{-5}	horsepower hours
liter-atmospheres (normal)	101.33	joules (abs)
liter-atmospheres (normal)	10.33	kilogram-meters
liter-atmospheres (normal)	2.4206×10^{-2}	kilogram calories
liter-atmospheres (normal)	2.815×10^{-5}	kilowatt-hours
liter/cu m-sec	60.0	cfm/1000 cu ft
liters/minute	5.885×10^{-4}	cubic feet/sec
liters/minute	4.403×10^{-3}	gallons/sec
liter/person-day	0.264	gpcd
liters/sec	2.119	cu ft /min
liters/sec	3.5316×10^{-2}	cu ft /sec
liters/sec	15.85	gallons/minute
liters/sec	0.02282	MGD
$\log_{10} N$	2.303	$\log_e N$ or ln N
$\log_e N$ or ln N	0.4343	$\log_{10} N$
lumens	0.07958	candle-power (spherical)
lumens	0.00147	watts of maximum visibility radiation
lumens/sq. centimeters	1	lamberts
lumens/sq cm/steradian	3.1416	lamberts
lumens/sq ft	1	foot-candles
lumens/sq ft	10.764	lumens/sq meter
lumens/sq ft/steradian	3.3816	millilamberts
lumens/sq meter	0.09290	foot-candles or lumens/sq
lumens/sq meter	10^{-4}	phots
lux	0.09290	foot-candles

Multiply	*by*	*to obtain*
lux	1	lumens/sq meter
lux	10^{-4}	phots
maxwells	0.001	kilolines
maxwells	10^{-8}	webers
megajoule	0.3725	horsepower-hour
megalines	10^{6}	maxwells
megohms	10^{12}	microhms
megohms	10^{6}	ohms
meters	10^{10}	angstrom units
meters	100	centimeters
meters	0.5467	fathoms
meters	3.280833	feet (U.S.)
meters	39.37	inches
meters	10^{-3}	kilometers
meters	5.396×10^{-4}	miles (naut.)
meters	6.2137×10^{-4}	miles (statute)
meters	10^{3}	millimeters
meters	10^{9}	millimicrons
meters	1.09361	yards (U.S.)
meters	1.179	varas
meter-candles	1	lumens/sq meter
meter-kilograms	9.807×10^{7}	centimeter-dynes
meter-kilograms	10^{5}	centimeter-grams
meter-kilograms	7.233	pound-feet
meters/minute	1.667	centimeters/sec
meters/minute	3.281	feet/minute
meters/minute	0.05468	feet/second
meters/minute	0.06	kilograms/hour
meters/minute	0.03238	knots
meters/minute	0.03728	miles/hour
meters/second	196.8	feet/minute
meters/second	3.281	feet/second
meters/second	3.6	kilometers/hour
meters/second	0.06	kilometers/min
meters/second	1.944	knots
meters/second	2.23693	miles/hour
meters/second	0.03728	miles/minute
meters/sec/sec	100.0	cm/sec/sec
meters/sec/sec	3.281	feet/sec/sec
meters/sec/sec	3.6	km/hour/sec
meters/sec/sec	2.237	miles/hour/sec
microfarad	10^{-6}	farads
micrograms	10^{-6}	grams
micrograms/cu ft	10^{-6}	grams/cu ft

Multiply	*by*	*to obtain*
micrograms/cu ft	35.314×10^{-6}	grams/cu m
micrograms/cu ft	35.314	microgram/cu m
micrograms/cu ft	35.314×10^{-3}	milligrams/cu m
micrograms/cu ft	2.2046×10^{-6}	pounds/1000 cu ft
micrograms/cu m	28.317×10^{-9}	grams/cu ft
micrograms/cu m	10^{-6}	grams/ cu m
micrograms/cu m	0.02832	micrograms/cu ft
micrograms/cu m	0.001	milligrams/cu m
micrograms/cu m	62.43×10^{-9}	pounds/1000 cu ft
micrograms/cu m	$\frac{0.02404}{\text{molecular weight of gas}}$	ppm by volume (20°C)
micrograms/cu m	834.7×10^{-6}	ppm by weight
micrograms/liter	1000.0	micrograms/cu m
micrograms/liter	1.0	milligrams/cu m
micrograms/liter	62.43×10^{-9}	pounds/cu ft
micrograms/liter	$\frac{24.04}{\text{molecular weight of gas}}$	ppm by volume (20°C)
micrograms/liter	0.834.7	ppm by weight
microhms	10^{-12}	megohms
microhms	10^{-6}	ohms
microliters	10^{-6}	liters
microns	10^{4}	angstrom units
microns	1×10^{-4}	centimeters
microns	3.9370×10^{-5}	inches
microns	10^{-6}	meters
miles (naut.)	6080.27	feet
miles (naut.)	1.853	kilometers
miles (naut.)	1.853	meters
miles (naut.)	1.1516	miles (statute)
miles (naut.)	2027	yards
miles (statute)	1.609×10^{5}	centimeters
miles (statute)	5280	feet
miles (statute)	6.336×10^{4}	inches
miles (statute)	1.609	kilometers
miles (statute)	1609	meters
miles (statute)	0.8684	miles (naut.)
miles (statute)	320	rods
miles (statute)	1760	yards
miles/hour	44.7041	centimeter/second
miles/hour	88	feet/min
miles/hour	1.4667	feet/sec
miles/hour	1.6093	kilometers/hour
miles/hour	0.02682	km/min

Multiply	*by*	*to obtain*
miles/hour	0.86839	knots
miles/hour	26.82	meters/min
miles/hour	0.447	meters/sec
miles/hour	0.1667	miles/min
miles/hour/sec	44.70	cms/sec/sec
miles/hour/sec	1.4667	ft/sec/sec
miles/hour/sec	1.6093	km/hour/sec
miles/hour/sec	0.4470	m/sec/sec
miles/min	2682	centimeters/sec
miles/min	88	ft/sec
miles/min	1.609	km/min
miles/min	0.8684	knots/min
miles/min	60	miles/hour
miles-feet	9.425×10^{-6}	cu inches
millibars	0.00987	atmospheres
millibars	0.30	inches of mercury
millibars	0.75	millimeters of mercury
milliers	10^3	kilograms
millimicrons	1×10^{-9}	meters
milligrams	0.01543236	grains
milligrams	10^{-3}	grams
milligrams	10^{-6}	kilograms
milligrams	3.5274×10^{-5}	ounces (avdp)
milligrams	2.2046×10^{-6}	pounds (avdp)
milligrams/assay ton	1	ounces (troy)/ton (short)
milligrams/cu m	283.2×10^{-6}	grams/cu ft
milligrams/cu m	0.001	grams/cu m
milligrams/cu m	1000.0	micrograms/cu m
milligrams/cu m	28.32	micrograms/cu ft
milligrams/cu m	1.0	micrograms/liter
milligrams/cu m	62.43×10^{-6}	pounds/1000 cu ft
milligrams/cu m	$\frac{24.04}{\text{molecular weight of gas}}$	ppm by volume (20°C)
milligrams/cu m	0.8347	ppm by weight
milligrams/joule	5.918	pounds/horsepower-hour
milligrams/liter	0.05841	grains/gallon
milligrams/liter	0.07016	grains/imp. gal
milligrams/liter	0.0584	grains/U.S. gal
milligrams/liter	1.0	parts/million
milligrams/liter	8.345	lb/mil gal
milligrams/sq cm	0.929	grams/sq ft
milligrams/sq cm	10.0	grams/sq meter
milligrams/sq cm	10^4	kilograms/sq km

Multiply	*by*	*to obtain*
milligrams/sq cm	10^4	milligrams/sq meter
milligrams/sq cm	2.048	pounds/1000 sq ft
milligrams/sq cm	89.21	pounds/acre
milligrams/sq cm	28.55	tons/sq mile
milligrams/sq meter	92.9×10^{-6}	grams/sq ft
milligrams/sq meter	0.001	grams/sq meter
milligrams/sq meter	1.0	kilograms/sq km
milligrams/sq meter	0.0001	milligrams/sq cm
milligrams/sq meter	8.921×10^{-3}	pounds/acre
milligrams/sq meter	204.8×10^{-6}	pounds/1000 sq ft
milligrams/sq meter	2.855×10^{-3}	tons/sq mile
millihenries	0.001	henries
milliliters	1	cubic centimeters
milliliters	3.531×10^{-5}	cu ft
milliliters	6.102×10^{-2}	cu in
milliliters	10^{-6}	cu m
milliliters	2.642×10^{-4}	gal (U.S.)
milliliters	10^{-3}	liters
milliliters	0.03381	ounces (U.S. fl)
millimeters	0.1	centimeters
millimeters	3.281×10^{-3}	feet
millimeters	0.03937	inches
millimeters	10^{-6}	kilometers
millimeters	0.001	meters
millimeters	6.214×10^{-7}	miles
millimeters	39.37	mils
millimeters	1.094×10^{-3}	yards
millimeters of mercury	1.316×10^{-3}	atmospheres
millimeters of mercury	0.0394	inches of mercury
millimeters of mercury (0°C)	0.5358	inches of water (60°F)
millimeters of mercury	1.3595×10^{-3}	kg/sq cm
millimeter of mercury (0°C)	133.3224	newton/meter^2
millimeters of mercury	0.01934	pounds/sq in
millimeters/sec	11.81	feet/hour
million gallons	306.89	acre-ft
million gallons	3785.0	cubic meters
million gallons	3.785	mega liters (1×10^6)
million gallons/day (MGD)	1.547	cu ft/sec
MGD	3785	cu m/day
MGD	0.0438	cubic meters/sec
MGD	43.808	liters/sec
MGD/acre	9360	cu m/day/ha
MGD/acre	0.039	cu meters/hour/sq meter

Multiply	*by*	*to obtain*
mils	0.002540	centimeters
mils	8.333×10^{-5}	feet
mils	0.001	inches
mils	2.540×10^{-8}	kilometers
mils	25.40	microns
mils	2.778×10^{-5}	yards
miner's in.	1.5	cu ft/min
miner's inches (Ariz., Calif. Mont., and Ore.)	0.025	cubic feet/second
miner's in. (Colorado)	0.02604	cubic feet/second
miner's inches (Idaho, Kan., Neb., Nev., N. Mex., N. Dak., S. Dak. and Utah)	0.020	cubic feet/second
minims (British)	0.05919	cubic centimeter
minims (U.S.)	0.06161	cubic centimeters
minutes (angles)	0.01667	degrees
minutes (angles)	1.852×10^{-4}	quadrants
minutes (angles)	2.909×10^{-4}	radians
minutes (angle)	60	seconds (angle)
months (mean calendar)	30.4202	days
months (mean calendar)	730.1	hours
months (mean calendar)	43805	minutes
months (mean calendar)	2.6283×10^{6}	seconds
myriagrams	10	kilograms
myriameters	10	kilometers
myriawatts	10	kilowatts
nepers	8.686	decibels
newtons	10^{5}	dynes
newtons	0.10197	kilograms
newtons	0.22481	pounds
newtons/sq meter	1.00	pascals (Pa)
noggins (British)	1/32	gallons (British)
No./cu.cm.	28.316×10^{3}	No./cu ft
No./cu.cm.	10^{6}	No./cu meter
No./cu.cm.	1000.0	No./liter
No./cu.ft.	35.314×10^{-6}	No./cu cm
No./cu.ft.	35.314	No./cu meter
No./cu.ft.	35.314×10^{-3}	No./liter
No./cu. meter	10^{-6}	No./cu cm
No./cu. meter	28.317×10^{-3}	No./cu ft
No./cu. meter	0.001	No./liter
No./liter	0.001	No./cu cm
No./liter	28.316	No./cu ft

Multiply	*by*	*to obtain*
No./liter	1000.0	No./cu meter
oersteds (abs)	1	electromagnetic cgs units of magnetizing force
oersteds (abs)	2.9978×10^{10}	electrostatic cgs units of magnetizing force
ohms	10^{9}	abohms
ohms	1.1126×10^{-12}	statohms
ohms	10^{-6}	megohms
ohms	10^{6}	microhms
ohms (International)	1.0005	ohms (absolute)
ounces (avdp)	16	drams (avoirdupois)
ounces (avdp)	7.2917	drams (troy)
ounces (avdp)	437.5	grains
ounces (avdp)	28.349527	grams
ounces (avdp)	0.028350	kilograms
ounces (avdp)	2.8350×10^{4}	milligrams
ounces (avdp)	0.9114583	ounces (troy)
ounces (avdp)	0.0625	pounds (avoirdupois)
ounces (avdp)	0.075955	pounds (troy)
ounces (avdp)	2.790×10^{-5}	tons (long)
ounces (avdp)	2.835×10^{-5}	tons (metric)
ounces (avdp)	3.125×10^{-5}	tons (short)
ounces (Br. fl)	2.3828×10^{-4}	barrels (U.S.)
ounces (Br. fl)	1.0033×10^{-3}	cubic feet
ounces (Br. fl)	1.73457	cubic inches
ounces (Br. fl)	7.6860	drams (U.S. fl)
ounces (Br. fl)	6.250×10^{-3}	gallons (Br.)
ounces (Br. fl)	0.07506	gallons (U.S.)
ounces (Br. fl)	2.84121×10^{-2}	liters
ounces (Br. fl)	480	minims (Br.)
ounces (Br. fl)	461.160	minims (U.S.)
ounces (Br. fl)	28.4121	mL
ounces (Br. fl)	0.9607	ounces (U.S. fl)
ounces (troy)	17.554	drams (avdp)
ounces (troy)	8	drams (troy)
ounces (troy)	480	grains (troy)
ounces (troy)	31.103481	grams
ounces (troy)	0.03110	kilograms
ounces (troy)	1.09714	ounces (avoirdupois)
ounces (troy)	20	pennyweights (troy)
ounces (troy)	0.068571	pounds (avdp)
ounces (troy)	0.08333	pounds (troy)
ounces (troy)	3.061×10^{-5}	tons (long)
ounces (troy)	3.429×10^{-5}	tons (short)

Multiply	*by*	*to obtain*
ounces (U.S. fl)	2.48×10^{-4}	barrels (U.S.)
ounces (U.S. fl)	29.5737	cubic centimeters
ounces (U.S. fl)	1.0443×10^{-3}	cubic feet
ounces (U.S. fl)	1.80469	cubic inches
ounces (U.S. fl)	8	drams (fluid)
ounces (U.S. fl)	6.5053×10^{-3}	gallons (Br.)
ounces (U.S. fl)	7.8125×10^{-3}	gallons (U.S.)
ounces (U.S. fl)	29.5729	milliliters
ounces (U.S. fl)	499.61	minims (Br.)
ounces (U.S. fl)	480	minims (U.S.)
ounces (U.S. fl)	1.0409	ounces (Br. fl)
ounces/sq inch	4309	dynes/sq cm
ounces/sq. inch	0.0625	pounds/sq inch
paces	30	inches
palms (British)	3	inches
parsecs	3.260	light years
parsecs	3.084×10^{13}	kilometers
parsecs	3.084×10^{16}	meters
parsec	19×10^{12}	miles
parts/billion (ppb)	10^{-3}	mg/L
parts/million (ppm)	0.07016	grains/imp. gal.
parts/million	0.058417	grains/gallon (U.S.)
parts/million	1.0	mg/liter
parts/million	8.345	lbs/million gallons
ppm by volume (20°C)	$\frac{\text{molecular weight of gas}}{24.04}$	micrograms/liter
ppm by volume (20°C)	$\frac{\text{molecular weight of gas}}{0.02404}$	micrograms/cu meter
ppm by volume (20°C)	$\frac{\text{molecular weight of gas}}{24.04}$	milligrams/cu meter
ppm by volume (20°C)	$\frac{\text{molecular weight of gas}}{28.8}$	ppm by weight
ppm by volume (20°C)	$\frac{\text{molecular weight of gas}}{385.1 \times 10^{6}}$	pounds/cu ft
ppm by weight	1.198×10^{-3}	micrograms/cu meter
ppm by weight	1.198	micrograms/liter
ppm by weight	1.198	milligrams/cu meter
ppm by weight	$\frac{28.8}{\text{molecular weight of gas}}$	ppm by volume (20°C)
ppm by weight	7.48×10^{-6}	pounds/cu ft
pecks (British)	0.25	bushels (British)
pecks (British)	554.6	cubic inches

Multiply	*by*	*to obtain*
pecks (British)	9.091901	liters
pecks (U.S.)	0.25	bushels (U.S.)
pecks (U.S.)	537.605	cubic inches
pecks (U.S.)	8.809582	liters
pecks (U.S.)	8	quarts (dry)
pennyweights	24	grains
pennyweights	1.555174	grams
pennyweights	0.05	ounces (troy)
pennyweights (troy)	4.1667×10^{-3}	pounds (troy)
perches (masonry)	24.75	cubic feet
phots	929.0	foot-candles
phots	1	lumen incident/sq cm
phots	10^4	lux
picas (printers')	1/6	inches
pieds (French feet)	0.3249	meters
pints (dry)	33.6003	cubic inches
pints (liq.)	473.179	cubic centimeters
pints (liq.)	0.01671	cubic feet
pints (liq.)	4.732×10^{-4}	cubic meters
pints (liq.)	6.189×10^{-4}	cubic yards
pints (liq.)	0.125	gallons
pints (liq.)	0.4732	liters
pints (liq.)	16	ounces (U.S. fluid)
pints (liq.)	0.5	quarts (liq.)
planck's constant	6.6256×10^{-27}	erg-seconds
poise	1.00	gram/cm sec
poise	0.1	newton-second/meter^2
population equivalent (PE)	0.17	pounds BOD
pottles (British)	0.5	gallons (British)
pouces (Paris inches)	0.02707	meters
pouces (Paris inches)	0.08333	pieds (Paris feet)
poundals	13,826	dynes
poundals	14.0981	grams
poundals	1.383×10^{-3}	joules/cm
poundals	0.1383	joules/meter (newton)
poundals	0.01410	kilograms
poundals	0.031081	pounds
pounds (avdp)	256	drams (avdp)
pounds (avdp)	116.67	drams (troy)
pounds (avdp)	444,823	dynes
pounds (avdp)	7000	grains
pounds (avdp)	453.5924	grams
pounds (avdp)	0.04448	joules/cm
pounds (avdp)	4.448	joules/meter (newtons)

Multiply	*by*	*to obtain*
pounds (avdp)	0.454	kilograms
pounds (avdp)	4.5359×10^5	milligrams
pounds (avdp)	16	ounces (avdp)
pounds (avdp)	14.5833	ounces (troy)
pounds (avdp)	32.17	poundals
pounds (avdp)	1.2152778	pounds (troy)
pounds (avdp)	4.464×10^{-4}	tons (long)
pounds (avdp)	0.0005	tons (short)
pounds (troy)	210.65	drams (avdp)
pounds (troy)	96	drams (troy)
pounds (troy)	5760	grains
pounds (troy)	373.2418	grams
pounds (troy)	0.37324	kilograms
pounds (troy)	3.7324×10^5	milligrams
pounds (troy)	13.1657	ounces (avdp)
pounds (troy)	12.0	ounces (troy)
pounds (troy)	240.0	pennyweights (troy)
pounds (troy)	0.8229	pounds (avdp)
pounds (troy)	3.6735×10^{-4}	tons (long)
pounds (troy)	3.7324×10^{-4}	tons (metric)
pounds (troy)	4.1143×10^{-4}	tons (short)
pounds (avdp)-force	4.448	newtons
pounds-force-sec/ft^2	47.88026	newton-sec/$meter^2$
pounds (avdp)-mass	0.4536	kilograms
pounds-mass/ft^3	16.0185	kilogram/$meter^3$
pounds-mass/ft-sec	1.4882	mewton-sec/$meter^2$
pounds of BOD	5.882	population equivalent (PE)
pounds of carbon to CO_2	14,544	BTU (mean)
pounds of water	0.0160	cu ft
pounds of water	27.68	cu in
pounds of water	0.1198	gallons
pounds of water evaporated at 212°F	970.3	BTU
pounds of water per min	2.699×10^{-4}	cubic feet/sec
pound-feet	13,825	centimeter-grams
pound-feet (torque)	1.3558×10^7	dyne-centimeters
pound-feet	0.1383	meter-kilograms
pounds-feet squared	421.3	kg-cm squared
pounds-feet squared	144	pounds-inches squared
pounds-inches squared	2926	kg-cm squared
pounds-inches squared	6.945×10^{-3}	pounds-feet squared
pounds/acre	0.0104	grams/sq ft
pounds/acre	0.1121	grams/sq meter
pounds/acre	1.121	kg/ha
pounds/acre	112.1	kilograms/sq km

Multiply	*by*	*to obtain*
pounds/acre	0.01121	milligrams/sq cm
pounds/acre	112.1	milligrams/sq meter
pounds/acre	0.023	pounds/1000 sq ft
pounds/acre	0.32	tons/sq mile
pounds/acre/day	0.112	g/day/sq m
pounds/cu ft	0.0160	g/mL
pounds/cu ft	16.02	kg/cu m
pounds/cu ft	16.018×10^9	micrograms/cu meter
pounds/cu ft	16.018×10^6	micrograms/liter
pounds/cu ft	16.018×10^6	milligrams/cu meter
pounds/cu ft	$\frac{385.1 \times 10^6}{\text{molecular weight of gas}}$	ppm by volume (20°C)
pounds/cu ft	133.7×10^3	ppm by weight
pounds/cu ft	5.787×10^{-4}	lb/cu in
pounds/cu ft	5.456×10^{-9}	pounds/mil-foot
pounds/1000 cu ft	0.35314	grams/cu ft
pounds/1000 cu ft	16.018	grams/cu m
pounds/1000 cu ft	353.14×10^3	micrograms/cu ft
pounds/1000 cu ft	16.018×10^6	microgram/cu m
pounds/1000 cu ft	16.018×10^3	milligrams/cu m
pounds/cubic inch	27.68	grams/cubic cm
pounds/cubic inch	2.768×10^4	kgs/cubic meter
pounds/cubic inch	1728	pounds/cubic foot
pounds/cubic inch	9.425×10^{-6}	pounds/mil foot
pounds/day/acre-ft	3.68	g/day/cu m
pounds/day/cu ft	16	kg/day/cu m
pounds/day/cu yd	0.6	kg/day/cu m
pounds/day/sq ft	4,880	g/day/sq m
pounds/ft	1.488	kg/m
pounds/gal	$454\,\text{g}/3.7851\text{L} = 119.947$	g/liter
pounds/1000-gal	120	g/1000-liters
pounds/horsepower-hour	0.169	mg/joule
pounds/in	178.6	g/cm
pounds/mil-foot	2.306×10^6	gms/cu cm
pounds/mil gal	0.12	g/cu m
pounds/sq ft	4.725×10^{-4}	atmospheres
pounds/sq ft	0.01602	ft of water
pounds/sq ft	0.01414	inches of mercury
pounds/sq ft	4.8824×10^{-4}	kgs/sq cm
pounds/sq ft	4.88241	kilograms/square meter
pounds/sq ft	47.9	newtons/sq m
pounds/sq ft	6.944×10^{-3}	pounds/sq inch
pounds/1000 sq ft	0.4536	grams/sq ft

Multiply	*by*	*to obtain*
pounds/1000 sq ft	4.882	grams/sq meter
pounds/1000 sq ft	4882.4	kilograms/sq km
pounds/1000 sq ft	0.4882	milligrams/sq cm
pounds/1000 sq ft	4882.4	milligrams/sq meter
pounds/1000 sq ft	43.56	pounds/acre
pounds/1000 sq ft	13.94	tons/sq mile
pounds/sq in	0.068046	atmospheres
pounds/sq in	2.307	ft of water
pounds/sq in	70.307	grams/square centimeter
pounds/sq in	2.036	in of mercury
pounds/sq in	0.0703	kgs/square cm
pounds/sq in	703.07	kilograms/square meter
pounds/sq in	51.715	millimeters of mercury
pounds/sq in	6894.76	newton/meter^2
pounds/sq in	51.715	millimeters of mercury at 0°C
pounds/sq in	144	pounds/sq foot
pounds/sq in (abs)	1	pound/sq in (gage) + 14.696
proof (U.S.)	0.5	percent alcohol by volume
puncheons (British)	70	gallons (British)
quadrants (angle)	90	degrees
quadrants (angle)	5400	minutes
quadrants (angle)	3.24×10^5	seconds
quadrants (angle)	1.571	radians
quarts (dry)	67.20	cubic inches
quarts (liq.)	946.4	cubic centimeters
quarts (liq.)	0.033420	cubic feet
quarts (liq.)	57.75	cubic inches
quarts (liq.)	9.464×10^{-4}	cubic meters
quarts (liq.)	1.238×10^{-3}	cubic yards
quarts (liq.)	0.25	gallons
quarts (liq.)	0.9463	liters
quarts (liq.)	32	ounces (U.S., fl)
quarts (liq.)	0.832674	quarts (British)
quintals (long)	112	pounds
quintals (metric)	100	kilograms
quintals (short)	100	pounds
quires	24	sheets
radians	57.29578	degrees
radians	3438	minutes
radians	0.637	quadrants
radians	2.063×10^5	seconds
radians/second	57.30	degrees/second
radians/second	9.549	revolutions/min
radians/second	0.1592	revolutions/sec

Multiply	*by*	*to obtain*
radians/sec/sec	573.0	revs/min/min
radians/sec/sec	9.549	revs/min/sec
radians/sec/sec	0.1592	revs/sec/sec
reams	500	sheets
register tons (British)	100	cubic feet
revolutions	360	degrees
revolutions	4	quadrants
revolutions	6.283	radians
revolutions/minute	6	degrees/second
revolutions/minute	0.10472	radians/second
revolutions/minute	0.01667	revolutions/sec
revolutions/minute^2	0.0017453	radians/sec/sec
revs/min/min	0.01667	revs/min/sec
revs/min/min	2.778×10^{-4}	revs/sec/sec
revolutions/second	360	degrees/second
revolutions/second	6.283	radians/second
revolutions/second	60	revs/minute
revs/sec/sec	6.283	rads/sec/sec
revs/sec/sec	3600	revs/min/min
revs/sec/sec	60	revs/min/sec
reyns	6.8948×10^{6}	centipoises
rod	.25	chain (gunters)
rods	16.5	feet
rods	5.0292	meters
rods	3.125×10^{-3}	miles
rods (surveyors' means)	5.5	yards
roods (British)	0.25	acres
scruples	1/3	drams (troy)
scruples	20	grains
sections	1	square miles
seconds (mean solar)	1.1574×10^{-5}	days
seconds (angle)	2.778×10^{-4}	degrees
seconds (mean solar)	2.7778×10^{-4}	hours
seconds (angle)	0.01667	minutes
seconds (angle)	3.087×10^{-6}	quadrants
seconds (angle)	4.848×10^{-6}	radians
slugs	14.59	kilogram
slugs	32.174	pounds
space, entire (solid angle)	12.566	steradians
spans	9	inches
spheres (solid angle)	12.57	steradians
spherical right angles	0.25	hemispheres
spherical right angles	0.125	spheres
spherical right angles	1.571	steradians

Multiply	*by*	*to obtain*
square centimeters	1.973×10^{5}	circular mils
square centimeters	1.07639×10^{-3}	square feet (U.S.)
square centimeters	0.15499969	square inches (U.S.)
square centimeters	10^{-4}	square meters
square centimeters	3.861×10^{-11}	square miles
square centimeters	100	square millimeters
square centimeters	1.196×10^{-4}	square yards
square centimeters-square centimeter (moment of area)	0.024025	square inch-square inch
square chains (gunter's)	0.1	acres
square chains (gunter's)	404.7	square meters
square chains (Ramden's)	0.22956	acres
square chains (Ramden's)	10000	square feet
square feet	2.29×10^{-5}	acres
square feet	1.833×10^{8}	circular mils
square feet	144	square inches
square feet	0.092903	square meters
square feet	929.0341	square centimeters
square feet	3.587×10^{-8}	square miles
square feet	1/9	square yards
square feet/cu ft	3.29	sq m/cu m
square foot-square foot (moment of area)	20,736	square inch-square inch
square inches	1.273×10^{6}	circular mils
square inches	6.4516258	square centimeters
square inches	6.944×10^{-3}	square feet
square inches	645.2	square millimeters
square inches	10^{6}	square mils
square inches	7.71605×10^{-4}	square yards
square inches-inches sqd.	41.62	sq cm-cm sqd
square inches-inches sqd.	4.823×10^{-5}	sq feet-feet sqd
square kilometers	247.1	acres
square kilometers	10^{10}	square centimeters
square kilometers	10.76×10^{6}	square feet
square kilometers	1.550×10^{9}	square inches
square kilometers	10^{6}	square meters
square kilometers	0.3861006	square miles (U.S.)
square kilometers	1.196×10^{6}	square yards
square links (Gunter's)	10^{-5}	acres (U.S.)
square links (Gunter's)	0.04047	square meters
square meters	2.471×10^{-4}	acres (U.S.)
square meters	10^{4}	square centimeters
square meters	10.76387	square feet (U.S.)
square meters	1550	square inches

Multiply	*by*	*to obtain*
square meters	3.8610×10^{-7}	square miles (statute)
square meters	10^6	square millimeters
square meters	1.196	square yards (U.S.)
square miles	640	acres
square miles	2.78784×10^7	square feet
square miles	2.590	sq km
square miles	2.5900×10^6	square meters
square miles	3.098×10^6	square yards
square millimeters	1.973×10^3	circular mils
square millimeters	0.01	square centimeters
square millimeters	1.076×10^{-5}	square feet
square millimeters	1.550×10^{-3}	square inches
square mils	1.273	circular mils
square mils	6.452×10^{-6}	square centimeters
square mils	10^{-6}	square inches
square rods	272.3	square feet
square yard	2.1×10^{-4}	acres
square yards	8361	square centimeters
square yards	9	square feet
square yards	1296	square inches
square yards	0.8361	square meters
square yards	3.228×10^{-7}	square miles
square yards	8.361×10^5	square millimeters
statamperes	3.33560×10^{-10}	amperes (abs)
statcoulombs	3.33560×10^{-10}	coulombs (abs)
statcoulombs/kilogram	1.0197×10^{-6}	statcoulombs/dyne
statfarads	1.11263×10^{-12}	farads (abs)
stathenries	8.98776×10^{11}	henries (abs)
statohms	8.98776×10^{11}	ohms (abs)
statvolts	299.796	volts (abs)
statvolts/inch	118.05	volts (abs)/centimeter
statwebers	2.99796×10^{10}	electromagnetic cgs units of magnetic flux
statwebers	1	electrostatic cgs units of magnetic flux
stilb	2919	footlambert
stilb	1	int. candle cm^{-2}
stilb	3.142	lambert
stoke (kinematic viscosity)	10^{-4}	$meter^2$/second
stones (British)	6.350	kilograms
stones (British)	14	pounds
temp. (degs. C.) + 273	1	abs. temp. (degs. K.)
temps (degs. C.) + 17.8	1.8	temp. (degs. Fahr.)
temps. (degs. F.) + 460	1	abs. temp. (degs. R.)
temps. (degs. F.) − 32	5/9	temp. (degs. Cent.)

Multiply	*by*	*to obtain*
toises (French)	6	paris feet (pieds)
tons (long)	5.734×10^5	drams (avdp)
tons (long)	2.613×10^5	drams (troy)
tons (long)	1.568×10^7	grains
tons (long)	1.016×10^6	grams
tons (long)	1016	kilograms
tons (long)	3.584×10^4	ounces (avdp)
tons (long)	3.267×10^4	ounces (troy)
tons (long)	2240	pounds (avdp)
tons (long)	2722.2	pounds (troy)
tons (long)	1.12	tons (short)
Tons (metric) (T)	1000	kilograms
Tons (metric) (T)	2204.6	pounds
Tons (metric) (T)	1.1025	tons (short)
tons (short)	5.120×10^5	drams (avdp)
tons (short)	2.334×10^5	drams (troy)
tons (short)	1.4×10^7	grains
tons (short)	9.072×10^5	grams
tons (short)	907.2	kilograms
tons (short)	32,000	ounces (avdp)
tons (short)	29,166.66	ounces (troy)
tons (short)	2000	pounds (avdp)
tons (short)	2.430.56	pounds (troy)
tons (short)	0.89287	tons (long)
tons (short)	0.9078	Tons (metric) (T)
tons (short)/sq ft	9765	kg/sq meter
tons (short)/sq ft	13.89	pounds/sq inch
tons (short)/sq in	1.406×10^6	kg/sq meter
tons (short)/sq in	2000	pounds/sq inch
tons/sq mile	3.125	pounds/acre
tons/sq mile	0.07174	pounds/1000 sq ft
tons/sq mile	0.3503	grams/sq meter
tons/sq mile	350.3	kilograms/sq km
tons/sq mile	350.3	milligrams/sq meter
tons/sq mile	0.03503	milligrams/sq cm
tons/sq mile	0.03254	grams/sq ft
tons of water/24 hours	83.333	pounds of water/hr
tons of water/24 hours	0.16643	gallons/min
tons of water/24 hours	1.3349	cu ft/hr
torr (mm Hg, 0°C)	133.322	newton/meter^2
townships (U.S.)	23040	acres
townships (U.S.)	36	square miles
tuns	252	gallons
volts (abs)	10^8	abvolts

Multiply	*by*	*to obtain*
volts (abs)	3.336×10^{-3}	statvolts
volts (internationalof 1948)	1.00033	volts (abs)
volt/inch	.39370	volt/cm
watts (abs)	3.41304	BTU (mean)/hour
watts (abs)	0.0569	BTU (mean)/min
watts (abs)	0.01433	calories, kilogram (mean)/minute
watts (abs)	10^7	ergs/second
watts (abs)	44.26	foot-pounds/minute
watts (abs)	0.7376	foot-pounds/second
watts (abs)	0.0013405	horsepower (electrical)
watts (abs)	1.360×10^{-3}	horsepower (metric)
watts (abs)	1	joules/sec
watts (abs)	0.10197	kilogram-meters/second
watts (abs)	10^{-3}	kilowatts
watt-hours	3.415	British Thermal Units
watt-hours	3.60×10^{10}	ergs
watt-hours	2655	foot-pounds
watt-hours	859.85	gram-calories
watt-hours	1.34×10^{-3}	horsepower-hours
watt-hours	3.6×10^3	joule
watt-hours	0.8605	kilogram-calories
watt-hours	367.1	kilogram-meters
watt-hours	10^{-3}	kilowatt-hours
watt (international)	1.0002	watt (absolute)
watt/(cm^2)(°C/cm)	693.6	BTU/(hr)(ft^2)(°F/in)
wave length of the red line of cadmium	6.43847×10^{-7}	meters
webers	10^3	electromagnetic cgs units
webers	3.336×10^{-3}	electrostatic cgs units
webers	10^5	kilolines
webers	10^8	lines
webers	10^8	maxwells
webers	3.336×10^{-3}	statwebers
webers/sq in	1.550×10^7	gausses
webers/sq in	10^8	lines/sq in
webers/sq in	0.1550	webers/sq cm
webers/sq in	1,550	webers/sq meter
webers/sq meter	10^4	gausses
webers/sq meter	6.452×10^4	lines/sq in
webers/sq meter	10^{-4}	webers/sq cm
webers/sq meter	6.452×10^{-4}	webers/sq in
weeks	168	hours
weeks	10,080	minutes

Multiply	*by*	*to obtain*
weeks	604,800	seconds
yards	91.44	centimeters
yards	3	feet
yards	36	inches
yards	9.144×10^{-4}	kilometers
yards	0.91440	meters
yards	4.934×10^{-4}	miles (naut.)
yards	5.682×10^{-4}	miles (stat.)
yards	914.4	millimeters
years (sidereal)	365.2564	days (mean solar)
years (sidereal)	366.2564	days (sidereal)
years (tropical, mean solar)	365.2422	days (mean solar)
years (common)	8760	hours
years (tropical, mean solar)	8765.8128	hours (mean solar)
years (leap)	366	days
years (leap)	8784	hours
years (tropical, mean solar)	3.155693×10^{7}	seconds (mean solar)
years (tropical, mean solar)	1.00273780	years (sidereal)

2. BASIC AND SUPPLEMENTARY UNITS

A *meter (m)* is 1,650,763.73 wavelengths in vacuo of the radiation corresponding to the transition between the energy levels $2p_{10}$ and $5d_5$ of the krypton 86 atom.

A *kilogram (kg)* is the mass of the international prototype in the custody of the Bureau International des Poids et Mesures at Sevres in France.

A *second (sec)* is the interval occupied by 9,192,631,770 cycles of the radiation corresponding to the transition of the cesium-133 atom when unperturbed by exterior fields.

An *ampere* is the constant current that if maintained in two parallel rectilinear conductors of infinite length of negligible circular cross section and placed at a distance of one meter apart in vacuo would produce between these conductors a force equal to 2×10^{-7} newton per meter length.

A *kelvin* ($^\circ K$) is the degree interval of the thermodynamic scale on which the temperature of the triple point of water is 273.16 degrees.

A *candle* is such that the luminance of a full radiator at the temperature of solidification of platinum is 60 units of luminous intensity per square centimeter.

A *mole (mol)* is the amount of substance which contains as many elementary units as there are atoms in 0.012 kg of carbon-12. The elementary unit must be specified and may be an atom, an ion, an electron, a photon, etc., or a given group of such entities.

A *radian* is the angle subtended at the center of a circle by an arc of the circle equal in length to the radius of the circle.

A *steradian* is the solid angle that, having its vertex at the center of a sphere, cuts off an area of the surface of the sphere equal to that of a square with sides of length equal to the radius of the sphere.

3. DERIVED UNITS AND QUANTITIES

The *liter* was defined in 1901 as the volume of 1 kilogram of pure water at normal atmospheric pressure and maximum density equal therefore to $1.000028\,dm^3$. This 1901 definition applied for the purpose of the 1963 Weights and Measures Acts.

By a resolution of the 12th Conference General des Poids et Mesures (CGPM) in 1964 the word *liter* is now recognized as a special name for the dm^3, but is not used to express high precision measurements. It is used widely in engineering and the retail business, where the discrepancy of 28 parts in 1 million is of negligible significance.

A *newton (N)* is the force that, when applied to a body of mass of one kilogram, gives it an acceleration of one meter per second per second.

Stress is defined as the resultant internal force per unit area resisting change in the shape or size of a body acted on by external forces, and is therefore measured in *newtons per square meter* (N/m^2).

A *bar* is a pressure equivalent to 100,000 newtons acting on an area of one square metor.

A *joule (J)* is the work done when the point of application of a force of one newton is displaced through a distance of one meter in the direction of the force.

A *watt* is equal to one joule per second.

Dynamic viscosity is the property of a fluid whereby it tends to resist relative motion within itself. It is the shear stress, i.e., the tangential force on unit area, between two infinite horizontal planes at unit distance apart, one of which is fixed while the other moves with unit velocity. In other words, it is the shear stress divided by the velocity gradient, i.e., $(N/m^2) \div (m/sec/m) = N\ sec/m^2$.

Kinematic viscosity is the dynamic viscosity of a fluid divided by its density, i.e., $(N\ sec/m^2)/(kg/m^3) = m^2/sec$.

Density of heat flow rate (or heat flux) is the heat flow rate (W) per unit area, i.e., W/m^2.

Coefficient of heat transfer is the heat flow rate (W) per unit area per unit temperature difference, i.e., $W/m^{2\circ}C$.

Thermal conductivity is the quantity of heat that will be conducted in unit time through unit area of a slab of material of unit thickness with a unit difference of temperature between the faces; in other words, the heat flow rate (W) per unit area per unit temperature gradient, i.e., $W/m^2(^\circ C/m) = W/m^\circ C$.

The *heat capacity* of a substance is the quantity of heat gained or lost by the substance per unit temperature change, i.e., $J/^\circ C$.

Specific heat capacity is the heat capacity per unit mass of the substance, i.e., $J/kg^\circ C$.

Internal energy is the kinetic energy possessed by the molecules of a substance due to temperature and is measured in joules (J).

Specific internal energy (u) is the internal energy per unit mass of the substance, i.e., J/kg. When a small amount of heat is added at constant volume the increase in specific internal

energy is given by: $\mathrm{du} = c_\mathrm{v}\, dT$, where c_v is the specific heat capacity at constant volume, and dT is the increase in absolute temperature.

Specific enthalpy (h) is defined by the equation: $h = u + pv$, where p is the pressure and v is the specific volume. Specific enthalpy is measured in J/kg. When a small amount of heat is added to a substance at constant pressure, the increase in specific enthalpy is given by: $-dh = cp\, dT$, where cp is the specific heat capacity at constant pressure.

The *specific latent heat* of a substance is the heat gained per unit mass without an accompanying rise in temperature during a change of state at constant pressure. It is measured in J/kg.

The *entropy* (S) of a substance is such that when a small amount of heat is added, the increase in entropy is equal to the quantity of heat added (dQ) divided by the absolute temperature (T) at which the heat is absorbed; i.e., $dS = dQ/T$, measured in J/°K.

The *specific entropy* (s) of a substance is the entropy per unit mass, i.e., J/kg°K.

A *volt* is the difference of electric potential between two points of a conductor carrying a constant current of one ampere when the power dissipated is one watt.

A *weber* (Wb) is the magnetic flux through a conductor with a resistance of one ohm when reversal of the direction of the magnetic flux causes the transfer of one coulomb in the conductor loop.

Tesla: The magnetic flux density is the normal magnetic flux per unit area and is measured in *teslas*.

A *lumen*, the unit of luminous flux, is the flux emitted within unit solid angle of one steradian by a point source having a uniform intensity of one candle.

A *lux* is an illumination of one lumen per square meter.

Luminance is the luminous intensity per unit area of a source of light or of an illumination. It is measured in candles per square meter.

4. PHYSICAL CONSTANTS

Standard temperature and pressure (S.T.P.)	$= 273.15^\circ$K and 1.013×10^5 N/m^2 $= 0^\circ$C and 1.013 bar $= 0^\circ$C and 760 mm Hg
Molecular volume of ideal gas at S.T.P.	= 22.41liters/mol
Gas constant (R)	$= 8.314$ J/mol$^\circ$K
$^{RT}(273.15^\circ\text{K})$	$= 2.271 \times 10^3$ J/mol
Avogadro constant	$= 6.023 \times 10^{23}$/mol
Boltzmann constant	$= 1.3805 \times 10^{-23}$ J/K
Faraday constant	$= 9.6487 \times 10^4\,^\circ$C/mol (= A s/mol)
Planck constant	$= 6.626 \times 10^{-34}$ J sec
Stefan-Boltzman constant	$= 5.6697 \times 10^{-8}$ W/m^2 K^4
Ice point of water	$= 273.15^\circ$K (0°C)
Triple point of water	$= 273.16^\circ$K (0.01°C)
Speed of light	$= 2.998 \times 10^8$ m/sec
Acceleration of gravity (standard) (Greenwich)	$= 9.80665$ m/s^2 $= 9.81188$ m/s^2 [take g as 9.81 m/s^2]
Universal constant of gravitation	$= 6.670 \times 10^{-11}$ Newton m^2/kg^2
Mass of hydrogen atom	$= 1.6734 \times 10^{-27}$ kg

5. PROPERTIES OF WATER

Temperature ($^\circ$F)	Specific weight, γ (lb/ft^3)	Mass density, ρ (lb-sec^2/ft^4)	Dynamic viscosity, $\mu \times 10^5$ (lb-sec/ft^2)	Kinematic viscosity, $\nu \times 10^5$ (ft^2/sec)	Surface energy, $\sigma \times 10^3$ (lb/ft)	Vapor pressure, ρ (lb/in.^{2})	Bulk modulus, $E \times 10^{-3}$ (lb/in.^{2})
32	62.42	1.940	3.746	1.931	5.18	0.09	290
40	62.43	1.938	3.229	1.664	5.14	0.12	295
50	62.41	1.936	2.735	1.410	5.09	0.18	300
60	62.37	1.934	2.359	1.217	5.04	0.26	312
70	62.30	1.931	2.050	1.059	5.00	0.36	320
80	62.22	1.927	1.799	0.930	4.92	0.51	323
90	62.11	1.923	1.595	0.826	4.86	0.70	326
100	62.00	1.918	1.424	0.739	4.80	0.95	329
110	61.86	1.913	1.284	0.667	4.73	1.24	331
120	61.71	1.908	1.168	0.609	4.65	1.69	333
130	61.55	1.902	1.069	0.558	4.60	2.22	332
140	61.38	1.896	0.981	0.514	4.54	2.89	330
150	61.20	1.890	0.905	0.476	4.47	3.72	328
160	61.00	1.896	0.838	0.442	4.41	4.74	326
170	60.80	1.890	0.780	0.413	4.33	5.99	322
180	60.58	1.883	0.726	0.385	4.26	7.51	318
190	60.36	1.876	0.678	0.362	4.19	9.34	313
200	60.12	1.868	0.637	0.341	4.12	11.52	308
212	59.83	1.860	0.593	0.319	4.04	14.7	300

6. PERIODIC TABLE OF THE ELEMENTS (COMPLIMENTS OF THE LENOX INSTITUTE OF WATER TECHNOLOGY)

Groups ⇨ / Periods & sub-shells ⇩	I IA	2 IIA	3 IIIB	4 IVB	5 VB	6 VIB	7 VIIB	8	9 VIII	10	11 IB	12 IIB	13 IIIA	14 VIA	15 VA	16 VIA	17 VIIA	18 O
I IS	1 H 1.00794 Hydrogen																1 H 1.00794 Hydrogen	2 He 4.00260 Helium
2 2s2p	3 Li 6.941 Lithium	4 Be 9.01218 Beryllium											5 B 10.811 Boron	6 C 12.011 Boron	7 N 14.0067 Nitrogen	8 O 15.9994 Oxygen	9 F 18.9984 Fluorine	10 Ne 20.179 Neon
3 3s3p	11 Na 22.9897 Sodium	12 Mg 24.305 Magnesium											13 Al 26.9815 Aluminum	14 Si 28.0855 Silicon	15 P 30.9738 Phosphorus	16 S 32.066 Sulfur	17 Cl 35.4527 Chlorine	18 Ar 39.948 Argon
4 4s3d4p	19 K 39.098 Potassium	20 Ca 40.078 Calcium	21 Sc 44.9559 Scandium	22 Ti 47.88 Titanium	23 V 50.9415 Vanadium	24 Cr 51.996 Chromium	25 Mn 54.938 Manganese	26 Fe 55.847 Iron	27 Co 58.933 Cobalt	28 Ni 58.69 Nickel	29 Cu 63.546 Copper	30 Zn 65.39 Zinc	31 Ga 69,723 Gallium	32 Ge 69.561 Germanium	33 As 74.9216 Arsenic	34 Se 78.96 Selenium	35 Br 79.904 Bromine	36 Kr 83.80 Krypton
5 5s4d5p	37 Rb 85.468 Rubidium	38 Sr 87.62 Strontium	39 Y 88.9059 Yttrium	40 Zr 91.224 Zirconium	41 Nb 92.9064 Niobium	42 Mo 95.94 Molybdenum	43 Tc (98) Technetium	44 Ru 101.07 Ruthenium	45 Rh 102.906 Rhodium	46 Pd 106.42 Palladium	47 Ag 107.868 Silver	48 Cd 112.411 Cadmium	49 In 114.82 Indium	50 Sn 118.710 Tin	51 Sb 121.75 Antimony	52 Te 127.60 Tellurium	53 I 126.90 Iodine	54 Xe 131.29 Xenon
6 6s4f5d6p	55 Cs 132.905 Cesium	56 Ba 137.327 Barium	57 La 138.906 Lanthanum	72 Hf 178.49 Hafnium	73 Ta 180.948 Tantalum	74 W 183.85 Tungsten	75 Re 186.207 Rhenium	76 Os 190.2 Osmium	77 Ir 192.22 Iridium	78 Pt 195.08 Platinum	79 Au 196.97 Gold	80 Hg 200.59 Mercury	81 Tl 204.383 Thallium	82 Pb 207.2 Lead	83 Bi 208.98 Bismuth	84 Po (209) Polonium	85 At (210) Astatine	86 Rn (222) Radon
7 7s5f6d	87 Fr (223) Francium	88 Ra (226) Radium	89 Ac (227) Actinium	104 Rf (261) Ruther-fordium	105 Ha (262) Dubnium	106 Sg (263) Seaborgium	107 Ns (262) Bohrium	108 Hs (265) Hassium	109 Mt (266) Meitnerium	110		112						

6 4f	58 Ce 140.116 Cerium	59 Pr 140.91 Praseody-mium	60 Nd 144.24 Neodymium	61 Pm (145) Promethium	62 Sm 150.35 Samarium	63 Eu 107.26 Europium	64 Gd 157.25 Gadolinium	65 Tb 158.925 Terbium	66 Dy 162.50 Dysprosium	67 Ho 104.930 Holmium	68 Er 167.26 Erbium	69 Tm 168.934 Thulium	70 Yb 173.04 Ytterbium	71 Lu 174.967 Lutetium
7 5f	90 Th 232.038 Thorium	91 Pa (231) Protactinium	92 U 238.029 Uranium	93 Np (237) Neptunium	94 Pu (244) Plutonium	95 Am (243) Americium	96 Cm (247) Curium	97 Bk (247) Berkelium	98 Cf (251) Californium	99 Es (252) Einsteinium	100 Fm (257) Fermium	101 Md (258) Mendelevium	102 No (259) Nobelium	103 Lr (262) Lawrencium

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