Handbook of Environmental Engineering 26

Lawrence K. Wang
Mu-Hao Sung Wang
Yung-Tse Hung *Editors*

# Waste Treatment in the Biotechnology, Agricultural and Food Industries

## Volume 1

Springer

# Handbook of Environmental Engineering 26

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The past 75 years have seen the emergence of a growing desire worldwide to take positive actions to restore and protect the environment from the degrading effects of all forms of pollution: air, noise, solid waste, and water. The principle intention of the Handbook of Environmental Engineering (HEE) series is to help readers formulate answers to the fundamental questions facing pollution in the modern era, mainly how serious is pollution and is the technology needed to abate it not only available, but feasible. In a highly practical manner, HEE offers educators, students, and engineers a strong grounding in the principles of Environmental Engineering, as well as providing effective methods for developing optimal abatement technologies at costs that are fully justified by the degree of abatement achieved. With an emphasis on using the Best Available Technologies, the authors of these volumes present the necessary engineering protocols derived from the fundamental principles of chemistry, physics, and mathematics, making these volumes a must have for environmental pollution researchers.

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# Preface

The past 75 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, thermal, radioactive, and noise. Since pollution is a direct or indirect consequence of waste, 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, or reusable form. Three major questions usually arise when a particular type of pollution has been identified: (1) How serious are the environmental pollution and natural resources crisis? (2) Is the technology to abate them or recycle them available? and (3) Do the costs of abatement justify the degree of treatment achieved for environmental protection and resources conservation? 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 above three questions.

The traditional approach of applying tried-and-true solutions to specific environmental and natural resources 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. A prerequisite to such planning is an understanding of the performance, potential, and limitations of the various methods of environmental protection and resources recovery available for environmental scientists and engineers. In this series of handbooks, we will review at a tutorial level a broad spectrum of engineering systems (natural environment, processes, operations, and methods) currently being utilized, or of potential utility, for pollution abatement, environmental protection, and natural resources conservation. 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 an empirical accumulation of facts. It is not intended, though, to neglect empiricism where such data lead quickly to the most economical 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 a bio-environmental engineer must understand science within the context of applications, 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 natural resources conservation or environmental protection. Throughout the series, methods of mathematical modeling, system analysis, 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 or models have been provided.

Our treatment of wastes from biotechnology, agricultural, and food industries 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 bio-environmental engineering systems, and exhibit greater flexibility and originality in the definition and innovative solution of bio-environmental system problems. In short, the bio-environmental engineers should, by conviction and practice, be more readily adaptable to change and progress.

Coverage of the unusually broad field of environmental science, technology, engineering, and mathematics (STEM) has demanded 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 materials 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 the 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 redundancy of units' usage will prove to be useful rather than being disruptive to the readers.

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

This book (*Waste Treatment in the Biotechnology, Agricultural and Food Industries, Volume 1*) and its sister books of the *Handbook of Environmental Engineering (HEE)* series have been designed to serve as a mini-series of bio-environmental engineering and management textbooks as well as supplemental reference books. We hope and expect they will prove of equally high value to advanced undergraduate and graduate students, to designers of sustainable biological resources systems, and to scientists and researchers. The editors welcome comments from readers in all of these categories. It is our hope that the bio-environmental engineering and management books will not only provide information on bio-resources engineering but will also serve as a basis for advanced study or specialized investigation of the theory and analysis of various biological systems.

This book, *Waste Treatment in the Biotechnology, Agricultural and Food Industries, Volume 1,* covers the topics on: treatment and management of livestock wastes; waste treatment in the pharmaceutical biotechnology industry using green environmental technologies; vermicomposting process for processing agricultural and food industry wastes; the impacts of climate change on agricultural, food, and public utility industries; innovative PACT-activated sludge, CAPTOR-activated sludge, activated bio-filter, vertical loop reactor, and PHOSTRIP processes; agricultural waste treatment by water hyacinth aquaculture, wetland aquaculture, evapotranspiration, rapid rate land treatment, slow rate land treatment, and subsurface infiltration; production and applications of crude polyhydroxyalkanoate-containing bioplastic from the agricultural and food-processing wastes; optimization processes of biodiesel production from pig and neem (Azadirachta indica A. juss) seeds blend oil using alternative catalysts from waste biomass; making castor oil a promising source for the production of flavor and fragrance through lipase-mediated biotransformation; and treatment and minimization of waste in baker's yeast industry.

The editors are pleased to acknowledge the encouragement and support received from Mr. Aaron Schiller, Executive Editor of the Springer Nature Switzerland AG, and his colleagues, 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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# Contents

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**Mu-Hao Sung Wang** has been an engineer of the New York State Department of Environmental Conservation, an editor of CRC Press, Springer Nature Switzerland, and Lenox Institute Press, and a university professor of the Stevens Institute of Technology, National Cheng-Kung University, and the Lenox Institute of Water Technology. Totally, she has been a government official and an educator in the USA and Taiwan for over 50 years. Dr. Wang is a licensed Professional Engineer, and a Diplomate of the American Academy of Environmental Engineers (AAEE). Her publications have been in the areas of water quality, modeling, environmental sustainability, solid and hazardous waste management, NPDES, flotation technology, industrial waste treatment, and analytical methods. Dr. Wang is the author of over 50 publications and an inventor of 14 US and foreign patents. She holds a BSCE degree from National Cheng-Kung University, Taiwan, ROC; an MS degree from the University of Rhode Island, RI, USA; and a PhD degree from Rutgers University, NJ, USA. She is the co-series editor of the *Handbook of Environmental Engineering* series (Springer Nature Switzerland), Coeditor of the *Advances in Industrial and Hazardous Wastes Treatment* series (CRC Press of Taylor & Francis Group) and the Coeditor of the *Environmental Science, Technology, Engineering and Mathematics series* (Lenox Institute Press). She is a member of AWWA, NYWWA, NEWWA, WEF, NEWEA, CIE, and OCEESA.

**Yung-Tse Hung** has been Professor of Civil Engineering at Cleveland State University, Cleveland, Ohio, USA, since 1981. He is a Fellow of the American Society of Civil Engineers, a licensed Professional Engineer in Ohio and North Dakota, and a Diplomate of American Academy of Environmental Engineers. He has taught at 16 universities in 8 countries. His research interests and publications have been involved with biological treatment processes, solid wastes, hazardous waste management, and industrial waste treatment. Dr. Hung is credited with over 470 publications and presentations, 28 books, 159 book chapters, in water and wastewater treatment. He received his BSCE and MSCE degrees from National Cheng-Kung University, Taiwan, ROC, and his PhD degree from the University of Texas at Austin, USA. He is the Editor-in-Chief of *International Journal of Environment and Waste Management, International Journal of Environmental Engineering,* and *International Journal of Environmental Engineering Science,* and Coeditor of the *Advances in Industrial and Hazardous Wastes Treatment* series (CRC Press of Taylor & Francis Group), and the *Handbook of Environmental Engineering* series (Springer). Dr. Hung is also the Chief Editor of the *Handbook of Environment and Waste Management series* (World Scientific Singapore) and the Permanent Executive Director and Ex-President of OCEESA (Overseas Chinese Environmental Engineers and Scientists Association).

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# Chapter 1
# Management and Treatment of Livestock Wastes


**Dale H. Vanderholm, Donald L. Day, Arthur J. Muehling, Lawrence K. Wang, Yung-Tse Hung, Erick Butler, Mu-Hao Sung Wang, and Haneen Yehya**

## Nomenclature

| | |
|---|---|
| AU | Number of 1000 lb animal units per animal type |
| $BOD_5$ | Five-day biochemical oxygen demand |
| BUW | Bedding unit weight, $lb/ft^3$ |
| $Ca^{+2}$ | Calcium cation |
| C | Targeted rate concentration |
| C* | Background rate concentration |
| Co | Initial concentration of conditions |

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| | |
|---|---|
| COD | Chemical oxygen demand |
| $CH_3COOH$ | Acetic acid |
| CO | Carbon monoxide |
| $CO_2$ | Carbon dioxide |
| D | Number of days in storage period |
| DS | Dissolved solids |
| DVM | Daily volume of manure production for animal type, $ft^3$/AU/day |
| FR | Volumetric void ratio |
| FS | Fixed solids |
| $H_2$ | Diatomic hydrogen |
| HLR | Hydraulic loading rate |
| k | First-order rate constant (cm/day) |
| $Mg^{+2}$ | Magnesium cation |
| $MMCTCO_{2e}$ | Million metric tons of $CO_2$ equivalent |
| $N_2$ | Diatomic nitrogen |
| $NH_3$-N | Ammonia-nitrogen |
| $NH_4$-N | Ammonium-nitrogen |
| NO | Nitrous oxide |
| OLR | Organic loading rate |
| $PO_4^{-3}$ | Phosphate ion |
| q | Hydraulic loading rate (cm/day) |
| SS | Suspended solids |
| TKN | Total Kjeldahl nitrogen |
| TP | Total phosphorus |
| TS | Total solids |
| TBV | Total bedding volume stored, $ft^3$ |
| TVM | Total volume of stored manure, $ft^3$ |
| TWW | Total wastewater stored, $ft^3$ |
| TVS | Total volatile solids |
| VMD | Volume of manure production for animal type for storage period, $ft^3$ |
| WB | Weight of bedding used for animal type, lb/AU/day |
| WV | Volume of waste stored, $ft^3$ |

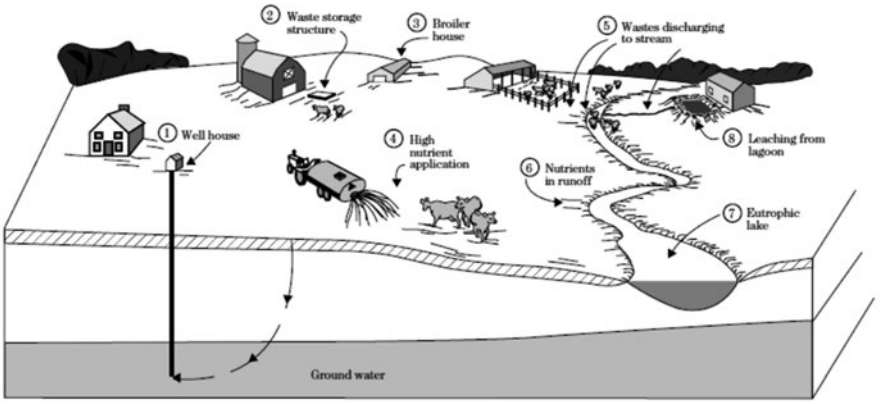
## 1.1 Introduction

In recent years, livestock waste management has been a rapidly changing technology. It is subject to government regulation and sensitive to population growth patterns, community attitudes, and land-use changes. It is influenced by variables such as soil type, topography, climate, crops, and livestock production practices. The evolution of larger and more concentrated livestock operations has accentuated the problems of waste management. Better management methods are necessary not only to hold down labor requirements and expense but also to minimize detrimental effects on the environment. Where animals are allowed to roam freely on pastures, such as is still done in many areas of the state, the manure from the livestock is

deposited directly on the land and recycled with a minimum hazard to the environment. Even pasture production of livestock, however, requires management to prevent overgrazing, overcrowding, loss of vegetative cover, and the development of potential nonpoint sources of pollution. The facilities that cause the greatest environmental threat, however, are those in which the livestock are confined permanently or frequently on a regular basis. Figure 1.1 provides the consequences of infiltrated livestock waste.

In general, the regulations do not stipulate how waste must be handled but rather delineate the unsatisfactory practices and acceptable methods for correcting unsatisfactory situations. The decision-making process, when a farmer has to deal with correcting a problem situation, is essentially left to the farmer as to the selection of the system or combination of systems to correct the problems.

The frequent use of the term "waste" in this chapter is not intended to imply that we are dealing with a material of no value. The intent is to convey the understanding that the material consists of more than just the feces and urine excreted by the animals, for example, hair, soil, spilled feed, and other materials. In actuality, there is much that can be recovered and reused from this material for supplying plant




1. Contaminated well: Well water contaminated by bacteria and nitrates because of leaching through soil. (See item 4.)
2. Waste storage structure: Poisonous and explosive gases in structure.
3. Animals in poorly ventilated building: Ammonia and other gases create respiratory and eye problems in animals and corrosion of metals in building.
4. Waste applied at high rates: Nitrate toxicity and other N-related diseases in cattle grazing cool-season grasses; leaching of $NO_3$ and microorganisms through soil, fractured rock, and sinkholes.
5. Discharging lagoon, runoff from open feedlot, and cattle in creek: (a) Organic matter creates low dissolved oxygen levels in stream; (b) Ammonia concentration reaches toxic limits for fish; and (c) Stream is enriched with nutrients, creating eutrophic conditions in downstream lake.
6. Runoff from fields where livestock waste is spread and no conservation practices on land: P and $NH_4$ attached to eroded soil particles and soluble nutrients reach stream, creating eutrophic conditions in downstream lake.
7. Eutrophic conditions: Excess algae and aquatic weeds created by contributions from items 5 and 6; nitrite poisoning (brown-blood disease) in fish because of high N levels in bottom muds when spring overturn occurs.
8. Leaching of nutrients and bacteria from poorly sealed lagoon: May contaminate ground water or enter stream as interflow.

**Fig. 1.1** Consequences of infiltrated livestock waste [1]

fertilizers, livestock feed additives, and conversion to energy. Practical management practices to realize these and other benefits are encouraged whenever possible.

The manual has components grouped together by function, and systems are composed of components with different functions. For this reason, some skipping around in the manual will be necessary when using it for planning purposes. The important thing is to insure that the components selected for the system are compatible and adequate for their purpose as well as to insure that the entire system accomplishes its management objective. English units of measurement are used in examples, although metric units are included in many tables.

Another point to consider in consistent planning is whether the failure of one component will result in the failure of the entire system or if adequate flexibility is provided to permit continued operation without disastrous effects when unforeseen events happen. Often simple emergency or contingency measures can be planned into a system at various points, thereby preventing difficult situations later.

Data presented on waste production and characteristics are values generated from different parts of the United States, making it nearly impossible to define consistent values. Where specific values for an individual system can be obtained, these should be used in preference to the manual values. The values found in this chapter are deemed to provide perspective on what occurs in livestock operations across the country.

Selecting a system and the individual components involved is a process that includes engineering, economics, regulatory considerations, personal preferences, and other factors. There is no single system which is best. Each component, facility, or process has advantages and disadvantages. Each of these factors mentioned in the previous sentence needs to be given consideration in order to develop the most suitable waste management system for a given situation.

The information provided in this chapter is intended to create a frame for planning and sizing waste management system components. If systems require further explanation, the reader should consult the resources for further direction on determining what constituents are necessary to create a more adequate design. It may also be necessary to obtain professional design assistance.

### *1.1.1 Federal Regulations*

Federal regulations have been mandated by the US Environmental Protection Agency (USEPA) since its establishment in 1970. For the purpose of livestock waste treatment, legislation is applicable for both air and water. Air pollution research began in 1955 prior to the formation of the USEPA when the Air Pollution Act was passed to support funding and research. In 1970, the Clean Air Act required air quality standards for existing facilities and the refusal of building new infrastructure if not compliance with current legislation [2]. In addition, legislation has the USEPA control air emissions from mobile and stationary sources and establishes the National Ambient Air Quality Standards (NAAQs). NAAQs regulate hazardous

air pollutants for the purpose of protecting the public health and environment and are incorporated with State Implementation Plans [3].

Nevertheless, agriculture persists with odor problems, and further mandates were added later through the Clean Air Act Amendments of 1990. In the amendments, the legislation headed by the USEPA and Secrecies of Agriculture and Energy required reduction emissions that produce acid rain and for the protection of ozone, ammonia volatilization from animal and other agricultural operations for water and soil acidification, and methane emissions from rice and livestock production for ozone depletion [2]. Figure 1.2 provides the various methods in which air pollution can be caused by the livestock industry.

Water legislation began as early as 1886 with the River and Harbors Act of 1886 and 1889. Following the induction of the USEPA, the passing of the Federal Pollution Control Act of 1972 placed federal government responsible for creating and enforcing standards for water pollution control and maintaining the integrity of the water supplies, where a goal of having 0% discharge by 1985 was set. However, the biggest impact to water treatment in livestock wastes was the Clean Water Act of 1977. The Clean Water Act of 1977 introduces stringent legislation on feedlots and also required National Pollution Discharge Elimination System (NPDES) permits [2].

The National Pollution Discharge Elimination System (NDPES) regulates the quantity of waste entering navigable waters and also point sources [5]. In regard to

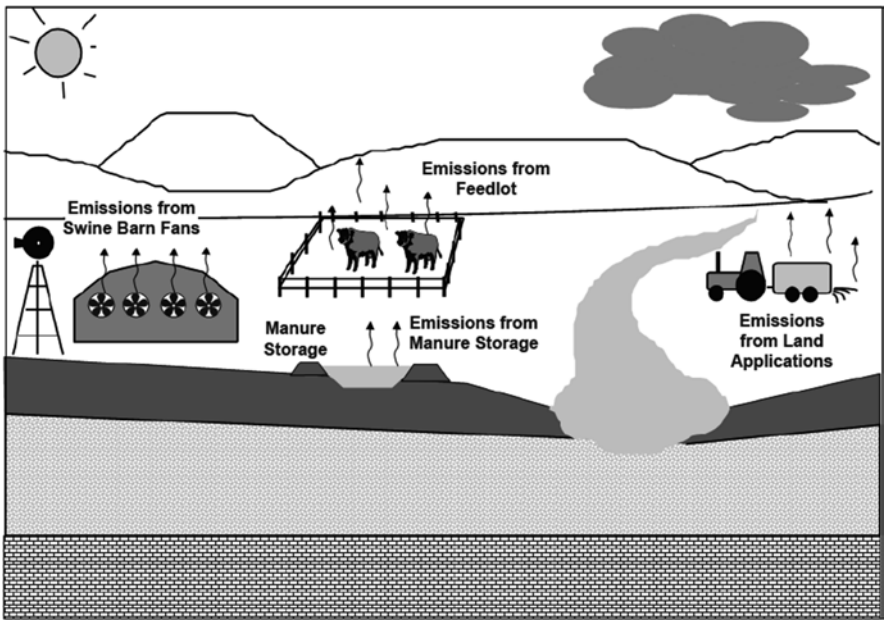



**Fig. 1.2** Pathways for manure contaminants in the air [4]

livestock wastes, the NPDES require permits when discharging in the following conditions [2]:

1. Feeding operations consisting of 1000 animals confined for a time greater than 45 days per year and pollution less than 25 year, 24 storm events
2. Feeding operations with 300 animals discharge through a manmade device into navigable waters either from a feed lot of a manmade device
3. Hatcheries and fish farm cold-water ponds that have a total of 20,000 lbs animal production with 5000 lbs of food discharging 30 days per year, or warm-water ponds discharging 30 days per year

There have been several revisions made to NPDES permit involving concentrated animal feeding operations (CAFOs) or feedlots. The 2003 revision makes permits necessary for both open lots and CAFOs, refines the definition of CAFO requirements, and incorporates a nutrition management plan that considers faculty and land application issues where the lack of compliance can require CAFOs to point source. Proposed revisions have been suggested in 2008 and 2011 from outcomes of lawsuits submitted by both the industry and environmental interest groups. For example, in 2011, a proposal was made where it would have been required for a CAFO or its affiliated state to release information. The proposal was not mandated as the USEPA decided to make additional measures to ascertain existing techniques to collect necessary information [6, 7].

### *1.1.2 State Regulations*

Regulations imposed by the state will vary. There are many resources available to the user to determine which regulations are appropriate for a given state. An investigation of specific state investigation will be up to the user. A list of each state's environmental agency with associated links is in Table 1.1.

## 1.2 Wastewater Characteristics

### *1.2.1 General Characteristics of Wastewater*

#### 1.2.1.1 Terminology

Prior to evaluating the properties of wastewater, it is important to understand the general terminology related to quantifying the characteristics of wastewater. Overall, wastes can be evaluated based on their physical and chemical properties. Tables 1.2 and 1.3 summarize the physical and chemical properties along with characteristics from excreted beef. The most important physical properties within waste include the weight, volume, and moisture content. These properties quantify the amount of

**Table 1.1** List of state environmental agencies with associated links

| State | State agency | Website |
|---|---|---|
| Alabama | Alabama Department of Environmental Management | http://www.adem.state.al.us/default.cnt |
| Alaska | Alaska Department of Environmental Conservation | http://dec.alaska.gov/ |
| Arizona | Arizona Department of Environmental Quality | http://www.azdeq.gov/ |
| Arkansas | Arkansas Department of Environmental Quality | http://www.adeq.state.ar.us/ |
| California | California Environmental Protection Agency | http://www.calepa.ca.gov/ |
| Colorado | Colorado Department of Public Health and Environment | https://www.colorado.gov/cdphe/ |
| Connecticut | Connecticut Department of Energy and Environmental Protection | http://www.ct.gov/deep/site/default.asp |
| Delaware | Delaware Department of Natural Resources and Environmental Control | http://www.dnrec.delaware.gov/Pages/Portal.aspx |
| Florida | Florida Department of Environmental Protection | http://www.dep.state.fl.us/ |
| Georgia | Georgia Environmental Protection Division | http://epd.georgia.gov/ |
| Hawaii | Hawaii Office of Environmental Quality Control | http://health.hawaii.gov/oeqc/ |
| Idaho | Idaho Department of Environmental Quality | http://www.deq.idaho.gov/ |
| Illinois | Illinois Environmental Protection Agency | http://www.epa.illinois.gov/index |
| Indiana | Indiana Department of Environmental Management | https://secure.in.gov/idem/index.htm |
| Iowa | Iowa Department of Natural Resources | http://www.iowadnr.gov/Environment.aspx |
| Kansas | Kansas Department of Health and Environment: Division of Environment | http://www.kdheks.gov/environment/ |
| Kentucky | Kentucky Department for Environmental Protection | http://dep.ky.gov/Pages/default.aspx |
| Louisiana | Louisiana Department of Environmental Quality | http://www.deq.louisiana.gov/portal/ |
| Maine | Maine Department of Environmental Protection | http://www.maine.gov/dep/ |
| Maryland | Maryland Department of the Environment | http://www.mde.state.md.us/Pages/Home.aspx |
| Massachusetts | Massachusetts Department of Environmental Protection | http://www.mass.gov/eea/agencies/massdep/ |
| Michigan | Michigan Department of Environmental Quality | http://www.michigan.gov/deq |
| Montana | Montana Department of Environmental Quality | http://www.deq.mt.gov/default.mcpx |

(continued)

**Table 1.1** (continued)

| State | State agency | Website |
|---|---|---|
| Minnesota | Minnesota Pollution Control Agency | http://www.pca.state.mn.us/ |
| Mississippi | Mississippi Department of Environmental Quality | http://www.deq.state.ms.us/ |
| Missouri | Missouri Department of Environmental Quality | http://dnr.mo.gov/env/index.html |
| Nebraska | Nebraska Department of Environmental Quality | http://www.deq.state.ne.us/ |
| Nevada | Nevada Division of Environmental Protection | http://ndep.nv.gov/ |
| New Hampshire | New Hampshire Department of Environmental Services | http://des.nh.gov/index.htm |
| New Mexico | New Mexico Environmental Department | http://www.nmenv.state.nm.us/ |
| New York | New York Department of Environmental Conservation | http://www.dec.ny.gov/ |
| North Carolina | North Carolina Department of Environment and Natural Resources | http://www.ncdenr.gov/web/guest |
| Noth Dakota | North Dakota Environmental Health | http://www.ndhealth.gov/EHS/ |
| Ohio | Ohio Environmental Protection Agency | http://www.epa.state.oh.us/ |
| Oklahoma | Oklahoma Department of Environmental Quality | http://www.deq.state.ok.us/ |
| Oregon | Oregon Department of Environmental Quality | http://www.oregon.gov/deq/pages/index.aspx |
| Pennsylvania | Pennsylvania Department of Environmental Protection | http://www.depweb.state.pa.us/portal/server.pt/community/dep_home/5968 |
| Rhode Island | Rhose Island Department of Environmental Management | http://www.dem.ri.gov/ |
| South Carolina | South Carolina Department of Health and Environmental Control | http://www.scdhec.gov/HomeAndEnvironment/ |
| South Dakota | South Dakota Department of Environment and Natural Resources | http://denr.sd.gov/ |
| Tennessee | Tennessee Department of Environment and Conservation | http://www.state.tn.us/environment/ |
| Texas | Texas Commission of Environmental Quality | http://www.tceq.state.tx.us/ |
| Utah | Utah Department of Environmental Quality | http://deq.utah.gov/ |
| Vermont | Vermont Department of Environmental Conservation | http://www.anr.state.vt.us/dec/dec.htm |
| Virginia | Virginia Department of Environmental Quality | http://deq.state.va.us/ |

| | | |
|---|---|---|
| Washington | Washington Department of Ecology | http://www.ecy.wa.gov/ |
| West Virginia | West Virginia Department of Environmental Protection | http://www.dep.wv.gov/Pages/default.aspx |
| Wisconsin | Wisconsin Department of Natural Resources | http://dnr.wi.gov/ |
| Wyoming | Wyoming Department of Environmental Quality | http://deq.wyoming.gov/ |

**Table 1.2** Physical and chemical properties of waste [2]

| **Physical properties** | |
|---|---|
| Moisture content | Component of a waste that can be removed by evaporation and drying |
| Total solids | Component of a waste that is left after evaporation |
| Volatile solids | Component of a waste that has been removed when a waste sample is placed in a muffle furnace at 1112 °F |
| Fixed solids | Component of a waste that remains after a waste sample is heated in a muffle furnace at 1111 °F |
| Suspended solids | Component of a waste removed by means of filtration |
| **Chemical properties** | |
| Five-day biological oxygen demand (BOD5) | Water quality index that measures the amount of oxygen needed for microorganisms to degrade material |
| Chemical oxygen demand (COD) | Water quality index that determines the amount of oxygen consumed by organic material |

**Table 1.3** Excreted beef waste characteristics [8]

| Components | Units | Beef cow in confinement | Growing calf confined (450–750 lb) |
|---|---|---|---|
| Weight | lb/da-a | 125 | 50 |
| Volume | $ft^3$/d-a | 2.0 | 0.8 |
| Moisture | %wet basis | 88 | 88 |
| TS | lb/d-a | 15 | 6.0 |
| VS | lb/d-a | 13 | 5.0 |
| BOD | lb/d-a | 3.0 | 1.1 |
| N | lb/d-a | 0.42 | 0.29 |
| P | lb/d-a | 0.097 | 0.055 |
| K | lb/d-a | 0.30 | 0.19 |

waste that must be handled and subsequently treated. Secondary physical properties evaluate categories that are found within a given waste. These secondary properties include total solids (TS), volatile solids (VS), fixed solids (FS), dissolved solids (DS), and suspended solids (SS) [2].

On the other hand, chemical properties are represented as nutrients or wastewater quality indices. Nitrogen (N), phosphorus (P), and potassium are the elements mainly considered as nutrients. These nutrients are further subdivided into subsequent forms that can be beneficial or detrimental to the handling of livestock. Figures 1.3 and 1.4 summarize nitrogen and phosphorus processes that occur within livestock waste. Five-day biochemical oxygen demand ($BOD_5$) and chemical oxygen demand (COD) are two of the many wastewater quality indices. These indices are evaluated within a laboratory and are important in determining the nature of the wastewater present. $BOD_5$ relates the amount of oxygen required to degrade waste

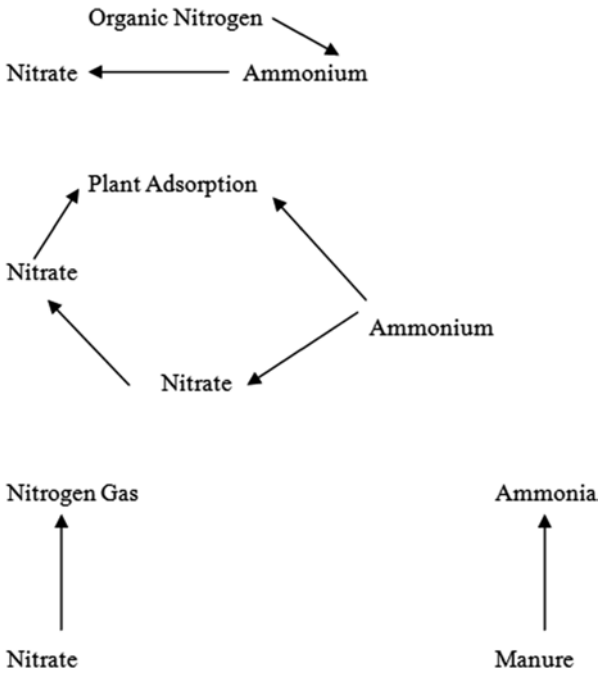



**Fig. 1.3** Nitrogen processes involved in manure management (from top to bottom: mineralization, nitrification, denitrification (bottom left), volatilization (bottom right)). (Adapted from [4])

by microorganisms in 5 days at 20 °C, while COD involves the consumption of oxygen by organic and inorganic constituents [2].

#### 1.2.1.2 Wastewater Characteristics

It can be said that the type of manure in wastewater produced varies not only on characteristics but also on the time of year. Based on the data collected between summer and winter for cattle manure and bedding, Loehr (1974) found that the ranges for parameters are different between summer and winter. For example, percent total solids (%TS) in winter have an average of 2.8% versus 2.3% in summer. In regard to biochemical and chemical oxygen demands ($BOD_5$ and COD), winter indicates higher values of BOD at 13,800 mg/L versus only 10,300 mg/L in summer. Nutrient presence is higher at 2350 mg/L as N for total nitrogen in summer, as compared with 1800 mg/L ion summer, and total phosphorus is 280 mg/L in winter, while only 190 mg/L in summer. These results can be reflected based on conditions such as precipitation and temperature [9].

In addition, having considered swine lagoon analysis in Missouri, liquid wastes are significantly higher in total solids, total nitrogen and ammonia, salts, and minerals as compared to sludge. In particular, liquid wastes contained 3091 mg/L, as

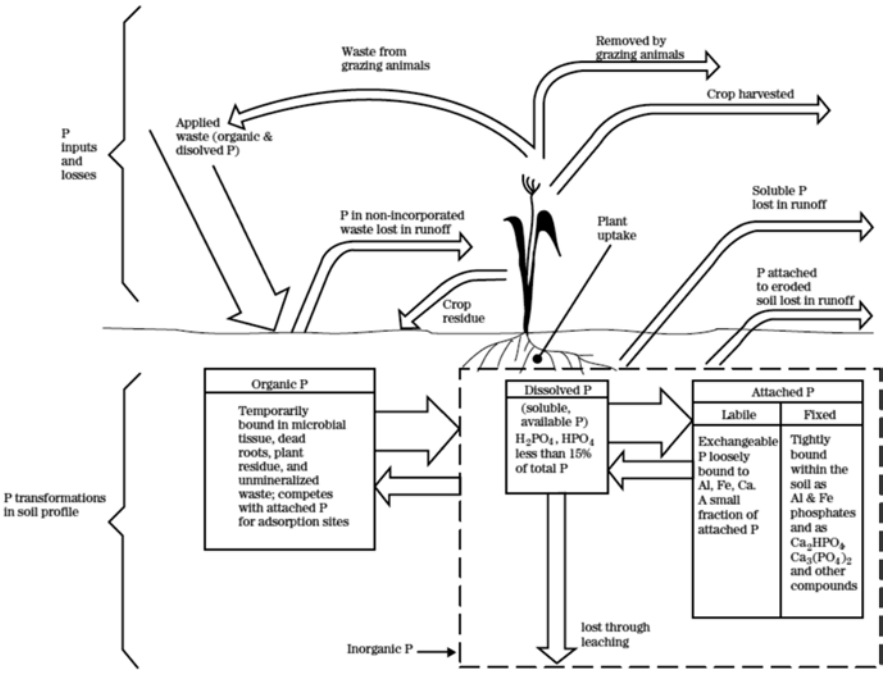



**Fig. 1.4** Phosphorus cycle in relation to waste application and transformation of phosphorus in the soil profile [1]

compared to only 203.843 mg/L in solids. This trend is also noticed in terms of salts (Na 470 mg/L, Ca 257 mg/L, and Mg 64 mg/L versus 4.627 mg/L, 6.176 mg/L, and 1.514 mg/L in liquid, respectively) [10].

Also, the waste characteristics of different industries vary. The supernatant for different animal wastes sampled from a lagoon and municipal waste treatment was compared. Poultry lagoons contained the highest concentration of wastes. The mean COD for poultry was 3700 mg/L, compared with 2050 mg/L and 1672 for the swine and dairy lagoons, respectively. This trend can be highly seen in $BOD_5$, TS, total volatile solids (TVS), suspended solids (SS), and ammonia nitrogen ($NH_3$-N), where the poultry lagoon contained the highest amounts of all three. Nevertheless, untreated municipal wastewater has significantly lower values for every category; in some cases such as COD values, the lowest animal waste value (1672 mg/L for dairy lagoons) was four times the COD than in municipal waste and almost ten times less than the highest (poultry) [11]. Tables 1.4 and 1.5 present characteristics of manure based on various livestock types. Table 1.6 presents wastewater characteristics of swine waste.

On the other hand, while waste constituents were higher in the animal waste, the untreated municipal wastewater contained higher amounts of trace metals, specifically cadmium, chromium, copper, and lead. In fact, examining copper, the range for copper was between 190 and 440 mg/L for poultry lagoons; however, in untreated

**Table 1.4** Total manure, nitrogen, phosphorus, and potassium excreted by different livestock species [12]

| Livestock type | Fresh manure (gal/day) | N (lb/day) | $P_2O_5$ (lb/day) | P (lb/day) | $K_2O$ (lb/day) | K (lb/day) |
|---|---|---|---|---|---|---|
| Beef cattle (1000 lb body weight) | 7.5 | 0.34 | 0.25 | 0.11 | 0.29 | 0.24 |
| Dairy cow (1000 lb body weight) | 11 | 0.41 | 0.17 | 0.074 | 0.32 | 0.27 |
| Swine (100 lb body weight) | 1 | 0.045 | 0.034 | 0.015 | 0.036 | 0.030 |
| Poultry (4 lb body weight) | 0.028 | 0.0029 | 0.0026 | 0.0011 | 0.0015 | 0.0012 |

**Note:** Livestock type is based on 1000 lb body weight

**Table 1.5** Manure characteristics per animal [13]

| Animal type | Average weight (lb) | Total manure production ($ft^3$/day) | Total solids production (lb/day) | Volatile solids production (lb/day) |
|---|---|---|---|---|
| Swine | | | | |
| Nursery pig | 35 | 0.04 | 0.39 | 0.30 |
| Growing pig | 65 | 0.07 | 0.72 | 0.55 |
| Finishing pig | 150 | 0.16 | 1.65 | 1.28 |
| Gestation sow | 275 | 0.15 | 0.82 | 0.66 |
| Sow and litter | 375 | 0.36 | 2.05 | 1.64 |
| Boar | 350 | 0.19 | 1.04 | 0.84 |
| Cattle | | | | |
| Dairy | 1000 | 1.39 | 12.00 | 10.00 |
| Beef | 1000 | 0.95 | 8.50 | 7.20 |
| Poultry | | | | |
| Layers | 4 | 0.0035 | 0.064 | 0.048 |
| Broilers | 2 | 0.0022 | 0.044 | 0.034 |

municipal wastewater, it was found that the range of copper was between 20 and 3360 mg/L, almost four times as much for the averages of these ranges. With the exception of arsenic and cadmium, poultry lagoons consistently had higher amounts of trace elements [11].

**Table 1.6** Swine waste characteristics [2]

| Component | Units | Grower 40–220 lb | Replacement gilt | Sow | | Boar | Nursing/ nursery pig 0–40 lb |
|---|---|---|---|---|---|---|---|
| | | | | Gestation | Lactation | | |
| Weight | lb/d/1000# | 63.40 | 32.80 | 27.20 | 60.00 | 20.50 | 106.00 |
| Volume | $ft^3$/d/1000# | 1.00 | 0.53 | 0.44 | 0.96 | 0.33 | 1.70 |
| Moisture | % | 90.00 | 90.00 | 90.80 | 90. 00 | 90.70 | 90.00 |
| TS | % w.b. | 10.00 | 10.00 | 9.20 | 10. 00 | 9.30 | 10.00 |
| | lb/d/1000# | 6.34 | 3.28 | 2.50 | 6.00 | 1.90 | 10.60 |
| VS | " | 5.40 | 2.92 | 2.13 | 5.40 | 1.70 | 8.80 |
| FS | " | 0.94 | 0.36 | 0.37 | 0.60 | 0.30 | 1.80 |
| COD | " | 6.06 | 3.12 | 2.37 | 5.73 | 1.37 | 9.80 |
| $BOD_5$ | " | 2.08 | 1.08 | 0.83 | 2.00 | 0.65 | 3.40 |
| N | " | 0.42 | 0.24 | 0.19 | 0.47 | 0.15 | 0.60 |
| P | " | 0.16 | 0.08 | 0.06 | 0.15 | 0.05 | 0.25 |
| K | " | 0.22 | 0.13 | 0.12 | 0.30 | 0.10 | 0.35 |
| TDS | | 1.29 | | | | | |
| C:N ratio | | 7 | 7 | 6 | 6 | 6 | 8 |

Average daily production for weight range noted. Increase solids and nutrients by 4% for each 1% feed waste more than 5%

### *1.2.2 Milk House Wastewater Characteristics*

Milk house wastewater is generated from various sources within the dairy industry. These sources include but are not limited to [14]:

1. Wash water from cleaning bulk tanks
2. Cleaning of milk pipelines
3. Cleaning of milking units
4. Cleaning equipment
5. Cleaning of milk house floor
6. Remnant within the milk pipelines, receiver, and bulk tanks
7. Chemicals
8. Water softener recharge
9. Manure
10. Bedding
11. Floor dirt and grit
12. Washing the udders of the cows

Typical milk house and diary wastewater characteristics are listed in Tables 1.7 and 1.8.

The Wisconsin National Resource Conservation Service (NRCS) describes three constituents within milk house wastewater—solids, phosphorus, and ammonia nitrogen and chlorides. Solids contain manure, primarily made of lignin and cellulose. These are a major producer of milk house wastewater. Solids usually have a

**Table 1.7** Characteristics of milk house wastewater [14]

| Parameter | Final effluent tank (mg/L) | Design (mg/L) |
|---|---|---|
| $BOD_5$ | 500–2600 | 1200 |
| Total Solids (TS) | 200–1000 | 450 |
| Fats, Oils, Grease | 90–500 | 225 |
| | 30–100 | 65 |
| Total Phosphorus | 21–100 | 55 |
| pH | 6.2–8.0 | 7.5 |
| Temperature | 53–70 °C | – |

**Table 1.8** Dairy waste characterization; milking center [15, 16]

| Component | Units | Milk house only | Milk house and parlor | Milk house, parlor, and holding area | Milk house, parlor, and holding area |
|---|---|---|---|---|---|
| Volume | $ft^3$/day/1000 head | 0.22 | 0.60 | 1.40 | 1.60 |
| Water volume | gal/day/1400 lb cow | 2.3 | 6.3 | 14.7 | 16.8 |
| Moisture | % | 99.72 | 99.40 | 99.70 | 98.50 |
| COD | lb/1000 gal | 25.30 | 41.70 | – | – |
| $BOD_5$ | lb/1000 gal | – | 8.37 | – | – |
| N | lb/1000 gal | | | | |
| P | lb/1000 gal | 0.58 | 0.83 | 0.23 | 0.83 |
| K | lb/1000 gal | 1.50 | 2.50 | 0.57 | 3.33 |

concentration range between 1600 and 7000 mg/L. Depending on the source, some solids can be comprised of high-concentration BOD. For example, it has been determined that raw waste milk can have a BOD concentration of 100,000 mg/L [15].

The presence of phosphorus has been attributed to daily cleaning operations such as pipeline washing or the presence of cleaning chemicals such as detergents and acid rinses, many of which can have 3.1–10.6% phosphorus by weight. Phosphorus in milking house centers is usually soluble and can cause eutrophication [15].

Ammonia is found in manure, urine, and decomposed milk. The discharge of milk house wastewater with substantial concentrations of ammonia can be toxic to fish. On the other hand, chlorides are also found in urine, milking system cleaners and sanitation, and water softening generation. The presence of chlorides can have an impact on the salinity of the wastewater being treated [15].

The daily operations within a milk house require daily cleaning of equipment and pipelines. The University of Minnesota Extension describes a four-stage cleaning process. Cleaning begins with rinsing the transfer lines to remove any raw milk

that may remain. Next, organic material is removed by a detergent with an active chlorine concentration of 100 mg/L. This detergent raises the pH above 11. Then, an acid rise is completed to reduce inorganic material. The pH is lowered to around 3.5 to prevent bacteria formation and neutralize any detergent residue that may remain. Finally, chlorine with a concentration of 200 mg/L is added to kill microorganisms in the line. The process of cleaning equipment and pipelines accounts for an additional source of wastewater that needs to be treated prior to any discharge [14].

#### 1.2.2.1 Treatment of Milk House Wastewater

There are several treatment methods for milk house wastewater. Table 1.9 lists several treatment methods that are being used in the state of Minnesota. For example, a viable option of treating milk house wastewater is through a two-stage septic system. It is important to note that wastewater entering into the tank does not include waste milk from cows. Waste milk will be disposed with manure. Treatment by the septic system is contingent on the strength of the wastewater, leaving the parlor and also time spent in the septic tanks [17, 18].

Wastewater is pretreated using two septic tanks consisting of inlet and outlet baffles. The tanks remove settable solids, fats, and grease and inhibit contamination throughout the remaining sections of the treatment plant. In the state of Minnesota, tank sizing is based on either a hydraulic retention time of 3 days or a volume of 1000 gallons, whichever is greater. In addition, Minnesota requires 4 ft of soil cover. Prior to exiting the septic tank, the wastewater passes through an effluent filter. The effluent filter prevents suspended solids from leaving the septic tank [17, 18].

Next, wastewater moves through a bark bed. The bark bed combines soil with bark and shredded wood. The depth of the bark bed is between 18 and 24 inches. The purpose of the mixture is to prevent the soil in colder climates and allows for more oxygen transfer, which in turn increases the rate of degradation at the soil-effluent interface. The sizing and application within the bark bed is determined by the soil type. Typical bark beds consist of a depth of 2 ft of soil to the bedrock or groundwater. Sizing of the bed is computed by taking the loading rate of the soil (contingent on soil type) and dividing it by the total wastewater volume. The loading rate is read from a table based on soil type. Presented values consider a $BOD_5$ concentration of 750 mg/L, flow rate of 5 gallons per day, and a $BOD_5$ loading rate of 0.0062 lbs/gallon. Bark beds can also be sized using hydraulic loading as well [19].

Another treatment method that can be employed is the use of constructed wetlands. Because constructed wetlands are not unique to milk house waste treatment, they will be discussed in Sect. 1.3.

Nevertheless, literature has discussed the efficiency of constructed wetlands for treating dairy wastewater. A three-celled surface wetland was used to treat dairy wastewater. The study compared the performance of the summer and winter seasons. The results found that total suspended solids (TSS), total phosphorus (TP), and total Kjeldahl nitrogen (TKN) were reduced in the summer as compared to the

**Table 1.9** Treatment methods for milk house wastewater treatment [17, 18]

| Treatment method | Description | Requirements |
|---|---|---|
| Chemical batch reactor | Coagulation and flocculation | Effluent BOD ≤205 mg/L<br>Discharge into infiltration/filtration system |
| Bark bed | Soil infiltration with 18–24 inches of barkwood<br>Pressure distribution system disperses effluent | Requirement of soil texture to a minimum of 3 ft bedrock. Treatment consists of three processes:<br>1. Primary treatment is completed by two septic tanks. Tanks are designated based on an HRT of 3 days or the volume whichever is greater<br>2. Infiltration area<br>3. Distribution system: The system consists of a pump, transferring pipe. Effluent traveling to the pipe must have a minimum velocity of 2 ft/s. The transferring pipe must have a diameter of 2 inches with a drainage slope of 1%. Distribution is done through gravel bed or a chamber system |
| Aeration and media filtration | Aerobic treatment or recirculating media filter | Treatment will consist of three processes:<br>1. Primary treatment will use two septic tanks. Design requirements similar to bark bed primary treatment<br>2. Aerobic treatment follows primary treatment where the goal must be less than 200 mg/L effluent BOD<br>3. Following aerobic treatment the discharge will enter an infiltration/filtration system |
| Irrigation | Treatment consists of water filled within the tank that will be dispersed onto crops | 1. A proper site for irrigation consists of a location where 20% of materials from 2 ft below the buffer zone pass through a #200 sieve<br>2. The irrigation area must have a minimum 3% slope, where the down gradient should be 50 ft away karst, surface water, or any private wells<br>3. Treatment consists of using a septic tank. Design requirements are similar to bark bed primary treatment<br>4. Wastewater moves to a 3-day holding dosing tank with piping for distribution and pumping |
| Vegetated treatment dosing system | Wastewater from a septic tank is distributed onto vegetation by a sloping elevated pipe where the upslope side of the pipe is enclosed | 1. Both siting and primary treatment use similar design criteria as previously mentioned<br>2. Treated waste from a septic system will travel through a distribution system to a dosing tank by a perforated pipe with perforations between 1/2 and 1 inch diameter. The pipe is elevated 1–1.5 ft above the ground<br>3. Determination of vegetated area is based on either a flow depth no greater than 0.5 ft using a treatment time of 15 min and a Manning constant of 0.24, or the smallest area that can handle a design loading rate no greater than 0.9 inches/week |

winter. In addition, $BOD_5$ removal was lower than 30 mg/L during the summer months as compared to the winter months. Finally, fecal coliform removal was approximately 31% [20].

To avoid eutrophication in a local surface water body, a three-celled parallel free water surface wetland was used to treat dairy wastewater. The treatment process began with the concrete settling pad for the purpose of eliminating solids prior to entry into the wetland. Following treatment into the constructed wetland, a three-sump pump transfers the wastewater into a holding pond. The authors concluded that $BOD_5$, conductivity, total dissolved solids (TDS), TSS, TKN, TP, phosphate, ammonia, nitrate, nitrite, and fecal coliform bacteria were generally reduced by the wetland. In addition, all parameters with the exception of nitrate and nitrate were diminished from the settling pad to the holding pond. Fecal coliform was reduced provided that cows were kept from grazing in the constructed wetlands [21].

#### 1.2.2.2 Conservation

Along with dairy wastewater treatment, water conservation is another important facet to properly handle wastewater. Water conservation is important because it provides the dairy plant owners an opportunity to reduce the cost for treatment. In general, wastewaters with high $BOD_5$ concentration discharged into a municipal wastewater treatment system incur high costs. It can also become expensive for onsite treatment as well; therefore, water conservation efforts provide owners an opportunity to save funds. In addition, methods have a positive impact on areas where water resources are currently being depleted and can also reduce the potential of stringent legislation. In the dairy industry, water reuse can reduce freshwater demand to 1 gal of water/1 gal of milk produced if proper management of goals is provided and maintenance is regularly scheduled [22].

## 1.3 Waste Treatment

### *1.3.1 Anaerobic Digestion*

Anaerobic digestion is the fermentation of organic waste by hydrolytic microorganisms into fatty acid chains, carbon dioxide ($CO_2$), and hydrogen ($H_2$). Short fatty acids are then converted into acetic acid ($CH_3COOH$), $H_2$, $CO_2$, and microorganisms. Acetic acid forms biogas, a combination of methane ($CH_4$), $CO_2$, and trace elements by means of methanogenic bacteria. Occasionally, biogas can form hydrogen sulfide by sulfate-reducing bacteria. In general, $CH_4$ in biogas produces between 55 and 80%, while approximately 65% is found in animal manure [23].

The processes in anaerobic digestion are driven by temperature, moisture, and solid content. There are three major temperature ranges defined—psychrophilic

(<20 °C), mesophilic (35–40 °C), and thermophilic (51–57 °C). Ideally an anaerobic digester should operate at temperatures greater than 35 °C. A moisture content of 60–99% is ideal, while solid content in the digester should be less than 15% [24].

Recently, there has been a big interest in anaerobic digestion for the purpose of energy conversion [25]. Since 1996, the Environmental Protection Agency has partnered with the US Department of Agriculture, the National Resource Conservation Service (NCRS), and the US Department of Energy to develop a program known as AgStar, an opportunity for monetary support in projects related to anaerobic digestive systems. In 1998, the program began by promoting seven farm digesters across the country [26].

There have been reports of profit being made on the energy that has been captured through the use of livestock manure. These values have greatly depended upon the monetary cost of electricity. For example, if one were to sell electricity in Wisconsin and California, a 1000-head dairy farm with manure production would be worth about $56,000 and $77,500, respectively [25]. Statistically speaking, it was found that in 2009, approximately 151 biogas systems that have been installed within the state of Wisconsin produced about 11.6 megawatts of electricity, enough for use by 10,000 homes. Within January 2007 and June 2008 alone, 150,000 kilowatt hours (kWh) of electricity were produced by farms that had 2000-head of animals and 440,000 kWh of electricity for those between 2000 and 4500 [27]. Figure 1.5 indicates the net value of dollars based on the digester per number of head of cattle. Figure 1.6 indicates the number of dairies operating at a given carbon price per operation size.

There are a plethora of reasons why AgStar has become a popular consideration for the development of biogas. Consider that the state of Wisconsin has spent between $16 and 18 billion each year for coal energy imports whereas about $853 million for transportation [27]. If the state of Wisconsin, rich in manure and crop remains and waste components from the dairy processing, fats, and greases can transport this material into fuels, it would create an infrastructure that would be safer and easier to be controlled as compared to the current energy options on the market today and additional revenue for farmers [27].

A recent 2013 study conducted by the US Environmental Protection Agency (USEPA) evaluating the AgStar program found that anaerobic digesters reduce greenhouse gases by 1.73 million metric tons of $CO_2$ equivalent ($MMCTCO_{2e}$). This is because methane is captured and burned before entering into the atmosphere. On the other hand, anaerobic digesters produced 840 million kWh in 2013. These benefits were contingent on the type of anaerobic digester applied. For example, the most commonly used digesters in the United States were complete mixed and mixed plug flow [28]. Biogas production is also dependent upon the type of livestock. Table 1.10 provides information concerning the daily production of biogas per animal type.

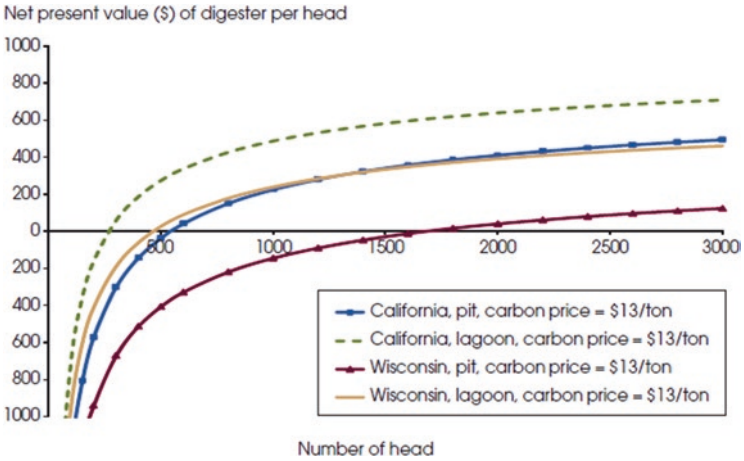
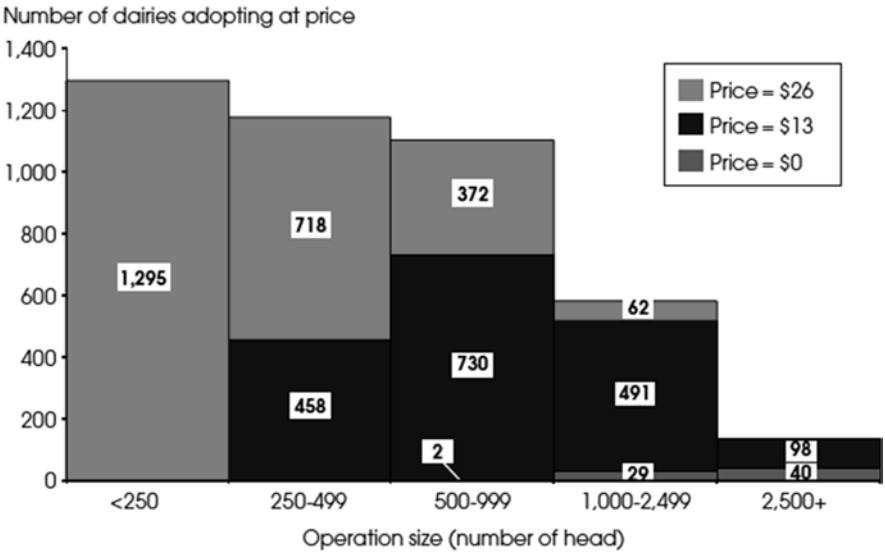



**Fig. 1.5** Net value in dollars of digesters per head vs. number of head [25]




Notes: Numbers at higher prices are additive to those for lower prices; for example, at a price of $13/ton, an additional 491 operations of size 1,000-2,499 head are predicted to adopt, for a total of 520 operations of this size. At a carbon price of $13/ton, no operation smaller than 250 head is predicted to adopt. At a carbon price of $0, no operation with fewer than 500 head and 2 operations 500-999 head are predicted to adopt.

**Fig. 1.6** Number of dairies operating at a given carbon price vs. operation size [25]

**Table 1.10** Biogas production by animal [23]

| Animal type | Average weight (kg) | Biogas/animal/d ($m^3$) |
|---|---|---|
| Dairy | 625 | 1.3 |
| Beef | 447 | 0.32 |
| Swine | 70 | 0.14 |
| Poultry | 1.2 | 0.0092 |

**Table 1.11** Characteristics of various anaerobic digester types [23]

| Anaerobic digestion system | OLR COD/$m^3$/kg | HRT (d) |
|---|---|---|
| Covered anaerobic lagoon | 0.05–0.2 | 60–360 |
| Plug flow digester | 1–6 | 18–20 |
| Mixed | 1–10 | 5–20 |

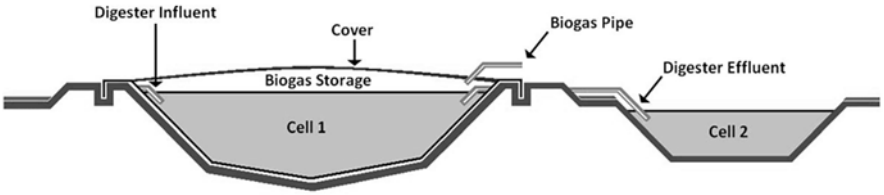



**Fig. 1.7** Covered lagoon digester [29]

### 1.3.1.1 Types of Anaerobic Digesters

There are six types of anaerobic digesters—covered anaerobic lagoons, plug flow, continually stirred tank reactor, fixed film, induced blanket reactor, and anaerobic sequencing batch reactors. Table 1.11 reports the characteristics of three of the six anaerobic digesters (covered anaerobic lagoons, plug flow digester, and mixed). The selection of the appropriate anaerobic digester is determined by appropriate parameters such as the geographic location. Covered anaerobic lagoons form biogas from manure stored in structures and are low cost, simplistic in design, and manageable. There are two types of covers—full and partial. Production of biogas by a covered anaerobic lagoon depends on the temperature. Therefore, covered lagoons are more appropriate in areas of warmer climate. Biogas production in a covered lagoon is collected in pipes at the top of the digester and then transported by using a low vacuum. From there, the remaining biogas is then flared. Additional characteristics of a covered anaerobic lagoon include high total solid (TS) concentration, organic loading rate (OLR) of 0.2–0.5 kg chemical oxygen demand (COD)/$m^3$ day, and a

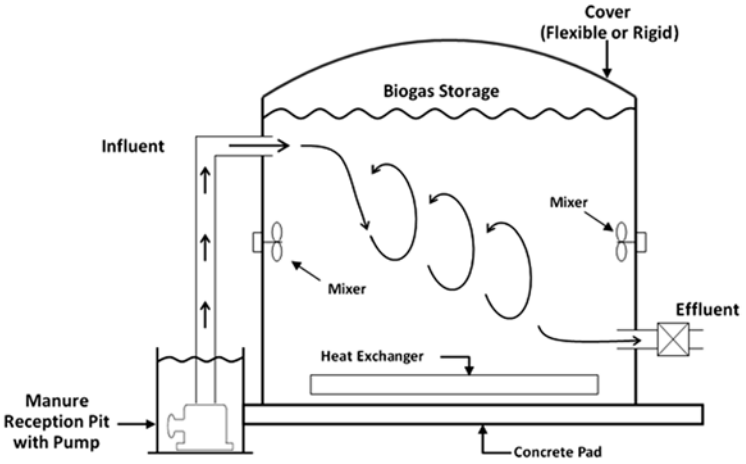



**Fig. 1.8** Complete mix digester [29]

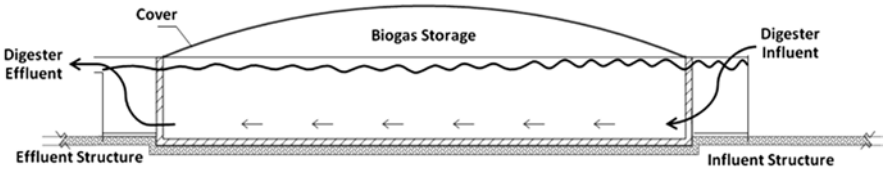



**Fig. 1.9** Plug flow digester [29]

hydraulic retention time (HRT) of 60–360 days [23]. Figures 1.7 and 1.8 are diagrams of a covered lagoon digester and a completely mixed digester.

On the other hand, manure in a plug flow digester enters undigested and leaves digested. A typical plug flow digester includes concrete and geosynthetic material for gas collection. Manure enters into the digester and is limited to 11–14% total solid concentration, 1–6 kg COD/m$^3$ day OLR, and an HRT between 20 and 30 days. In a continually stirred tank reactor, manure enters into a tank and is mixed to maintain a consistent concentration throughout the reactor. Unlike a plug flow digester which is limited to 6 kg COD/m$^3$ OLR, the maximum allowable organic loading rate for total solids entering into a continually stirred tank reactor is 10 kg COD/m$^3$ day. In addition, the hydraulic retention time is shorter than a plug flow reactor ranging between 5 and 20 days [23]. Figure 1.9 is a diagram of a plug flow digester.

A fixed film digester is an attached growth reactor with fixed film media. When waste enters into the fixed film digester, anaerobic biomass attaches to the fixed film media. Typical fixed film digesters have a low HRT between 0.5 and 4 days. Influent manure in a fixed film digester has an OLR between 5 and 10 kg COD/m$^3$ day with a solid concentration less than 1% [23].

Finally, an induced blanket reactor forms a sludge blanket by digesting the waste. Manure moves upward from the bottom of the reactor to the top. Inside the blanket,

manure moves upward contacting with anaerobic biomass to become digested. At the top of the tank, the biogas is created while the sludge blanket moves back to the bottom of the reactor. There are two types of blanket reactors—upflow anaerobic sludge blanket (UASB) digester and induced blanket reactor (IBR). UASB involves low concentration of solids, while IBR usually handles high solid concentrations [30].

The cost of an anaerobic digester application has been contingent on the type. In the design and construction of a system, the price involves the initial cost of the system and its operation and maintenance (O & M). The US Department of Agriculture (USDA) reported values on 38 different digesters. The overall cost of an anaerobic digester has been estimated to be between $114,000 and 326,000. Operation and maintenance (O & M) was found to be contingent on the type of waste. The O & M for swine waste was 2.3% of the initial cost for the system, while dairy was 7% [23].

Within the last 5 years, other anaerobic digestion processes have been tested. A specific type of anaerobic digestion design is known as a temperature-phased anaerobic digestion reactor. Temperature-phased anaerobic digestion (TPAD) is a system that completes treatment in two stages at two temperatures—during the first stage, the digester operates at a temperature at the highest thermophilic temperatures, approximately 55 °C while the second stage at the lower ended mesophilic conditions or approximately 35 °C. When using a TPAD for livestock waste, the advantages are significant as the digester is capable of increasing a higher probability of bioconversion and methane production, with lower hydraulic retention times (HRT) and also size reduction [31]. Harikishan and Sung (2003) used a TPAD process to treat livestock wastewater for the purpose of analyzing dairy cattle manure. Having organic loadings of 1.87–5.82 g VS/L/day, 36–41% of volatile solids were removed, converting 0.52–0.62 L methane/g VS. In addition, fecal coliform and *Salmonella* counts meet USEPA Class A standards [31].

Other authors have researched and found results under different conditions. King et al. (2011) used a 3-year pilot in-storage psychrophilic anaerobic digester (ISPAD) to consider swine manure and if it is able to handle psychrophilic conditions and be able to complete anaerobic digestion and successfully produce methane. Results based on the microbial community analysis were able to produce methane, provided that volatile solids (VS) had a rate of 44.6 $dm^3$/kg day at 35°, 9.8 $dm^3$/kg day at 18°, and 8.5 $dm^3$/kg day at 8° and an organic matter content of 24% [32]. Rao et al. (2010) used a self-mixed anaerobic digester (SMAD) combined with a multistage high-rate biomethanation process where the authors were capable of reducing volatile solids (VS) by 58% and producing a methane yield of 0.16 $m^3$/kg, with a loading rate of 3.5 kg VS/$m^3$ day and a hydraulic retention time (HRT) of 13 days. The authors considered using the opportunity to reduce the loading rate and the hydraulic retention time and percent treatment [33].

## *1.3.2 Constructed Wetlands*

### 1.3.2.1 Description

The purpose of a constructed wetland is to provide a low-maintenance treatment system that creates a quality effluent for areas that have a high volume of wastewater. Constructed wetlands house wastewater within wide channels. These channels also support plant life that grows by using the nutrients from the wastewater. There are four major processes employed in constructed wetlands—sedimentation, filtration, plant uptake (oxygen is provided at the plant root for waste decomposition), and biological decomposition (plants provide adequate binding sites for microorganisms) [15].

The basic idea of a wetland is to maintain moist conditions for pollutants to be trapped and broken down by the plant that are contained within them. In addition, constructed wetlands take advantage of combining anaerobic and aerobic conditions that persist through the wetland. The majority of constructed wetland design consists of using either subsurface flow or surface flow. Surface flow wetlands consist of having a "free water zone" about 30 cm deep on top of a soil layer where the majority of plant growth would occur. The advantage of designing a wetland by this manner is that it would place microbial growth in the best advantage to occur in the areas where the water and its contaminants would be. Subsurface flow wetlands, also known as "root zone method," remove the "free water zone" for the purpose of allowing direct contact between plant material and contaminants present [34]. There are several design parameters that are necessary for treatment—hydraulic loading rate, length-to-width ratio, bottom slope, water depth, and vegetation [35].

The water depth of a constructed wetland is usually between 20 and 40 cm deep. The advantage of using surface constructed wetlands is the biological and physical methods that are employed within the system. Microbial activity (biological) degrades much of the organic materials, while colloids are either settled within the wetland or can become filtered out (physical). Nitrogen is capable of being removed by means of nitrification (the formation of nitrate from ammonium nitrogen) and denitrification (the formation of atmospheric nitrogen from nitrates) [2], while ammonia is volatilized by the use of algal photosynthesis. If any phosphorus is removed, it is by means of wetland plants eventually by either absorption or precipitation [36].

### 1.3.2.2 Constructed Wetland Types

Literature recognizes three major types of constructed wetlands—free water surface (FWS), vegetated submerged or subsurface system, and floating aquatic plant (FAP) systems [38]. Figures 1.10, 1.11, and 1.12 are drawings of each type of constructed wetland.

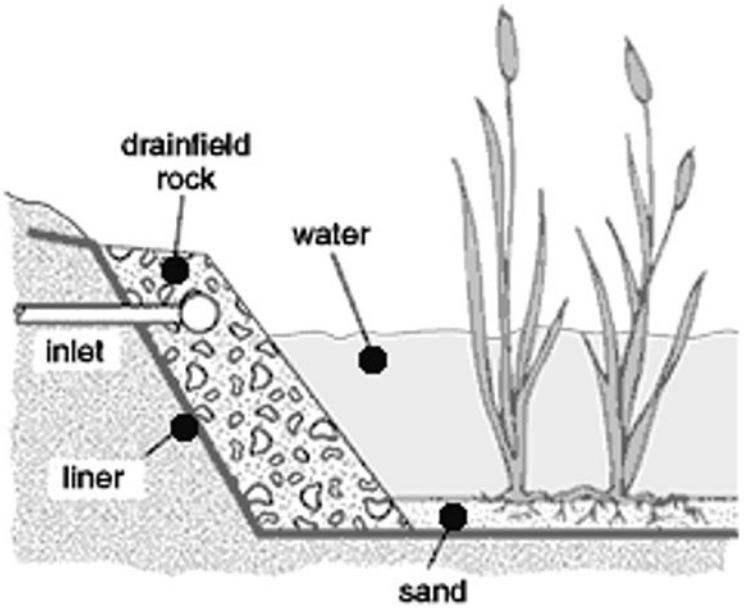



**Fig. 1.10** Free water surface (FWS) constructed wetland [37]

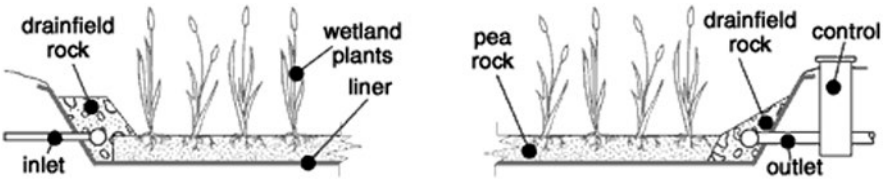



**Fig. 1.11** Subsurface constructed wetland [37]

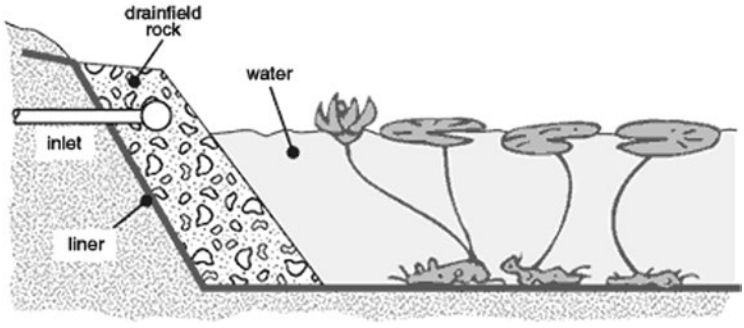



**Fig. 1.12** Floating aquatic plant (FAP) constructed wetland [37]

In a free water surface system, the wastewater depth is usually shallow, anywhere between 6 and 18 inches with a flat-bottom slope. Because of their shallow depths, FWS wetlands usually degrade wastewater under aerobic conditions. When wastewater enters an FWS, it moves above the sediment, having direct contact with the plants at the surface. However, the efficiency of FWS treatment is contingent upon the presence of microorganism located throughout the surface. Nevertheless, microorganisms attach themselves to plant stems and/or litter below the water surface, or at the soil/plant-root matrix, creating the proper environment for wastewater treatment. Prior to entry of an FWS, a pretreatment system to remove settling and floating solids is recommended or ammonia [38]. FWS-constructed wetlands have been proven to reduce $BOD_5$ and TSS to 30 mg/L, ammonia, and ammonium-nitrogen to 10 mg/L [39]. In addition, to the effluent quality, FWS wetlands are very common in livestock operations because they are inexpensive and can be in operation year round [38].

Under the National Resource Conservation Service guidelines, an FWS is to be designed based on a 25-year storm event depending on the state. Sometimes a detention pond downstream may be necessary to meet this requirement. The sizing of an FWS is done by using one of the two methods—presumptive method or the field test method. The presumptive method assumes a $BOD_5$ concentration, while the field test method is based on an actual daily measurement of $BOD_5$ from the given livestock operation [39]. The presumptive method approximates a pollutant entering into a wetland by reviewing the $BOD_5$ or nitrogen concentration and then applies the value to an areal loading rate (typically 65 lb $BOD_5$/acre/day). The presumptive method has been well-known since the Tennessee Valley Authority (TVA) introduced it in 1989 [38].

The field test method requires a collection of samples and analysis based on $BOD_5$ and total nitrogen (TN). Some of the important factors examined include average daily flow, temperature, and decay rate constant. The data collection from the field test is used to determine the size of the wetland. The purpose of the field test method is to ensure that the design of the wetland does not exceed discharge limits [38].

On the other hand, in vegetated submerged systems, wastewater flows within the sediment bed, having more contact with the plant roots. The sediment bed is usually made of rock, gravel, and soils. Vegetation is usually planted at the top of the wetland [38]. Because wastewater flows at lower depths, wastewater is usually degraded at anaerobic conditions. The slope of this wetland ranges between 2 and 6%. Sizing of submerged systems is contingent on flow rate and influent and desired outflow $BOD_5$ [39]. Vegetated submerged systems are not as prolific as surface flow wetlands. This is because the sediment beds can easily accumulate solids. Also, the beds can be very expensive to construct. Nevertheless, vegetated submerged systems can be used to treat wastewater with low flows and solids [38].

Finally, floating aquatic plant systems comprise of one or more ponds. The ponds are designed for plants to grow and float at the top of the ponds. Each pond is designed for a depth between 3 and 5 ft for the purpose of avoiding non-desired plant species to grow and become prominent within the system and gives the plant

access to nutrients within the wastewater. There are several factors for appropriately harvesting. These include the number, size, and arrangement of ponds and the technique for harvesting. There are two major plant species in FAP systems—water hyacinths and duckweed [38].

#### 1.3.2.3 Constructed Wetland Design

Constructed wetland design usually consists of first-order models under plug flow conditions, alternating between looking for values of BOD, TSS, ammonium, and fecal coliforms [34]:

$$\ln\left[\left(C - C^*\right) \div \left(C_o - C^*\right)\right] = -\frac{k}{q} \tag{1.1}$$

where

$C_0$ = initial concentration of conditions
$C$ = targeted rate concentration
$C^*$ = background rate concentration
$k$ = first-order rate constant (cm/d)
$q$ = hydraulic loading rate (cm/d)

An alternative method to designing a constructed wetland would be the use of regression equations for one had the desire to consider looking at multiple components at one time.

Stone et al. (2004) used constructed wetlands, particularly marsh-pond-marsh wetland system at North Carolina A & T University. Six wetland systems with the dimensions of 11 × 40 m treated nitrogen by removing % concentration of ammonia nitrogen of 30% but only removing 8% phosphorus treatment. First-order kinetics were 3.7–4.5 m/day for total N and 4.2–4.5 m/day for P, much lower than the typical model rate constant [40].

In addition, the Environmental Protection Agency has tracked several constructed wetlands that have been used for the purpose of waste treatment. Seven locations to treat three different waste types—swine, dairy, and poultry—were constructed. For swine wastewater, a project in Duplin County, North Carolina, was undertaken for the purpose of removing Total Kjeldahl Nitrogen (TKN), as it was observed that a major factor affecting treatment was loading rates of TKN (3 kg/ha/d TKN) and was able to remove between 91 and 96% TKN, while 10 kg/ha/day only removed approximately 73%. A wetland in Essex, Ontario, reduced TSS (97%), $BOD_5$ (97%), and 99% fecal coliforms, and 95% *E. coli* from dairy farm milk house wastewater. Auburn University used a constructed wetland for poultry lagoon that considered a series of five wetlands at 3.1 cm/day, a loading rate of 145 kg/ha-day for chemical oxygen demand (COD), and 30 kg/ha-day total TKN at a maximum of 49.8% $BOD_6$, 60.7% COD, and 36.8% $PO_4$ [41].

### *1.3.3 Lagoons*

A lagoon is an earthen basin that treats wastewater and stores both liquids and solids [2]. Lagoons can store wastewater, manure, or rainfall runoff [42]. Lagoons are capable of reducing BOD and chemical oxygen demand (COD), nitrogen, and odors [2]. Lagoons can take a round, square, or rectangular shape with a typical length-to-width ratio of 3:1 [43]. In addition, lagoons can be situated as a single or multiple-stage lagoon system. A single lagoon is divided into three major volumes—sludge storage, treatment, and effluent storage. Above the effluent storage is a freeboard for the purpose of protecting the lagoon from storm situations [44]. Figure 1.13 provides a cross-sectional area of a lagoon.

In the sludge storage, sludge settles at the bottom of the lagoon and is digested at the top of the layer. Over time, sludge will accumulate within this layer until it becomes equal to the liquid present. The treatment volume is located above sludge storage consisting of manure at the bottom. Biological degradation converts sludge into organic acids and other compounds. The products of organic acids include methane and carbon dioxide, hydrogen sulfide, ammonia, and volatile organics. Treated wastewater not leaving the lagoon is stored in the effluent storage section. Effluent is stored for the purpose of watering crops [44].

Lagoons are designed based on a 25-year, 24-h storm event. This value is contingent on the location of the lagoon as the 25-year, 24-h storm event varies across the country. The design loading into the lagoon is determined by the number, size, and the species of animal, along with the geographical location of the lagoon. Prior to

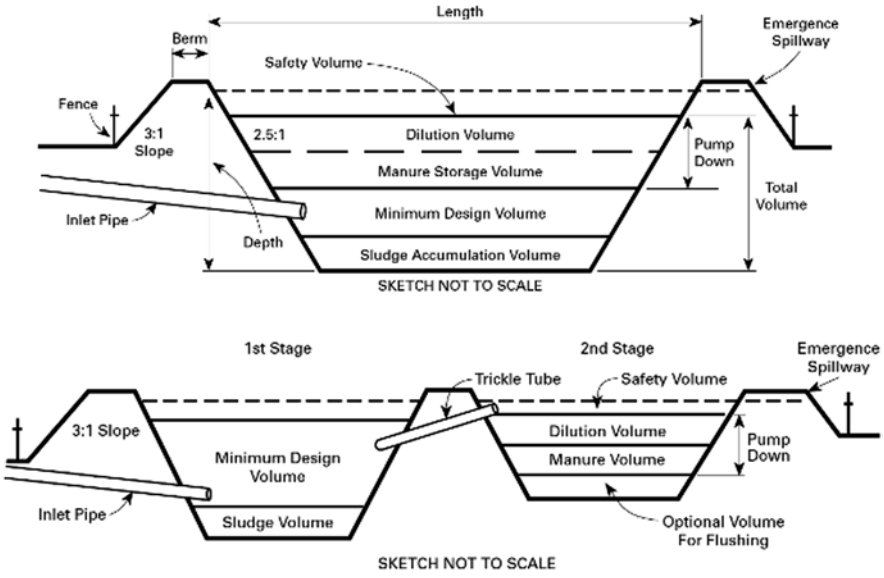



**Fig. 1.13** Cross-sectional area of lagoons [13]

land application, dewatering the lagoon is very important. Frequency of dewatering is contingent on the salt concentration and the soil type [45].

The sizing of a lagoon is based on the volume, depth, and pH. The volume of a lagoon is contingent on the loading rate of volatile solids per 1000 cubic foot. This is a function of temperature. The depth of a lagoon is predicated on the precipitation and evaporation rates where the lagoon is located. A typical minimum depth is 6 ft but can be 10 ft for colder climates. However, these values are general and are contingent on the type of lagoon constructed. The optimum pH should be maintained at 6.5 to avoid inhibiting methane bacteria. Anytime the pH is below 6.5, lagoons will experience a high organic loading [2].

Before construction of a lagoon, it is imperative that a soil and groundwater study is done. This is to ensure that sensitive areas are protected from any discharged from the lagoon. These areas would be any region that leads to surface runoff. Avoid areas that are geologically unstable [42]. Pretreatment of wastewater may be beneficial to reduce odor if the $BOD_5$ loading rate is 50 lb $BOD_5$/AC/day and the depth of the pond is between 6 and 20 ft [43]. In addition, lagoons should be in close proximity if manure is scraped into the lagoon or below the manure source [42].

Lagoon maintenance is important for controlling odors. Lagoons should be analyzed for the presence of algal blooms. Algal blooms occur in basins that have high loading of nutrients (nitrogen and phosphorus). If a lagoon is void of algal blooms, ensure that aerobic lagoons do not become anaerobic. Anaerobic conditions can produce products that can cause odors. The operator should also check and if necessary provide adequate dilution of waste prior to entry into the lagoon and avoid overloading [46]. This can be accomplished by using a combination of runoff and wash water [45]. If odors still persist, lime addition to the lagoon can reduce the presence of odors [46].

Lagoon operators should also evaluate the species of algae and check for the presence of weeds and grasses and protect them from erosion and unauthorized access. A healthy lagoon should have green algae. Blue-green and filamentous algae can clump within a lagoon blocking the sun. Gray, black, or purple algae are very unhealthy for a lagoon. The presence of weeds can cause a lagoon to short circuit, thereby affecting the flow of wastewater within the unit. Grass covers on the slopes and level surfaces of the lagoon can be beneficial but should be mowed and properly fertilized and should be checked for food, trash, or scum on or near the premise. These items should be discarded. Trees or any bushes should not be present near the berm of a lagoon and should be removed [46]. This will also protect the embankments [44]. In the event of erosion, operators should determine the source and make necessary adjustments to the lagoon if necessary. Unauthorized activity can be avoided by placing fences and warning signs adjacent to the lagoon [46].

Finally, operators should also monitor the sludge storage and sludge depth. Remove excess sludge that has accumulated within the lagoon [44].

#### 1.3.3.1 Anaerobic Lagoons

Anaerobic lagoons are the most common lagoon used for treatment of livestock wastewater. One of the biggest reasons is because anaerobic bacteria have a higher rate of organic decomposition as compared with aerobic bacteria [42]. This is because anaerobic bacteria operate in environments without molecular oxygen a condition that does not require constant maintenance. Generally, anaerobic lagoons are usually very deep. Ranges for depth can vary on the region [46]. For example, the University of Missouri Extension and the State of Mississippi state that lagoons can have depths between 8 and 20 ft [42, 43]. Based on treatment desired, lagoons can be designed to be completed as single stage with no secondary treatment, or in multiple stages where further treatment is completed by additional lagoons [45]. Figure 1.14 is a diagram of a two-stage anaerobic lagoon system.

Anaerobic lagoon can be circular, square, or rectangular. A length-to-width ratio of 3:1 for rectangular anaerobic is desired, with earthen dike and banks slopes between 2:1 and 3:1 [42, 43, 45]. Anaerobic lagoons should have a 1 foot spillway below the top of the berm where inlets should be located on the longest side of the lagoon [42].

During the wastewater treatment process, anaerobic lagoons separate into top and bottom layers. At the top of the lagoon, less dense materials such as oils float to the top of the lagoon, while sludge settles the bottom. The presence of oils and other materials prevents oxygen entry, maintaining anaerobic conditions within the system [46].

Anaerobic lagoons are sized based on the volatile solid (VS) loading rate. These values can be expressed in 1000 $ft^3$/day or lb VS/1000 $ft^3$-day. These numbers are affected by the climate. For example, in South Carolina, the volatile solids' loading rate is 5 lb VS/1000 $ft^3$-day, while Iowa has a VS loading rate of 3.5 lb VS/1000$^3$-day [48].

Nevertheless, anaerobic lagoons are problematic because of odors. These odors are a product of hydrogen sulfide, ammonia, organic acids [49], and methane. Odors can also be caused by winter to fall and summer to fall turnover within the lagoon or during land application [42]. There are many solutions that can resolve persisting odor problems in a pond. Anaerobic lagoons can be covered to prevent the release of methane gas exiting the system. Anaerobic lagoons can also have induced aerobic

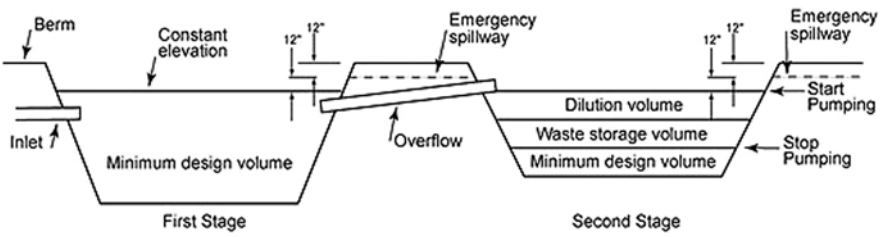



**Fig. 1.14** Two-stage anaerobic lagoons for livestock manure treatment [50]

**Fig. 1.15** Floating aerator [50]

layers at the top of the lagoon. This can be done by including a floating cover or aerating the top of the lagoon at very low rates [44]. Figure 1.15 is a floating aerator.

#### 1.3.3.2 Aerobic Lagoons

Aerobic lagoons degrade organic matter by the application of dissolved oxygen throughout the lagoon. Because dissolved oxygen persists throughout the lagoon, odors are not present within the system. In order to maintain aerobic conditions, aerobic ponds are shallow but require a large land requirement. These ponds are more commonly found in warm and sunny climates. There two subcategories of aerobic lagoons—naturally and mechanically aerated [46]. Figure 1.16 is a diagram of an aerobic lagoon.

Naturally aerobic, oxidation ponds reduce organic materials within wastewater by using either oxygen from the atmosphere or algae by means of photosynthesis [46]. Wind on the pond surface also mixes with the water within the oxidation pond [44]. These ponds are very shallow with a minimum depth between 1 and 5 ft with a maximum of 5 and 6 ft [46]. The main design parameter is the organic loading rate, which is typically 50 lbs $BOD_5$/acre of surface area [49]. Nevertheless, naturally aerobic lagoons are not often used for the treatment of livestock wastewater.

Mechanically aerated lagoons mix oxygen throughout the lagoon by mechanical means. The need for supplying energy can make these lagoons expensive. In many cases, solar or wind power supply the power to operate aeration equipment. Also the lagoons can be designed to have anaerobic segments to reduce energy requirement [46]. Compared with naturally aerobic, mechanically aerated lagoons do not have a large area requirement but usually have a depth of 10 ft However, in addition to

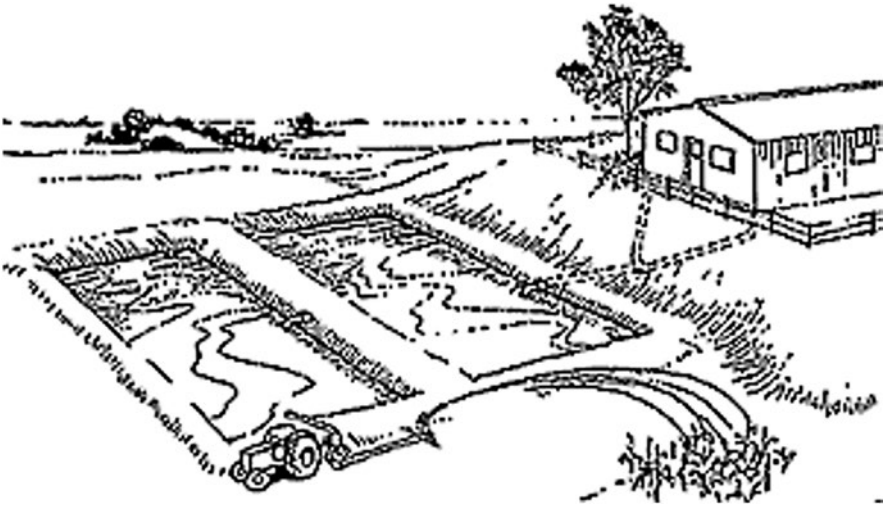


**Fig. 1.16** Two-cell aerobic lagoon to treat swine waste [51]

being more expensive, mechanically aerated lagoons tend to generate more sludge, have a high tendency for foaming, and may require additional treatment such as a septic tank to collect and remove solids [49].

#### 1.3.3.3 Facultative Lagoons

Facultative lagoons are basins that operate in both aerobic and anaerobic conditions. These lagoons can be arranged as a two-staged pond system where each pond has a depth of 4 ft or as a single-pond system with a depth of 6 ft [43]. Facultative lagoons usually have three layers. At the top is an aerobic layer. This layer receives sunlight and wind, promoting the process of photosynthesis, and provides oxygen. The middle layer is a facultative layer. In this layer of the lagoon, anaerobic and aerobic conditions exist. The extent as to which condition is prominent is contingent on the geographical location of the lagoon [46]. Bacteria that can thrive in anaerobic or aerobic conditions (facultative bacteria) are commonly found in this layer. The bottom layer is anaerobic. This layer contains an accumulation of sludge from lagoon activities [46]. Because of the layering of the lagoon, odors can be minimized [52].

### *1.3.4 Thermal and Biological Chemical Treatment for Biogas Producton*

#### 1.3.4.1 Description

Recent developments have occurred where there has been a call for the conversion of livestock wastes that can be used for energy, specifically biofuels. To summarize, biochemical processes are transforming organic materials to fuels by means of various processes such as anaerobic and photosynthesis. Following a biochemical process, the remaining solid and slurry within the reactor becomes viable as a reusable resource such as fertilizer [53].

Thermochemical processes convert organic matter into gas, fuels, or other carbon residuals by the use of high temperatures to physically convert the bonds of organic matter. Some of the major chemical conversion procedures include combustion, pyrolysis, gasification, and liquefaction [53].

#### 1.3.4.2 Pyrolysis

Pyrolysis ultimately transfers a given biomass into either char or a volatile gas that can form bio-oil or combustible pyrolytic oils. Slow pyrolysis methods have been used to form char, an entity that has the benefit of producing energy for coal combustion plants, or as an addendum to soil. Some authors have found that chars from various pyrolytic processes are capable of having better absorption than those made from granular activation carbon [53].

There are two major types of pyrolysis—fast and slow/moderate. Fast pyrolysis is a pyrolytic process that consists of using high heat rate and residence time. The resultant products include low molecular weight or an insoluble organic compound such as tar. Reactor examples include bubble fluidized bed, circulating fluidized bed, and vacuum reactor. The requirements within fast pyrolysis includes a particle size less than 1 mm. Slow/moderate pyrolysis is the antithesis of fast as it requires a long vapor residence time and low heat rate. The resultant products are charcoal, depending on the concentration of lignin and hemicelluloses. Examples include rotary kiln and moving bed reactor [54].

Pyrolysis applications have been experimented with various manure types. It has been determined that the effectiveness of char production was based upon manure type and the conditions, as it was observed that organic materials differ between two different waste types [55].

#### 1.3.4.3 Direct Liquefaction

Direct liquefaction is another thermochemical process that converts organic material, specifically lignin components, into various organic oils. Ideal conditions for liquefaction would be having very high pressures (5–20 MPa) and low temperatures (250–350 °C). Following the process, the remainders of direct liquefaction are non-reactive and stable, which are then converted into oil-based compounds with high molecular weights [53].

The process of liquefaction begins when the bonds of organic material are broken into simpler compounds, resulting into the forms of chars, instead of the process of oils. To prevent the formation of chars, solvents are typically added to slow down higher-order solid-state reactions, reducing condensation and the subsequent char formation. Examples of the solvents that are used include dioxane, MDSO, DMF, acetone, and methyl alcohol [54].

#### 1.3.4.4 Gasification

Gasification operates at high temperatures and atmospheric pressure within the range of 800–1300 °C for the purpose of producing chars and a low energy fuel. The gasification process has three components—first, pyrolysis, or the conversion of organic materials into both tars and hydrogen-based combustible fuels. Second, exothermic reactions with the presence of oxygen can occur to remove the bonds within the organic material at high temperatures. Third, methanation or the formation of methane from hydrogen and carbon monoxide proceeds where the conditions consist of lower temperatures [53].

A fixed bed 10 kW power, counter-current atmospheric pressure gasifier was capable of achieving a gas product made from either high ash feedlot manure (HFB) or poultry litter biomass (HLB) that consisted of the following product: $H_2$, $5.8 \pm 1.7\%$; CO, $27.6 \pm 3.6\%$; $CH_4$, $1.0 \pm 0.5\%$; $CO_2$, $6.7 \pm 4.3\%$; and $N_2$, $59.0 \pm 7.1\%$. Ideal processes included air-blown gasification for the purpose of having a higher energy fuel [56]. If application of a catalyst such as nickel or aluminum would better assist in the formation of gas production by preventing tar cracking, it would be preferable [57].

Priyadarsana et al. (2005) completed gasification studies for the production of both cattle manure and chicken litter biomass under batch mode where it was determined that the molar composition of gas was 27–30% CO, 7–10% $H_2$, 1–3% $CH_4$, 2–6% $CO_2$, and 51–63% $N_2$ based on the use of air mass flow rate of 1.48 and 1.97 kg/g, where particle sizes are 9.4 and 5.15 mm, respectively [58].

### *1.3.5 Composting*

There are many reasons to compost. Composting is done to reduce organic material, degrade dead livestock, and reduce disease transmission at a low cost. There are several factors that affect the quality of composting—carbon-to-nitrogen ratio, moisture content, temperature, and the type of composting materials [59]. A proper carbon-to-nitrogen ratio reduces the odors while the temperature affects the microbial degradation [60]. The temperature affects degradation processes. During the winter season, degradation can be reduced in some places by 20% [61]. Composting materials include sawdust, wood chips, and litter. Composting consist of microorganisms (bacteria and fungi) degrading organic materials within the compost pile to simple products [59].

The general composting values are shown in Table 1.12 below. These values are based on manure composting. Composting consist of primary and secondary processes. In primary composting, the temperature is raised and the organic material is degraded. As composting progresses, degradation begins to slow and the temperature is reduced. Eventually, degradation ends and the material is left idle [60] in a process known as curing. Curing maintains the conditions within the pile. It also allows items such as bones to be degraded [61].

There are two types of composting facilities—bins and piles or windrows. These are contingent on the type of livestock industry. Bins are used in poultry and swine. Beef and dairy cattle use piles or windrows [59]. Windrows or piles place materials into rows at triangular cross-sections. They are usually combined with bulking agents [62]. Aeration occurs by turning the piles by using frontend loader or compost turners [63]. Piles constructed in arid regions will need to receive outside moisture. This can be done by using a high-pressure nozzle from holding ponds or lagoon wastewater. On the other hand, piles in areas with precipitation may need to be covered to prevent odor production [63]. Bins can be designed to have dimensions of 6 ft by 8 ft with a wall height between 5 and 6 ft Bins can be made of 2 × 6 or 2 × 8 lumber or using plywood with a 2 × 6 to provide support behind the plywood [64]. The foundation of bins can be made up of pallets, gravel, concrete, and bare soil [65].

There are two entities that can be composted—manure and dead livestock. Dead animal composting is an option to remove livestock carcasses without having

**Table 1.12** Factors that affect composting [59]

| Factor | Value |
|---|---|
| **C:N ratio** | **25–40:1 (optimum: 30:1)** |
| Moisture | 40–65% (**optimum:** 50%) |
| Temperature | 43–66 (**optimum:** 54–60 C), >71 not ideal |
| Site selection | 1–5% (2–3% account for runoff and erosion) |

detrimental effects on the environment [59]. Dead animal composting maintains aerobic conditions, provided gases and liquids are taken away from the system [60]. Livestock operators should consider state requirements to decide what the state requirement of handling dead animals is. For example in the state of Kansas, composting facilities of dead livestock require a roof and floor to sustain moisture and avoid groundwater contamination with a fence surrounding the facility [59]. The process of composting is contingent on the size of the carcass materials [60].

A dead animal composting pile begins with a layer of sawdust 1–2 ft in depth. The dead livestock are then spread evenly across the sawdust layer [60]. Animals are laid on the side in an attempt to maximize the space for livestock [61]. Another layer of sawdust 2 ft in depth covers the dead animals. This second layer of sawdust maintains heat, prevents odors from escaping, and collects liquids and air to encourage microbial activity within the pile [60]. The amount of sawdust needed is contingent on the type of livestock to be composted. A rule of thumb for sawdust application is that in every 1000 lb of carcass, apply 7.4 $yd^3$ of sawdust in the dimensions of 9 ft × 10 ft [61]. When livestock need to be added to the composting pile, the top sawdust layer is removed, exposing the dead animal layer. At this point, the new animals are added and then covered up with a new sawdust layer. To maintain the quality of the pile, it advised that the pile is turned every 90 days. Once composting is complete, the products can be applied to the land or reused in other capacities [60]. This will usually happen anywhere between 4 and 12 months of composting time [61].

While composting dead livestock is advantageous, there are several concerns involved. These can include leachate of fluids from the carcasses entering into the surface and groundwater and disease-spreading pathogens [65]. Therefore, it is necessary to consider the best place to site the place for composting dead livestock. Changes can include placing the facilities away from the water table, away from low permeable soils, and downwind from neighbors. Facilities should also be constructed away from livestock to suppress disease potential. Livestock operators should also have an emergency plan in case of outbreak [61]. For additional protection, the livestock operator can create a barrier wall to prevent access to the composting pile. The barrier can be 4 ft high using four steel t-posts with concrete floors, wooden walls, and a metal roof [65].

### *1.3.6 Vermicomposting*

An alternative method of treating wastes that has been used related to composting is as vermicomposting. Vermicomposting is a method where earthworms digest a small portion of organic matter where the majority becomes waste in a form known as worm casts. The processes involved in earthworm digestion are typically physical or mechanical, grinding and mixing, and biological or microbial decomposition in nature. In vermicomposting, waste is added to the system. It must be added into the system in thin layers for the purpose of increasing degradation. There is great

competition between earthworms and microorganisms for the carbon sources. Application of waste can change—it will either increase or decrease productivity [66].

Vermicomposting treatment technology has been used extensively in animal excretion, sewage, and agroindustrial wastes but not animal manures. Therefore, Loh et al. (2004) treated cattle and goat manures using the earthworm, *Eisenia foetida*. The experiments found that total C, P, and K were high in goat manure worm casts as compared to cattle, whereas cattle worm casts were richer in N content. In addition, cattle manure had a higher biomass and reproductive performance as well along with a higher cocoon production per worm [66]. Other studies have been compiled on cow, buffalo, horse, donkey, goat, and animal [67], dairy [68], and pig [69, 70] to name a few. Within continuous feeding reactors, two different types of pig slurry were compared with 500 earthworms (*Eisenia foetida*); microbial biomass was specifically measured with 3 kg of pig slurry; loss of C was not related to pig slurry rate; rate of manure-earthworm relationships was investigated [71].

### *1.3.7 Summary*

There are many treatment methods that can be considered for the handling of wastes that persist within the livestock industry. An operator must consider what is available in regard to space and the desired treatment needed in order to make an appropriate decision on selecting the proper treatment method.

## 1.4 Land Application of Livestock Wastes

### *1.4.1 Description*

Land application is a waste management technique that involves recovery of nutrients from manure by plants for the purpose of producing a crop [2]. The classification of manure depends on the percent of dry matter present and the type of livestock waste industry. Manure can be in liquid (less than 5% dry matter), semiliquid (5–10% dry matter), or solid (greater than 15% dry matter) form. Generally, beef and poultry industry handles solid manure, while dairy and swine manure is usually in liquid form [72].

Regardless of the industry, the nutrient content is the primary focus for application. Nutrient content within the manure is affected by the type of animal species, the process for handling of manure, livestock housing, bedding system, diet, temperature, and the nutrients present. The primary nutrients of concern are nitrogen, phosphorus, and potassium. The nitrogen presence affects the type of plants and quality of the produce. There are two important forms of nitrogen that must be

considered—organic nitrogen and ammonium nitrogen. When organic nitrogen enters into soils, it is mineralized into inorganic nitrogen. Mineralization is contingent on the temperature and time of year. Warm and moist soils are better for the degradation of organic nitrogen as compared with cool and dry soils. Ammonium-nitrogen is converted to organic nitrogen by plants in a process known as nitrification. Moreover, 25–50% of organic nitrogen is converted to ammonium-nitrogen. However, improper application of manure can lead to volatilization or the conversion of ammonium-nitrogen to ammonia-nitrogen. This becomes problematic because ammonia-nitrogen dissipates into the atmosphere. On the other hand, potassium and phosphorus must be converted to inorganic forms in order for it to be of use by plants [73]. Manure can also be problematic because it can produce various gases. These gases can have grave effects depending on the concentration. Table 1.13 summarizes the major gases found in manure. Previous treatment methods can affect land application. Table 1.14 discusses the various treatment processes and their effects on land application. Therefore, the type of handling equipment, time, and rate of application should be considered if an operator is to consider land application.

## *1.4.2 Manure Handling Equipment*

The equipment necessary for handling manure depends on the type of manure. Each operator must make a decision of handling manure that best distributes the nutrients to the crops being planted. Depending on the type of manure handled, there are unique pieces of equipment that are used in order to safely move the manure onto the field.

### 1.4.2.1 Solid Manure

Solid manure is incorporated at the surface by using spreaders that are trucked-mounted or trailer-towed. Regardless of the type of spreader, manure can be spread at the side or the rear. Nevertheless, rear manure spreaders are more likely used today [72]. For example, livestock operators in the state of Missouri primarily use

**Table 1.13** Manure gases [74]

| Gas | Effects (percent indicates percent or concentration in ppm) |
|---|---|
| Ammonia ($NH_3$) | Eye irritation (<1%)<br>Coughing, irritation of throat, eyes, lungs (3–5%) |
| Carbon dioxide ($CO_2$) | Difficulty breathing, drowsiness, headaches (3–6%)<br>Death (>30%) |
| Methane ($CH_4$) | Asphyxiation (5–15%) |
| Hydrogen sulfide ($H_2S$) | Dizziness irritation, headache (50 ppm)<br>Death |

**Table 1.14** Various wastewater and biosolid treatment processes and methods and their effects on land application processes [75]

| Process/ Method | Process definition | Effects on biosolids | Effect on land application process |
|---|---|---|---|
| Wastewater treatment process | | | |
| Thickening | Low force separation of water and solids by gravity, flotation, or centrifugation | Increases solid content by removing water | Lowers transportation costs |
| Stabilization method | | | |
| Digestion (anaerobic and/ or aerobic) | Biological stabilization through conversion of organic matter to carbon dioxide, water, and methane | Reduces biological oxygen demand, pathogen density, and attractiveness of the material to vectors (disease-spreading organisms) | Reduces the quality of biosolids |
| Alkaline stabilization | Stabilization through the addition of alkaline materials (e.g. lime, kiln dust) | Raises pH. Temporarily decreases biological activity. Reduces pathogen density and attractiveness of the material to vectors | High pH immobilizes metals as long as pH levels are maintained |
| Heat drying | Drying of biosolids by increasing temperature of solids during wastewater treatment | Destroys pathogens, eliminates most of water | Greatly reduces sludge volume |
| Chemical and physical processes that enhance the handling of stabilized biosolids | | | |
| Conditioning | Processes that cause biosolids to coagulate to aid in the separation of water | Improves sludge dewatering characteristics. May increase dry solids mass and improve stabilization | The ease of spreading may be reduced by treating biosolids with polymers |
| Dewatering | High force separation of water and solids. Methods include vacuum filters, centrifuges, filter and belt presses, etc. | Increases solids concentration to 15–45%. Lowers nitrogen (N) and potassium (K) concentrations. Improves ease of handling | Reduces land requirements and lowers transportation costs |
| Advanced stabilization method | | | |
| Composting | Aerobic, thermophilic, biological stabilization in a windrow, aerated static pile, or vessel | Lowers biological activity, destroys most pathogens, and degrades sludge to humus-like material | Excellent soil conditioning properties. Contains less plant available N than other biosolids |

rear-end box-type spreaders with beaters. These spreaders can consist of a conveyor with chains to move manure from the front of the spreader to the beaters or a front endgate that moves the manure to the beaters [76]. Once it is moved to the rear, the beaters scatter the manure onto the ground [72].

Rear-end box-type spreaders can have single, horizontal, or double vertical beaters. However, each beater type is limited in its ability to properly distribute

nutrients. Single beaters cannot spread manure homogenously onto the land. Horizontal beaters only spread manure in areas of close proximity to the trailer. Double vertical beaters spread manure very wide and thin. Overall, rear-end box-type spreaders have a problem with spreading manure homogenously onto the land. They are also very heavy and have the potential to compact soils if land application is done in the fall and spring. Similar to box-type spreaders, truck-mounted spreaders apply manure using double beaters in various horizontal or vertical configurations. Regardless of application, solid manure handling should be applied within 24 h. This is to ensure the minimization of nutrient loss, the presence of odors, nutrient runoff, and compaction [72].

Since the application of solid manure can generate odors, there are methods that can be done to suppress odors in manure land application. These include placing a cover over solid manure not being applied, using chemical treatment such as alum (also advantageous for preventing ammonia volatilization), and considering the wind direction when applying onto the surface. There are also mechanisms that can be employed that can better spread the manure upon entry on the field. These include a tandem disk or a field cultivator. Solid manure can also be pretreated by drying or composting [77].

#### 1.4.2.2 Semisolid Manure

Semisolid manure is handled by using spreaders with an endgate. The configuration can range from side discharge or a V-shaped hopper. Each of these can be handled by power takeoff (PTO) or ground wheel tractor spreaders or a truck-mounted spreader. The process of application consists of moving the manure by augers to be flung at the point of emission on the spreader. Manure is flung either by using a rotating or flail-type expeller. A rotating expeller directly flings manure, while in a flail-type expeller, manure travels from a hopper onto a rotating shaft with chain-suspended hammers. Once the manure is on the hammers, it is tossed onto land [76].

#### 1.4.2.3 Liquid Manure

Liquid manure can be applied at or below the surface. Surface application of liquid manure is completed by fixed sprinklers, hand-carried sprinkler, traveling guns, or central pivot irrigators [76]. Factors that control application by irrigation equipment are nozzle size and pressure. These affect the size of the drops applied to the surface. Larger-sized drops are greatly preferred to control the loss of nitrogen and decrease odors [77]. A recommended size is greater than 150 μm. Other ways include adding dilution water or drop nozzles [78]. Surface application of manure is preferred in areas where odors and nutrient loss are minimal [76]. Figures 1.17 and 1.18 provide diagrams of irrigation systems.

Subsurface application injects liquid manure below the surface where it is then applied below the soil surface by a self-propelled application. Manure can also be

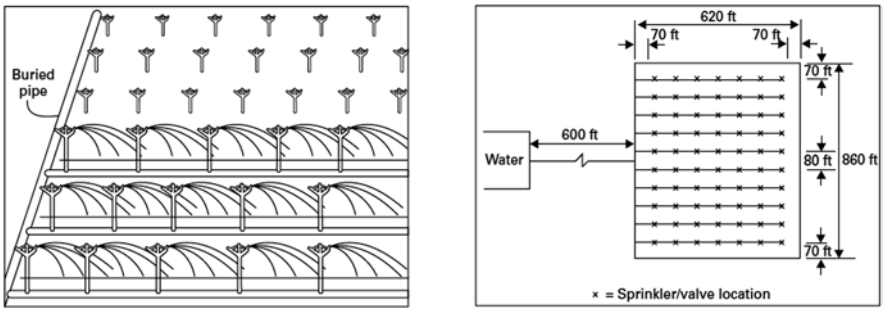



**Fig. 1.17** Irrigation system to apply liquid manure [76]

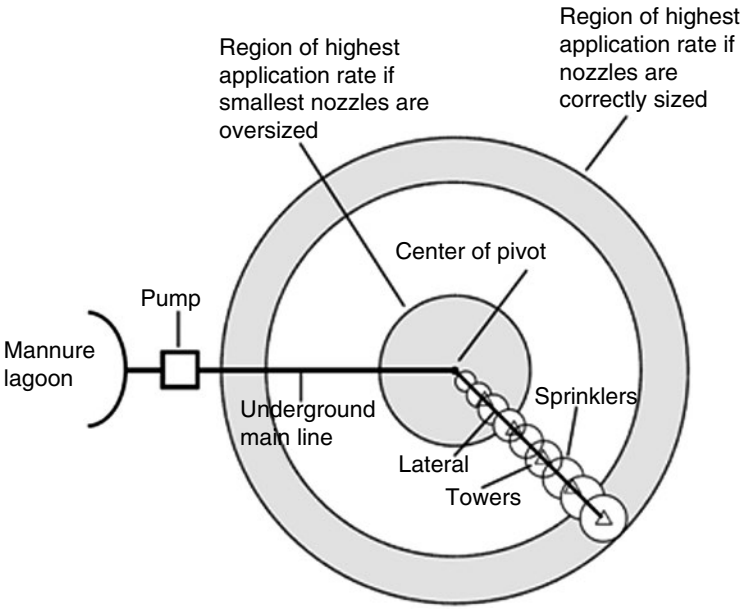



**Fig. 1.18** Center pivot irrigation system [79]

transferred by a drag hose or a tractor-drawn applicator. The method chosen is determined by the size of the operation. Usually larger operations opt to use a drag hose or a tractor-drawn applicator. When liquid waste is applied below the surface, injectors have chisels that break up material or sweeps that uniformly apply the liquid manure below the root surface to avoid leaching [76]. Chisel-type knives also prohibit odors and volatilization, while sweep-knife injection reduces volatilization, denitrification, and material degradation [73].

Subsurface is preferred to surface application for several reasons. First, subsurface reduces the potential of ammonia-nitrogen emissions [76], greenhouse gases, and odors [72]. For example, research has shown odors to be reduced by 90% when

incorporating a subsurface method [78]. Second, subsurface application reduces runoff potential, availing more nitrogen to plants. Third, subsurface injection spreads the manure so it does not have an impact on the surface of the soil. Despite its many advantages, subsurface application is energy intensive; requires more maintenance, time, and management; has higher equipment costs; and is incapable of being used on rocky soils. Therefore, assessment should be made to determine whether or not subsurface injection is a more viable option than any surface application method [80].

### *1.4.3 Time of Application*

The time when manure is applied determines nutrient availability to plants. Spring is the best season for manure application because nutrients are broken down into the soils during the growing season. Organics are quickly broken down in the soils, increasing nitrogen availability. Summer applications are appropriate if growing hay, pasture, and warm-season grasses and if application is completed by travel guns or the central pivot system. Applying manure during the fall is only appropriate if temperature stays below 50 °F [80]. This is because manure is immobilized and remains in the soil [73, 80], leading to more time for degradation. But when the temperature is above 50°, nitrification, leaching, and denitrification occur [73]. Winter application of manure is never recommended as manure hardly enters the soil and has a higher potential for runoff [80] into surface waters. If manure application is a necessity in the winter, apply at low concentrations or during periods of snow melt [73].

### *1.4.4 Rate of Application*

The amount of nitrogen, type of manure, how manure is applied and used, and additional economic or environmental are the factors that determine how frequently manure will be applied to a given crop. The University of Minnesota Extension provides four steps to determine the process by properly determining the rate of application [73]:

1. Determine the nutrient needs of the crop.
2. Analyze the nutrient content within the manure.
3. Uncover the nutrient available to the crop.
4. Compute the rate of application.

### *1.4.5 Summary*

In summary, the purpose of land application attempts to resolve the issue of losing nutrients that are vital to the growth of crops. Manure should be applied uniformly to land to avoid the volatilization of nitrogen into the atmosphere. It should also maintain the potassium (K) and phosphorus (P) on the field. The time of application should be considered in order to have nutrients maintained within the soils and avoid any subsequent losses that occur during improper times of application [81]. Manure application should be done to avoid the presence of odors [76] and other potential issues. The rate of application depends on the crop's needs.

## 1.5 Storage of Livestock Wastes

### *1.5.1 Description*

Most often, the treatment of livestock waste is done for the purpose of recycling products back within the system. This can include land application for growing plants. However, there may be times when the conditions are not conducive for reusing treated wastes. Therefore, livestock wastes must be stored until the appropriate conditions take place. There are several factors that should be considered when deciding whether or not to store manure: first, if the soil is saturated, wet, frozen, or snow covered or if the soil will compact under the weight of manure handling equipment; second, if the temperature and/or humidity create a proper environment for the generation of odors; third, if a livestock operation may not have the proper equipment or personnel available to apply manure at the present moment; fourth, if the cropping schedule may require temporary storage; and finally, if there is a higher volume of manure and wastewater than what can be handled [82]. There are several methods for storing wastes. These methods are employed usually based on the time of storage and type of waste treated—i.e., solid, semisolid, and liquid wastes.

### *1.5.2 Storage Time*

Livestock wastes can be stored either on a short-term or long-term basis. When wastes are stored for 60–90 days or up to 180 days, it is termed as short-term storage. Short-term storage is a viable option when poor weather conditions persist, or when setup is not appropriate to properly handle manure. Short-term storage is also used in mild climates or when growing crops [83]. However, it is very seldom for operators to store liquid manure on a short-term basis. Dairy wastes are the most appropriate to be stored short term.

There are many methods for storing manure on a short-term basis. These can include stacking within a field, covered with a plastic sheet, or storage in a detention pond. Manure can also be scraped into open lots in mounds or inside pole sheds. Regardless of the method, the operator should choose to avoid any contamination of water supplies or exposure to bacteria from the manure [84].

Long-term storage can last for approximately 180 days. Facilities are available to hold solid, semisolid, or liquid wastes. For example, walls and slabs can stack solid manure, while semisolid pumps or scrapers help transport waste into areas designated for storage. Liquid waste is usually transported by pumps or pipes [84]. Sometimes manure can be held for longer than 180 days. For example, waste is stored for 6 months for the purpose of application on annual row or small grain crops. In the center and upper Midwest, storage can happen for a full year if fall applications are unsuccessful because of wet conditions [82].

### *1.5.3 Facilities to Store Livestock Waste*

There are many facilities that can be used to store manure. However, the practicality of each facility depends if the operator is storing solid, semisolid, or liquid manure. Table 1.15 provides an estimated cost for manure storage facilities.

#### 1.5.3.1 Solid Manure Storage

The objective in storing solid manure is to reduce the volume, odor, and potential for runoff. Solid manure is stored based on climate and industry. Because the evaporation rate is greater than precipitation, arid regions can store solid manure in a different fashion as compared to regions that retain precipitation. Arid regions simply store manure in stacks or piles. In the beef and dairy industry, manure is composted using windrows or piles, while in the poultry industry, the manure is contained inside stack houses. On the other hand, non-arid regions require the solid

**Table 1.15** Estimated costs for manure storage facilities. Numbers based on 500,000 gallon capacity [85]

| Storage type | Cost ($/1000 gallon) |
|---|---|
| Naturally lined earthen basin | 25–36 |
| Clay-lined earth basin using clay onsite | 50–70 |
| Clay-lined earth basin using clay from off-farm borrow site (depending on hauling distance) | 80–100 |
| Earthen basin with plastic liner | 100–140 |
| Earthen basin with concrete | 120–280 |
| Aboveground pre-cast concrete tank | 200–250 |
| Aboveground concrete tank poured in place | 230–270 |

manure to be walled with a concrete bottom and covered with a roof. If solid manure is not housed in this manner, it could also be composted [83]. However, there are alternatives for non-arid region storage of solid manure. Purdue University Extension states that if manure is dried and bedding is added to form a solid, it can be stored on concrete pads. Concrete pad storage of manure reduces the potential of groundwater leaching and runoff provided the operator constructs a roof [86].

#### 1.5.3.2 Semisolid Manure Storage

Pits are a main way to store semisolid manure. Pits in general are a viable option for waste storage because they can reduce waste volume and reduce the production of odors provided they are properly maintained. Pits can be fabricated from concrete or a coated metal or can be completely made of earth. Manure is transferred into them by means of slated floors. Fabricated pits can be constructed for a location completely above, partially above, or below the surface of the ground. The process of transferring semisolid manure is by scraping or flushing the manure from its source. Equipment used for transferring can include collection sump pumps or by gravity, depending where the pit is located. Semisolid manure should be agitated before transfer to ensure all suspended solids are relocated into the pit [83].

Pits made from earthen structures are capable of housing large quantities of semisolid wastes. Therefore, operators will need to ensure ample space is available if a pit from earth is to be used [83]. The incorporation of manure at the bottom of the pit protects the pit from leaching nutrients. This is especially advantageous for very clayey soils. Pits are also lined to protect leaching from the walls. The change in fluid levels can alter the stability of the pit, leading to the formation of cracks [86]. In addition, earthen structures require vegetative cover. Maintenance is then necessary for its upkeep. As with fabricated pits, manure entering into an earth-structured pit also requires agitation. Transporting semisolid wastes into the pit is easily done with the use of a built-in access ramp. This can make hauling and transporting waste very time-consuming. Nevertheless, earthen pits can be a culprit for odor production so proper maintenance is necessary. Despite the time-consuming hauling and the high potential for odors, earthen pits are less expensive as compared to fabricated pits [83].

#### 1.5.3.3 Liquid Manure Storage

Facilities that can store liquid manure can include lagoons, runoff holding ponds, and storage tanks. Table 1.16 provides a detailed description of the solid content within liquid manure systems. Lagoons are a beneficial option for storing liquid manure because they can house liquid manure for 6–24 months [86], can be cost-effective per animal, and reduce odors [83]. Lagoons provide a mechanism for liquid waste to be treated prior to land application [86]. Lagoons require a higher volume than treatment of semisolid manure and must consider the temperature,

**Table 1.16** Solid content for liquid manure systems [76]

| System | Solid content |
|---|---|
| **Manure pit** | |
| Swine | 4–8% |
| Cattle | 10–15% |
| **Holding pond** | |
| Pit overflow | 1–3% |
| Feedlot runoff | <1% |
| Dairy bard wastewater | <1% |
| **Lagoon, single or first stage** | |
| Swine | ½–1% |
| Cattle | 1–2% |
| Lagoon, second stage | <1/2% |

climate, and volume of wastewater to be housed. Biological activities in the lagoon are maintained by replenishing the lagoon with dilution water and prevent salt buildup. This should be monitored during high rates of evaporation [86]. Lagoons should also be monitored to avoid a buildup of settled solids [87]. More information on lagoons can be found in Sect. 1.2.

Runoff holding ponds are typically used for storage during rainfall events. This means that any liquid manure housed must be pumped out following the event [83]. Holding ponds are designed to be smaller than lagoons. This reduces the rate of degradation within the pond. Erosion and overflow is controlled by installing a 12-inch spillway. To maintain liquids within the holding ponds, a settling basin is set up to collect 60–75% of the solid manure. This allows waste removal to be completed by irrigation systems [86].

Storage tanks for liquid waste can be made from glass, concrete, or earth. Similar to pits, storage tanks can be placed above, partially above, or underground. A storage tank is divided into five major sections—residual volume, manure storage, wash water, rainfall and evaporation, and safety volume depth. The residual volume comprises of 6–12 inches from the bottom of the tank. Above the residual volume houses the manure. The manure is pumped into this section of the tank and can be stored for 3–6 months. The wash water stores wash or freshwater. If the tank is open, the net rainfall and evaporation section collects any rainfall that may occur. Finally, the safety volume depth provides adequate space to handle a 25-year, 24-h storm event. Depending on the type of material, storage tanks will have a different depth [88].

### 1.5.4 Storage Area Design

The storage of manure has been published by the US Department of Agriculture (USDA) and follows the following calculation based on storage volume [2]:

$$VMD = AU \times DVM \times D \tag{1.2}$$

where.

$VMD$ = volume of manure production for animal type for storage period, $ft^3$
$AU$ = number of 1000 lb animal units per animal type
$DVM$ = daily volume of manure production for animal type, $ft^3$/AU/day
$D$ = number of days in storage period

The second equation calculates the bedding storing volume:

$$BV = (FR \times WB \times AU \times D)/BUW \tag{1.3}$$

wheres

$FR$ = volumetric void ratio (values range from 0.3 to 0.5)
$WB$ = weight of bedding used for animal type, lb/AU/day
$BUW$ = bedding unit weight, lb/$ft^3$
Sometimes this equation is multiplied by 0.5 as a volumetric void ratio.

Sizing for a liquid and slurry waste storage can be calculated from the following equation:

$$WV = TVM + TWM + TBV \tag{1.4}$$

where

$WV$ = volume of waste stored, $ft^3$
$TVM$ = total volume of stored manure, $ft^3$
$TWW$ = total wastewater stored, $ft^3$
$TBV$ = total bedding volume stored, $ft^3$

### 1.5.5 Summary

The type of manure affects the manure facility chosen. Within the types of manure, there are various facilities that can house manure. Each facility should be analyzed carefully before installation. This ensures that the proper facility is constructed based on the needs of the operation.

## 1.6 Feedlot Runoff Control Systems

### *1.6.1 Description*

Section 1.1 of this chapter indicates that feedlots are required to have NPDES permits as defined in the Clean Water Act of 1977 [89]. This limits the amount of discharge that can occur at a particular location. A major source of discharge from feedlots is runoff. There are several different systems that properly contain runoff. Many of systems have been discussed in prior sections, and therefore information concerning the significance for runoff control will only be presented. Runoff control protects a feedlot from the presence of weeds, odors, and insects. The collected water provides an alternative source for fertilizers and irrigation water [90].

### *1.6.2 Runoff Control Systems*

#### 1.6.2.1 Descrtiption

The processes of a runoff control systems are multifaceted. A runoff control system captures and reroutes rain or snowmelt. It can also provide a method to treat runoff before it is to be discharged. There are two major subsets of runoff control systems—full containment and discharge runoff control systems. Full containment systems (also known as clean water diversion systems) include the use of terraces, channels, and roof gutters [89].

#### 1.6.2.2 Clean Water Diversion

The purpose of diversion is to control runoff entry into holding ponds and settling basins [94]. In addition, precipitation is prevented from invading manure storage systems, preventing the potential for creating polluted runoff [90].

#### 1.6.2.3 Discharge Runoff Control

Discharge runoff control systems include settling basins and runoff holding ponds. Settling basins are a runoff control system that separate liquid from solids. The separation of liquids from solids allows liquids to be further treated by methods such as storage ponds. Solids settle to the bottom while the liquids remain at the top. There are several processes that will cause solids to separate from liquids. These include risers, slotted board, or porous dams. Settling basins consist of channels or boxes made of concrete or earth. Cleaning the basin is necessary to allow for solid

placement. The cleaning of the basin should be done if 50% of the basin is filled with solids. Solids are taken from the basin and led away from the feedlot. If cleaning is not permissible, an alternative method is to increase the size of the basin by 25–50%. Scrapers, high-pressure water systems, and metal screens prevent the system from being clogged. Figure 1.19 is a diagram of a solid-liquid separator [90]. Figure 1.20 depicts a system to handle runoff. Figure 1.21 provides a diagram of a settling basin.

Runoff holding ponds receive and store liquid runoff from settling basins. This process can happen 15–30 min before entry into a settling basin [92]. In general, they are smaller than holding ponds. This means that when wastewater is collected, it will only remain in the ponds for a short period of time. They must be dewatered by using equipment such as a sprinkling systems or perforated pipes. However, if holding ponds are constructed in arid regions, dewatering is not necessary as evaporation will be sufficient. Water removed from the holding pond can be applied onto crops [90]. Figure 1.22 is a diagram of a holding pond.

In general, holding ponds are designed based on a 25-year, 24-h storm [90].The volume chosen for the pond is also contingent upon the time of storage permitted [92].

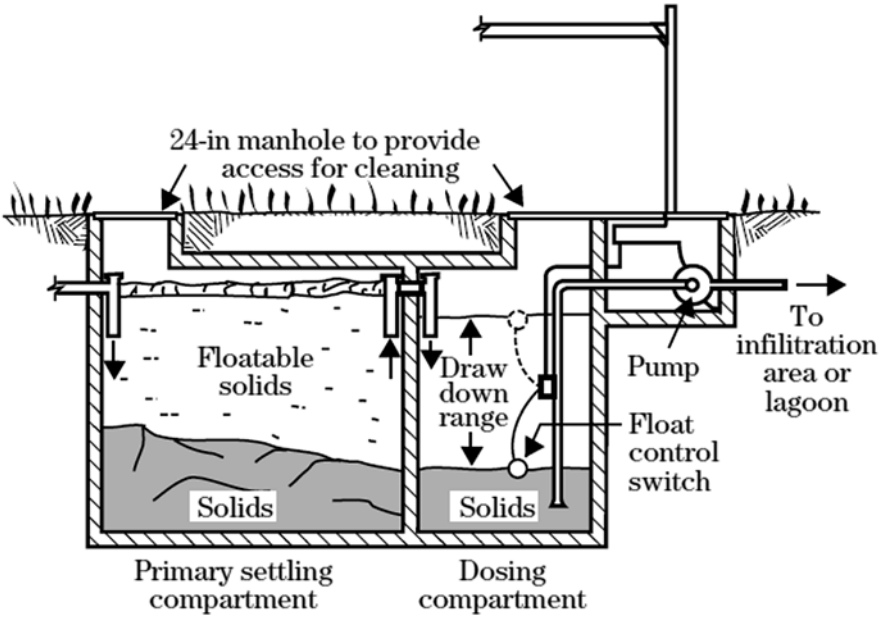



**Fig. 1.19** Solid-liquid separator [8]

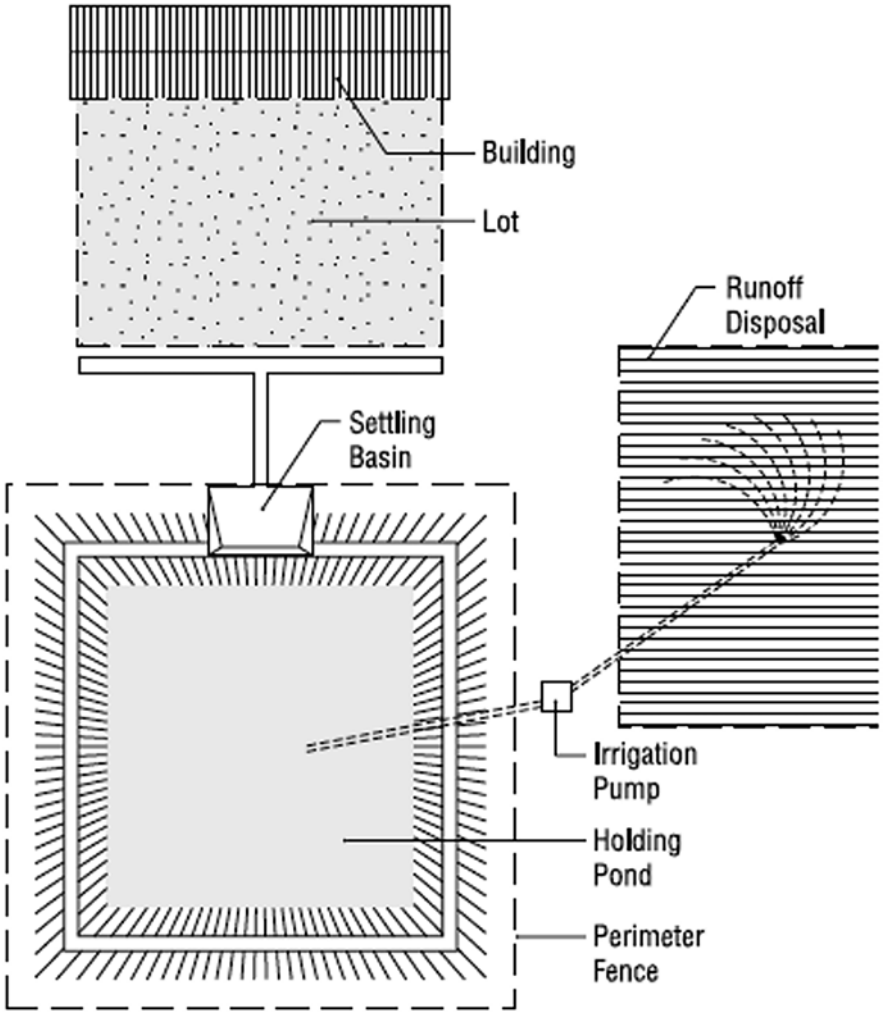
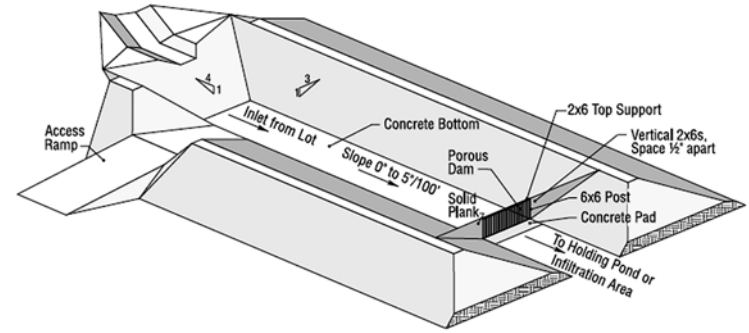


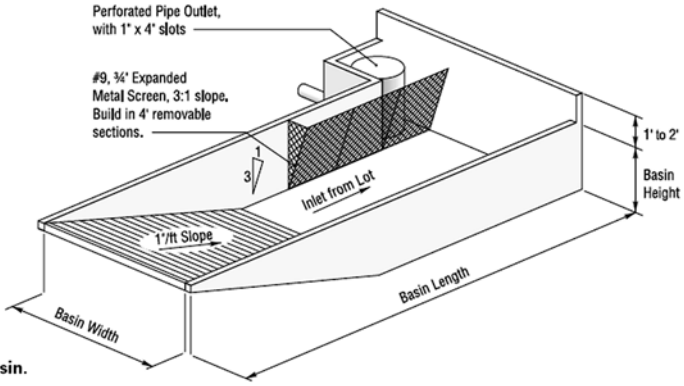

**Fig. 1.20** Lot runoff handling system for milking wastewater [91]

#### 1.6.2.4 Vegetative Filter Strips

Another method to control feedlot runoff includes vegetative filter strips. Vegetative filter strips (VFS) are a feedlot runoff control system consisting of vegetation. This vegetation is grown in close proximity to the feedlot, reducing constituents such as sediments, nutrients, and pesticides [93] and COD [94]. In a VFS system, vegetation uptakes pollutants from runoff prohibiting transport beyond the feedlot. The removal of these particulates from the runoff results in clean water [95]. Associated processes include settling, filtration, dilution, pollution absorption, and infiltration [96]. VFS systems are capable of removing 60–70% suspended solids, 70–80% nitrogen




a. Earthen sidewall settling basin.




b. Concrete settling basin.

**Fig. 1.21** Settling basins for manure management [91]. (**a**) Earthen sidewall setting basin. (**b**) Concrete setting basin

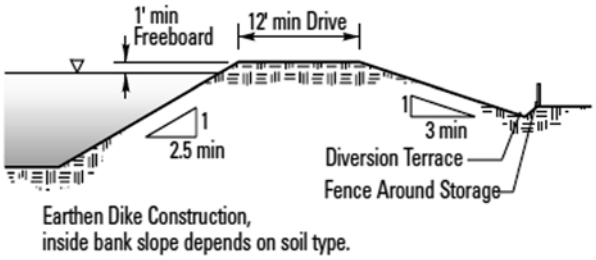



**Fig. 1.22** Holding pond for storing milk house wastewater [91]

[94], 7–100% phosphorus, and 64–87% pathogen removal [97]. VFS systems create a mechanism that can reduce non-point pollution runoff. Several factors affect the efficiency of a VFS system. These include the type of pollutant, soil type, vegetation, state of flow, and current plant status [93].

The nature of the pollutant is important in determining its ability to be treated by vegetative filter strips. Vegetative filter strips are capable of reducing particulate-bound pollutants in comparison with soluble particulates. Various processes incorporated within VFS are able to be removed by the system as compared with soluble particulates, which can only be removed by sedimentation. The type of soil is important because of the various processes that occur within soil. Sandy-loam soils with a depth between 3 and 13 ft or clay soil (26–145 ft) are ideal for VFS. Vegetation should be dense and rough and must be able to reduce the surface velocity so that collected solids are kept within the system. Flow entering into the VFS system should be overland sheet flow as compared to concentrated flow. Overland sheet flow prevents sediments from leaving the VFS system, lowering the velocity of the wastewater within the system [93]. Sheet flow is also uniform throughout the system and is shallow [97]. Channelized or channel flow differs from overland flow because runoff flows through a narrow channel such as a gated terrace or a waterway. This presents a problem because water flows a velocity that is higher than one in channelized flow. Channelized flow also requires more land because the strip will need to be longer to accommodate the channel [96]. Loading into the VFS system is also inconsistent. As a result, channelized flow includes a reduction in treatment and an increase in erosion [97].

There are two types of VFS systems—vegetated infiltration basin (VIB) and vegetative treatment area (VTA). A VTA system plants vegetation downslope from crops or livestock housing. On the other hand, VIB is similar to a VTA with the exception of a berm for runoff collection. Included within the treatment system is the presence of aerobic bacteria to treat nitrogen by means of nitrification. When wastewater enters into the VIB system, nutrients are absorbed into the soil and are used by plants. Runoff is collected through tiles in the system where it is transferred to other wastewater treatment systems [97].

A VFS system is most effective if it has a depth less than 1.5 ft. In this scenario, uptake of pollutants by plants is more feasible. Pollutant removal efficiency is also affected by the length of the VFS—the longer the VFS, the more efficient the treatment [93]. A recommended length for a VFS system is 100 ft or 1 ft/animal unit, whichever value is greater. However, the ground slope will affect the length of the system chosen. A 0–2% slope can have a minimum length of 100 ft, while a 6% slope a minimum of 300 ft [94]. Other recommendations for design include 200 ft length for a 1-year, 2-h storm, 300 ft for a 0.5% slope to 860 ft for a 4% slope. VFS treatment system should include a pretreatment step to settle solids from the runoff [97].

There are many types of vegetation that can be used with a VFS system. The University of Kentucky Extension states the type of vegetation planted within the system is contingent on the season. Five plants are suggested—tall fescue, orchardgrass, timothy, Bermuda grass, and gamagrass. Tall fescue is an option because it is capable of using nutrients when planted. However, it cannot be used for grazing. Orchardgrass not only removes nutrients but is capable of being used for grazing,

albeit only up to 4 inches, unlike tall fescue. Timothy grass is a viable option for horses and cattle to graze provided grazing is limited to 4 inches. Bermuda grass is a quality choice because it is capable of reducing nutrients and also drought resistant. Bermuda grass can grow up to 8 inches, while grazing is limited to 3–4 inches. Planting gamagrass will absorb nutrients deep from within the treatment system [95].

### *1.6.3 Summary*

In summary, this section presents several feedlot runoff control systems that are available to divert runoff coming from a feedlot. Feedlot operators must consider the characteristics of each control system and consult the state legislation in order to understand what are the design requirements and limitations to use the treatment method chosen by the feedlot.

## 1.7 Odors and Gases

### *1.7.1 Odors: Origin and Nature*

Dispersed odors in the air travel and can cause great discomfort for those that live in close proximity to livestock operations. There are three major causes for odor compounds in livestock operations—"the livestock themselves, animal housing facilities, feedlots, and feed storage facilities; manure storage structures; and application of livestock manure to agricultural land" [98]. Particular examples include anaerobic degradation of organics in manure, feed, and silage. Odors caused by anaerobic digestion increase in intensity when temperatures are warm. Also if manure becomes wet, it can also be a major cause for odors [99]. In feedlot operations, incomplete fermentation of nutrients by bacteria in manure produces odors [100].

Odors can spread in the air as a gas. Dust particles can also be agents to carry odors. When particles that cause odors come into contact with dust particles, they are absorbed and carried along. The effectiveness of odors spreading is contingent on the weather. Very humid days maintain the odors in the area, while dry and windy days will disperse them [99]. Rainfall can also increase the emission of odors. If rainwater remains on the ground surface, anaerobic conditions can occur on the manure [100].

### *1.7.2 Sources of Odors*

The major sources of odors are gases, anaerobic decomposition of manure, and other various compounds. The compounds that can provide the biggest issue include volatile fatty acids, mercaptans, esters, carbonyls, aldehydes, alcohols, ammonia, and amines [101]. A major proponent of odors is the formation of volatile fatty acids.

The reason why volatile fatty acids cause so many odors is because of the volatile organic compounds that are present within the manure. Volatile fatty acid presence within manure varies between animal types. For example, the majority of compounds found in pig manure include acetic, propionic, n-butyric, iso-butyric, n-valeric, iso-valeric, n-caproic, and iso-caproic acids. These organic compounds vary with the amount of carbon atoms present within the system, where butyric, valeric, and caproic being the highest amount of odor. Other potential dangers for volatile fatty acids increase toxic pathogens within soil base [102].

One can state that the majority of VFAs have carbon numbers between 2 and 9. In addition, the presence of *Eubacteria*, *Peptostreptococcus*, *Bacteroides*, *Streptococcus*, *Escherichia*, *Megasphaera*, *Propionibacterium*, *Lactobacilli*, and *Clostridium* are also noted for contributing to the major problems associated with volatile fatty acids [103]. Volatile fatty acids are generated during the process of fermentation, when carbohydrates are broken down from sugars into pyruvate, which is then fermented into volatile fatty acids in anaerobic conditions. Therefore, the lack of aerobic conditions such as incomplete microbial decomposition or other anaerobic treatment methods are the major cause of this potential issue [98].

Aromatic compounds are a major concern within animal manure due to the presence of indole, skatole, p-cresol, phenols, and 4-ethylphenol. Under anaerobic conditions, bacteria such as *Bifidobacterium*, *Clostridium*, *Escherichia*, *Eubacteria*, and *Propionibacterium* use aromatic amino acids such as tyrosine, phenylalanine, and tryptophan [98].

Sulfate-reducing bacteria typically cause the presence of hydrogen sulfide due to the reduction of amino acids cysteine and methionine. Sulfur-reducing bacteria typically use sulfate as a terminal electron acceptor transforming sulfate compounds into hydrogen sulfate. The most common bacteria heavily involved in this process are *Desulfovibrio desulfuricans*, *Veillonella*, *Megasphaera*, and the enterobacteria [98].

Ammonia emissions causing odor are commonly attributed to ammonia volatilization. The reason behind such a problem can be traced back to the animal species, diet, and age. For example, urea, the nitrogen compound within urine, typically forms ammonium and bicarbonate ions by means of urease enzymes. Nitrogen found in feces is broken down by bacteria, where it transfers from proteins to amino acids and eventually into ammonium. The time in which this occurs depends on the temperature, concentration, and pH [104].

One of the more common entities that is emitted through livestock waste is the presence of hydrogen sulfide. Hydrogen sulfate odor emissions commonly occur from the anaerobic decomposition of sulfur [105]. One of the most common

methods of forming hydrogen sulfate is due to the efforts of sulfate-reducing bacteria [106].

### *1.7.3 Odor Prevention*

There are various methods to prevent the spreading of odors. These can include animal nutrition management, manure treatment and handling, waste treatment methods, and better livestock operation management. Tables 1.17 and 1.18 provide various methods to mitigate odors.

#### 1.7.3.1 Animal Nutrition Management

One of the best ways to reduce odors is to alter animal nutrition. If livestock feed contains more crude protein concentration or blood meal, it will lead to the production of odors. Studies have shown that feeding livestock crystalline amino acids or peppermint as compared to a diet heavy with crude protein can reduce odorous manure. Barley-based diets can also reduce odors by 25% as compared with a diet dominated by sorghum [50, 107]. Fecal starches, proteins, and lipids should be eliminated as much as possible. This will prevent incomplete fermentation, which is the main cause of odors [100].

In addition to changing the diet of the animals, the operator should consider a change in feeding schedule. An appropriate feeding schedule could be feeding the animals at sunrise, noon, and sunset. This can not only eliminate the presence of odors but also control the emission of dust in the atmosphere from cattle that move

**Table 1.17** Odor emission strategies for livestock housing [50]

| Method | Description |
|---|---|
| Filtration and biofiltration | 1. Filtration traps 45% 5–10 μm particles; 40–70% particles greater than 10 μm |
| Biofilters | 1. Biofilters trap and biologically degrade particles; remove odorous emissions<br>2. Biofilters can remove 90% odors, including 90% hydrogen sulfide and 74% ammonia |
| Impermeable barriers | 1. Dust particles retain odors preventing movement<br>2. Impermeable barriers such as windbreak walls or dams are very effective |
| Oil sprinkling | 1. Application of vegetable oil can control dust movement<br>2. Study applying oil reduced hydrogen sulfide concentrations by 40–60% |
| Landscaping | 1. Application of trees and shrubs<br>2. Landscaping reduces particulate movement and inserts dilute the concentration of emissions |

**Table 1.18** Examples of odor emission strategies for manure storage [50]

| Method | Description |
|---|---|
| Solid separation | 1. Removal of large materials, typically the size of a screen opening<br>2. Removal of large material reduces the loading rates, thereby producing less odors during decomposition of remaining material<br>3. Solid separation uses processes such as sedimentation, screening, filtration, or centrifugation |
| Anaerobic digestion | 1. Under anaerobic conditions, odors are biologically reduced from manure<br>2. Anaerobic digestion encapsulates manure maintaining odors |
| Additives | 3. Application of additional enzymes or chemicals to dilute manure under anaerobic conditions |
| Impermeable cover | 1. Coverage of a manure storage area will control odors from gases<br>2. Impermeable covers can control wind and radiation |
| Permeable covers | 1. Coverage of a manure storage area to control the contact between manure and radiation and wind velocity<br>2. Emission rates are reduced<br>3. Permeable covers create an aerobic zone, encouraging aerobic microorganism growth |
| Aeration | 1. Application of oxygen by mechanical means to maintain aerobic conditions<br>2. Aeration can cause an increase in ammonia emissions |
| Composting | 1. Composting provides an aerobic environment reducing the creation of odors<br>2. A more viable option for those that handle solid manure because of high maintenance required to maintain suitable decomposition conditions |

their hooves on the ground. As a reminder, dust can be used as an agent to transfer odors [100].

#### 1.7.3.2 Manure Treatment and Handling

Another method for reducing odors is to consider the treatment and handling of manure. First, operators can incorporate additives to manure. Additives can be chemical or biological. Additives can be applied to overpower the presence of an odor, reduce the ability for odors to be smelt, absorb constituents in manure that cause odors, or slow microbial degradation to reduce odors [101]. Choices for additives are based on the product and the rate and frequency of application [50]. Manure can also be chemically treated. The University of Arkansas Extension recommends several options for chemical treatment. These include sodium bisulfate (PLT), ferric sulfate granular (Ferric-3), alum, and zeolite [107].

Next, solid separation can be used to better hand manure. Solid separation processes include sedimentation, screening, filtration, and centrifugation. This process attempts to remove constituents that cannot pass through a specified screen size. The removal of these materials decreases biological degradation and thereby reduces

odors [50]. Solid separation also reduces odors by reducing the organic loading. Usually solids are separated before entering a treatment basin such as a lagoon. Some of the materials removed include cattle waste fiber and grit. There are several machines employed for solid separation. These include vibrating screens, sloping stationary screens, or pressure-rolling mechanical separator. Solid separation can occur within a gravity settling basin, earthen settling basin, rectangular metallic, or a concrete settling tank [49].

Finally, operators can make strategic choices in how they apply manure to land for the sole purpose of preventing the spread of odors. Spreading manure can be done in the morning or when the sun is present and on days when the direction of the wind is away from the neighbors [101]. Manure can also be applied during the early evening for better wind dispersion [50]. It is best for the livestock operators to choose the weekdays as opposed to weekends when neighbors will most likely not be at home [107]. When manure is applied, it should be applied quickly, in large quantities, and based solely on the needs of the crop [50]. Operations should employ a liquid waste management schedule [107].

If liquid manure is applied by irrigation equipment, operators can make choices on nozzle size of the sprayers. An alternative would be using a low-rise, low-pressure, trickling system. Application of liquid manure should be done in close range to avoid the spread of odors [50]. Instead of the land application of manure by irrigation, operators can also make the decision to inject manure directly into soils as compared to choosing surface application [107].

When solid manure is not directly applied, operators can select to cover the manure before use. There are two types of covers—impermeable and permeable. Impermeable covers prevent manure storage facilities from the emission of odors into the atmosphere. The covers can also reduce the effects of wind and radiation. Impermeable covers can reduce odors by 90%. Cover efficiency is contingent on the presence of wind and snow [50].

On the other hand, permeable covers (biocovers) are used to cover places for anaerobic digesters or manure storage facilities [50, 107]. Biocovers can consist of straw, cornstalks, peat moss, foam geotextile fabric, or Leka rock [50]. Biocovers can also include use closed-cell polyurethane foam with or without zeolite. Biocovers remove radiation from the surface of the manure storage facility and also reduce the impact of the wind blowing [107]. Biocovers contain an aerobic zone where aerobic microorganisms thrive on the presence of chemical constituents within the manure. These microorganisms reduced the odors. The reduction of odors is contingent upon the material used. Covers that are primarily made of straw reduce odors by 50%, while 85% of odors are reduced when the cover consists of a floating mat or corrugated materials [50].

As an alternative to biocovers, manure storage facilities can be aerated to supply molecular oxygen. This will assist in reducing odors. Nevertheless, aeration can be dangerous because nitrogen is volatilized into the atmosphere as ammonia. Therefore, great care should be taken to prevent this from occurring [50].

#### 1.7.3.3 Waste Treatment Methods

There are many waste treatment methods that can reduce the potential of creating odors. First, operators can install filters to separate odor-causing particles within the air. There are two potential filters available—mechanical and biofilters. Mechanical filtration devices are capable of removing odors from particles. There are indications that 45% of odors are caused by particles with a size between 5 and 10 μm, while 80% are caused by particles greater than 10 μm. Mechanical filtration has been proven to reduce odors between 40 and 70% [50].

Biofilters capture particles where aerobic bacteria degrade them to create products that do not cause odors [50]. Biofilters are supplied air by natural ventilation. The presence of air and adequate environmental conditions allows for the bacteria to grow within the system [99]. Bacteria grow on media consisting of wood chips or compost [107]. For these reasons, biofilters are inexpensive as compared to mechanical filtration. Efficiency of a biofilter is contingent on oxygen concentration, temperature, residence time, and moisture content [50]. The design of biofilters is contingent on the volume of air needed to be treated [107]. Biofilters have been successful in removing 40% of hydrogen sulfide [50]. It has also been reported that biofilters remove 90% of odors [107]. Biofilters are also capable of filtering odor-causing liquids from manure storage [99].

By means of Rockwool packing material, Yasuda et al. (2010) was able to produce 8.2–12.2 mg N/100 g sample of nitrification and 1.42–4.69 mg N/100 g of denitrification [108]. Ro et al. (2008) found that a polyvinyl alcohol (PVA)-powered activated carbon biofilter removed 80% ammonia-nitrogen with hydrogen sulfide removal at 97% [109]. Kastner et al. (2004) found that ammonia-nitrogen concentration ranging between 25 and 95% was removed in waste from swine production, where the major factors that depended on the treatment efficiency were residence inlet time and ammonia concentration [110].

Second, anaerobic digestion is a feasible treatment method to reduce odors. The biological degradation of constituents under anaerobic conditions can reduce the odors significantly in organic material. The products from anaerobic digestion can be safely placed in a liquid storage facility [99]. A study using anaerobic digestion for degradation of dairy waste reported a 50% reduction in odors provided the waste remained in the digester for 20 days. While anaerobic digestion is an expensive method, it can be viable for some operators [50]. Anaerobic digestion can profitable as it produces biogas [99]. More information about anaerobic digestion is presented in Sect. 1.2 of this chapter.

Various enzymes such as peroxidase, specifically horseradish peroxidase (HRP) and trosinate [111], are used to control odors. Horseradish peroxidase (HRP) has become a new method in research for deodorization because of the quantity of peroxidase within the plant, which is capable of transforming aromatic compounds into free radicals or quinones, which ultimately form non-odor compounds [112].

Govere et al. (2007) experimented with pilot-scale reactors with volumes between 20 and 120 L using

minced horseradish comparing effectiveness between the addition of either calcium peroxide or hydrogen peroxide to deodorize swine wastewater. From the results, it was determined that the addition of horseradish was capable of completely removing odors [112].

The management of lagoons serves as a way of reducing odors. A healthy lagoon will degrade organic materials into constituents that do not produce odors. Odors can be reduced in a lagoon if manure contains a dilution of 1–2%. Lagoons should also be refrained from having a high solid concentration. When high solids are present, a lagoon is overloaded. Overloaded lagoons change the conditions from aerobic to anaerobic, thereby creating odors [47, 99].

#### 1.7.3.4 Livestock Operations Management

Livestock operators can mitigate the spread of odors by providing better management of the buildings and facilities. This can include disposing unused or even moldy feed, fix leaks and if necessary replace or repair pipes, and designate a location to dispose dead animals. Another alternative is to increase ventilation within these areas. Ventilation can be supplied by mechanical or natural means. Mechanical methods of ventilation include fans and fresh air inlets. If cost is a barrier, an alternative is to use natural methods. Openings, change in roof slope, and rearranging the orientation of the building are ways that a livestock operator can generate natural ventilation within a building or facility. Despite the fact that it saves energy, natural ventilation may be inhibited by environmental circumstances, so the operator should make a wise decision on which method should be chosen [101].

In addition to ventilation, livestock operators can introduce landscape onto the premises to contain odors. Landscaping provides an opportunity to prevent the constituents that cause odors from further leaving the operation. These constituents are either dispersed or diluted. Landscaping also gives an aesthetic appeal to the area. Trees and shrubs are the two most common entities planted [50].

The design and maintenance of feedlot pens should be reviewed to better prevent odor mitigation. Feedlot pens should maintain a dry surface to prevent the formation of anaerobic conditions on the surface. This means that each pen should be designed to have proper drainage. Having pens maintain a slope between 4 and 6% will provide adequate drainage and prevent pens from accumulating standing water. Also, pen scraping should occur once every 3–4 months [100].

#### 1.7.3.5 Summary

With many people leaving municipalities and inner-ring suburbs for rural and farmland communities, the discussion on odor mitigation will continue to increase. Therefore, it is important for livestock operators to develop good relationships with the residents living in close proximity to livestock operation facilities. Regardless of

the method(s) chosen, the ultimate goal should be to provide neighbors the ability to feel as liberated as possible from the presence of odors.

### *1.7.4 Greenhouse Gas Emissions*

Recent developments have discussed the relationship between greenhouse gas emissions and livestock. This chapter will discuss some of the current issues related to the relationship between greenhouse gas and livestock waste. The purpose of discussion is not to take sides but rather present what is currently found in literature.

Greenhouse gases consist of carbon dioxide ($CO_2$), methane ($CH_4$), and nitrous oxide ($N_2O$). Carbon dioxide is considered a primary greenhouse gas because in general only 9% of greenhouse gas emissions are caused by $CH_4$ and $N_2O$ [113]. However, in the livestock sector, $CH_4$ production is 21 times the carbon dioxide, while $N_2O$ 310 times the $CO_2$ emissions. This is because animals produce methane during the process of enteric fermentation, while nitrous oxides are formed during the degradation of manure when nitrification and denitrification occurs. In general, greenhouse gases maintain the temperature of the Earth to 15 °C. The current debate with greenhouse gases involves global warming and climate change. This debate has been whether or not greenhouse gases cause a change in climate [114]. It was reported that from 2001 to 2010, greenhouse gas emissions from crop and livestock operations increased by 14% [113]. In 2012, it was estimated that the agriculture industry released 526 million metric tons of carbon dioxide equivalent (MMT of $CO_{2e}$) plus 62 MMT of $CO_{2e}$ related to operating electric products [114].

According to the USEPA, greenhouse gases have caused 9% of the total greenhouse gas emissions in the United States, while the United Nations (UN) have stated 18% of global emissions have been caused by greenhouse gases. There are many sources of greenhouse gases reported. The United Nations mentions that greenhouse gas emissions are caused by livestock feeding, manure management, livestock processing, and transportation of livestock products. On the contrary, the USEPA states that greenhouse gases have affected crop and livestock production. Other sources have stated that deforestation (34%) and ruminant digestion (25%) are additional factors that must be considered [114].

According to the University of Missouri Extension's paper titled "Agriculture and Greenhouse Gas emissions," there are four major areas that have been major contributors to greenhouse gases in the agriculture sector—crop and soil management, livestock manure management, enteric fermentation, and agricultural carbon sequestration. These values are contingent on the US production of greenhouse gases in 2012, data produced by the USEPA [114].

1. **Crop and soil management.** Agricultural crop and soil management produced 307 MMT of $CO_{2e}$ or 48% of the total greenhouse gas emissions within the agricultural sector. Ninety-eight percent of all emissions from greenhouse gas were because of $N_2O$. This has been attributed to the fact that cropland has produced

more $N_2O$ than lands that are grasslands. In addition, fertilization, manure application, crop residue collection, nitrogen-fixed crops and forage, and soils with organic materials are major practices that lead to $N_2O$ emissions. $N_2O$ emissions occur in the Corn Belt, cropped land in California and the Mississippi Valley, rice production, and burnt fields.

2. **Livestock manure management.** Manure management accounted for 71 MMT of $CO_{2e}$ in greenhouse gas emissions. Most of the greenhouse gases produced in livestock manure are $CH_4$. The major causes of greenhouse gases include anaerobic decomposition of liquids and slurry. $N_2O$ in manure management is caused by manure, urine, and aerobic and anaerobic degradation. The dairy cattle industry produced 47% of $CO_2$ emissions, while the beef cattle industry was responsible for 71% of $CH_4$.
3. **Enteric fermentation.** As previously stated, enteric fermentation causes the majority of $CH_4$ emissions. Enteric fermentation produced a greenhouse gas total of 141 MMT of $CO_{2e}$. Varying factors determine the production of enteric fermentation. These include the number of livestock and the type of feed.
4. **Agricultural Carbon Sequestration.** Land use and forestry was responsible for 979 MMT of $CO_{2e}$ or 15% of overall greenhouse gas emissions. A relationship between land use and carbon sequestration was made. This relationship analyzed the carbon sequestration of land in 2012 and its state 20 years before. Land that remained grassland was capable of sequestering carbon where loses only occurred because of drought. This has also been the case when land was converted into grasslands. On the contrary, land that remained cropland or converted into cropland carbon was not sequestered. However, land that remained cropland was able to sequester carbon provided the organic content remained between 1 and 6%.

A more recent study was completed by Caro et al. to assess the global greenhouse gas emissions between 1961 and 2010. Analysis compared the livestock greenhouse gas emissions between developing and developed countries. The results from the study concluded that global greenhouse gas emissions increased by 51%, where the primary source of greenhouse gas emissions was caused by enteric fermentation. In general, the generation of greenhouse gases decreased overall. However, there was a difference in the trends for developing and developed countries. Greenhouse gas emissions in developed countries increased in the 1970s and then gradually decreased by 23%. On the contrary, greenhouse gas emissions increased in developing countries by 117%. The authors attributed increase to changes in economic and ideological changes. The signature year for these changes occurred in 1989. These countries transitioned from being focusing heavy on importing to exporting. With regard to the various livestock industries, the beef cattle industry was accountable for 54% of greenhouse gases, while only 17% was due to the dairy industry [113].

The development of numbers has created an interesting stir within the scientific community. Various authors have published papers that attempt to support the values generated by entities recognizing global climate change (e.g. USEPA, UN, and the International Panel on Climate Change (IPCC)). However, authors such as

Herrero et al. request for a reduction in ambiguity and more consistency in methodologies used to quantify greenhouse gas emissions within livestock. The areas of concern includes the exclusion of $CO_2$ production by livestock, quantifying emissions due to land use and land change, global warming potential of methane, and the overall allocation of processes to livestock. With a more accurate picture, the authors state that the discussion of greenhouse gas emissions in livestock can improve [115]. Regardless of an individual's stance on greenhouse gas emissions and global warning, the discussion of the livestock industry's role in greenhouse gas emission will continue.

## 1.8 Pathogens in Livestock Industries

Pathogens are an issue within the livestock industry. The impact from pathogenic outbreak cause a loss in productivity for the livestock operation by becoming detrimental to the animals, the business, and employees. Pathogens can also be harmful to the public and the environment. Survival of pathogens is predicated on the temperatures, the pH, the amount of microbial activity, the routes of transfer, and the applicable host. The routes of transfer for pathogens include fecal-to-oral, foodborne, aerosol, or human-to-animal contact. The applicable hosts can range from humans, farm animals, and other carriers such as flies. There are four major categories of pathogens—viruses, bacteria, mycotic agents, and parasites [62].

For example, contact with viruses for a period of time can lead to illness or death and can limit the product from livestock. Viruses are classified as enveloped and non-enveloped viruses. Enveloped viruses persist within animal manure and can stay for a long period of time without treatment and storage, while non-enveloped viruses are incapable of being destroyed with any treatment method. On the other hand, mycotic agents are not a major concern within the livestock industry and are usually dangerous in soils or self-contained with the body of an animal or human [62]. Examples of each pathogen category are listed in Table 1.19.

Livestock operators can know the quantity of pathogens within its waste by using organisms known as fecal indicator organisms. Fecal indicator organisms are surrogate organisms used in the laboratory as a method for quantifying pathogenic presence. Typically, *E. coli* has been used as a fecal indicator organism, but recent studies have used other organisms such as coliphages and *C. perfringens* spores. An adequate choice for a fecal indicator organism must fulfill a series of criteria. Fecal indicator organisms must:

1. Exist in the same conditions as pathogen.
2. Have a life span similar to pathogens.
3. Withstand disinfectants and unfavorable conditions.
4. Be easily detectable.
5. Be distributed randomly.
6. Portray similar risks in humans as pathogens.

**Table 1.19** Examples of each type of pathogen [122]

| Pathogen | Example |
|---|---|
| Viruses | Animal enteroviruses, rotaviruses, hepatitis E |
| Bacteria | *Aeromonas hydrophila*<br>*Aerobacter*<br>*Bacillus anthracis*<br>*Chlamydia*<br>*E. coli*<br>*Salmonella* |
| Mycotic agent | *Histoplasmosis capsulatum*<br>*Pneumocystis carinii* |
| Parasites | Protozoa<br>*Ascaris and Ascariasis*<br>*Cryptosporidium parvum*<br>*Giardia*<br>*Toxoplasmosis* |

**Table 1.20** Pathogen treatment methods [122]

| Pathogen | Method |
|---|---|
| Dry techniques | Composting |
| Physical treatment | Sand filtration or dry beds<br>Sedimentation and screening |
| Biological treatment | Lagoon<br>Anaerobic digestion<br>Sequencing batch reactor<br>Constructed wetlands<br>Overland flow |
| Disinfection | Chlorine<br>Ozone<br>Chlorine dioxide<br>Lime stabilization<br>Pasteurization |

As an alternative, testing for microorganisms can include culture-specific microorganisms, antibiotic resistance patterns, molecular fingerprinting, genotype, and chemical indicators [62].

There are various treatment methods that can be used to reduce the pathogens within livestock waste. The treatment of livestock waste can use dry techniques, physical treatment, biological treatment, and chemical treatment. Examples of treatment techniques found within each category are shown in Table 1.20. Many of these methods have been discussed in grave detail in the previous sections [62].

The presence of pathogens can have a major impact on livestock operations. While this section is not extensive, it does attempt to provide a summary of major pathogen categories, their associated impacts, and the potential treatment methods.

## 1.9 Livestock Waste Management Computer Software

Within the recent century transition, there has been the presence of computer modeling tools that are capable of being used to predict livestock wastes. For example, the Animal Waste Management Software Tool (AWM) is a computer program designed to determine parameters such as waste storage facilities, waste treatment lagoons, and utilization [2]. Other options include the collaboration between the University of South Carolina's Earth Science and Resource Institute and the Natural Resources Conservation Service (NCRS) in South Carolina to develop a suite of products that include the geospatial tools [ArcGIS] and a nutrient management planning software AFOPro© [116].

Ideas on the use of software for livestock waste management have not been limited to just the United States. A program known as Integrated Swine Manure Management (ISMM) is an integrated decision support system (DSSs) used by Canadian province decision-makers to control manure, considering various criteria such as environmental, agronomic, social and health, greenhouse gas emission, and economic factors [117]. The introduction of computer software for livestock management can be very significant for those that are planning to provide a consistent method of managing livestock. Nevertheless, it is still important to remember that computer software is a "tool" but does not replace proper education and understanding of what is needed for proper livestock waste within the given area.

## 1.10 Recent Advances in Livestock Waste Treatment and Management

### *1.10.1 Latest Technology Development, Market-Driven Strategies, and US Policy Changes*

In the United States, the major hurdles to reducing the impact of livestock waste pollution on the nation's watersheds are outdated American wastewater treatment policies. Under the prevailing US legislation, the 1972 Clean Water Act (CWA), the majority of wastewater treatment efforts have targeted "point sources of water pollution" with a measurable wastewater discharge. The CWA defines point sources as discharge pipes from industrial plants, utilities, or municipal sewage treatment facilities. Many new environmental process technologies, such as improved chemical coagulation/precipitation, clarification (dissolved air flotation and improved settlers), filtration, membrane bioreactor, advanced oxidation processes, etc., have been developed for water pollution control [118–130], but have not been seriously considered for agricultural waste treatment.

Agricultural wastes, such as livestock manure, farm's storm runoff water, etc., are considered the non-point sources of water pollution, and are not subject to CWA regulations. In the nearly one half of a century since the passage of the CWA, the

American agricultural industry has grown considerably. More than 70% of today's livestock production takes place not on small-scale family farms but on large-scale Concentrated Animal Feeding Operations (CAFO) facilities. However, CAFOs still use small-farm strategies for disposing of animal waste, and about half the crops in the United States are fertilized this way. An ineffective waste strategy, coupled with little meaningful regulation, poses a major hurdle for the rehabilitation of US watersheds. The agricultural water pollution problem must be dealt with its original source. It is the opinion of Director Craig Scott of Bion Environmental Technologies that spending billions of dollars to upgrade downstream wastewater treatment plants and to construct large-scale stormwater projects that recollect and treat the nutrients after they have been released to contaminate the environment is not an acceptable solution from either a cost or a common sense perspective [127–130]. The new market-driven strategies are treating the CAFO wastes with the best available technologies (BAT) and still considering both technical and economical feasibilities.

There are clear signs that the US Federal Government will provide funding for nutrient control and climate control strategies and private sector solutions. In December 2018, the USEPA and the US Department of Agriculture (USDA) notified state and tribal regulators that they are committed to working with all stakeholders to adopt market-based approaches in the fight to clean up America's watersheds and prevent livestock waste from further contributing to the crisis. The agencies said this commitment could include technical and financial support for water quality credit trading programs and public-private partnerships [127–130].

In January 2019, former US President Donald Trump signed bipartisan legislation for federal funding to combat toxic algae blooms in the country's water resources. In February 2019, the USEPA issued a memorandum updating its water quality trading policy and supporting market-based approaches to reduce nutrient pollution in the nation's waterways. The announcement stated "USEPA efforts seek to modernize the agency's water quality trading policies to leverage emerging technologies and facilitate broader adoption of market-based programs."

There is further proof that under the leadership of US President Joseph R. Biden, the US federal policymakers are serious about building the nation's infrastructure (including water and waste treatment). Controlling global warming, climate change, and greenhouse gases are all on the horizon.

### *1.10.2 Livestock Water Recycling (LWR) System*

Livestock Water Recycling (LWR) is one of the world's leading providers of manure treatment technology aiming at reducing greenhouse gas emissions, concentrating and segregating nutrients for strategic fertilizer application, and recycling clean, reusable water.

The LWR system is a proven and fully operating technology that reduces the overall volume of manure, concentrates nutrients, and delivers a renewable, high-quality water source. According to the manufacturer, the company's vision has

always been to help livestock farmers increase farm efficiencies while becoming even more environmentally sustainable, and its LWR system provides a minimum of 20–30% return on investment [118].

The LWR company is focused on developing scalable solutions that can be applied quickly and commercialized for maximum return on investment.

LWR system is a patented process technology that uses both mechanical and chemical treatments to remove manure contaminants and segregate valuable fertilizer nutrients at large livestock operations. Figures 1.23 and 1.24 show the LWR system's process flow diagram and process equipment, respectively [118]. As the livestock manure effluent flows through the LWR process, solids are sequentially removed by chemical precipitation, clarification, conventional filtration, and membrane filtration. The result is valuable segregated fertilizer nutrients and clean water that can be reused around the barns.

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The detailed process, descriptions, principles, design criteria, operational procedures, terminologies, etc. of each unit process (chemical precipitation, clarification, conventional filtration, membrane filtration, etc.) can be found in the literature [119–126]. Either sedimentation or flotation can be used for clarification [119, 122].

The nutrient and water recovery capacity of the LWR system is so far the highest on the market. LWR system extracts up to 75% of the water from livestock manure while concentrating dry solids (8%) and segregating nutrients for recycling (17%). By concentrating and segregating, the farm plant managers are given more control over their nutrient application, which minimizes their farm's field work. The result is clean, potable water, dry solids that are rich in both phosphorus and organic nitrogen and a concentrated stable ammonium and potassium liquid. At present, LWR system has the highest nutrient and water recovery capacity on the market, lowest

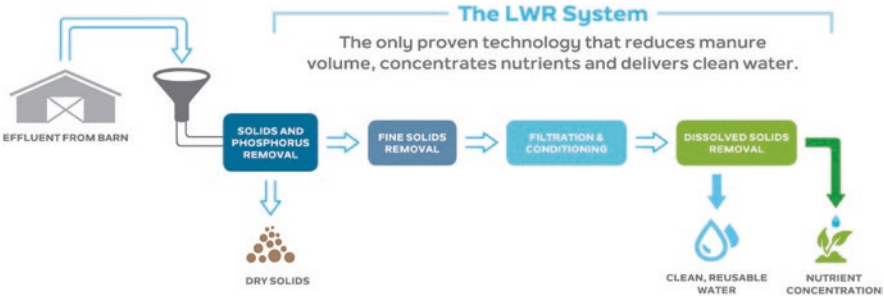


**Fig. 1.23** Flow diagram of a Livestock Water Recycling (LWR) system [118]

**Fig. 1.24** Process equipment of a Livestock Water Recycling (LWR) system [118]

electrical consumption on the market, and highest number of installations in the water and nutrient recovery market, which are all incredible.

### *1.10.3 BET Advanced Technologies To Benefit From Policy Changes*

There are a few commercial-ready technologies available today that can address the problem of excess nutrient runoff from large-scale agricultural operations. Section 1.10.2 has introduced the Livestock Water Recycling (LWR) system, which is now commercially available for livestock waste treatment.

Another advanced technology in the sector is Bion Environmental Technologies' comprehensive environmental management system, which is also designed for the largest CAFO livestock facilities and focused on maximizing resource recovery.

Bion Environmental Technologies' patented 2G (second-generation) technology has been commercially proven to substantially reduce pathogens from livestock waste while eliminating up to 90% of greenhouse gases and ammonia emissions and 95% of nitrogen and phosphorus. The waste management system harnesses the power of naturally occurring bacteria to convert nitrogen and phosphorus into solid forms that are removable by other processes [127–130]. Figure 1.25 shows the flow diagram of Bion Environmental Technologies' comprehensive environmental management system.

Livestock waste treatment technology not only provides clean water solutions but also creates new sources of revenue, including the production of value-added products such as fertilizers. Bion's patented 3G technology recovers stable concentrated ammonium bicarbonate, a quick-release nitrogen fertilizer, from livestock waste without the use of chemicals. This product is well suited for a wide range of applications in the organic markets. According to Markets and Markets researchers, the market for global organic fertilizers is expected to grow from US$6.3 billion in 2017 to US$11.15 billion by 2022.

In 2019, Bion plans to apply to the USDA's Organic Materials Review Institute for use of its ammonium bicarbonate product in organic food production. The company has already applied for a Patent Cooperation Treaty for international recognition of its ammonium bicarbonate production process.

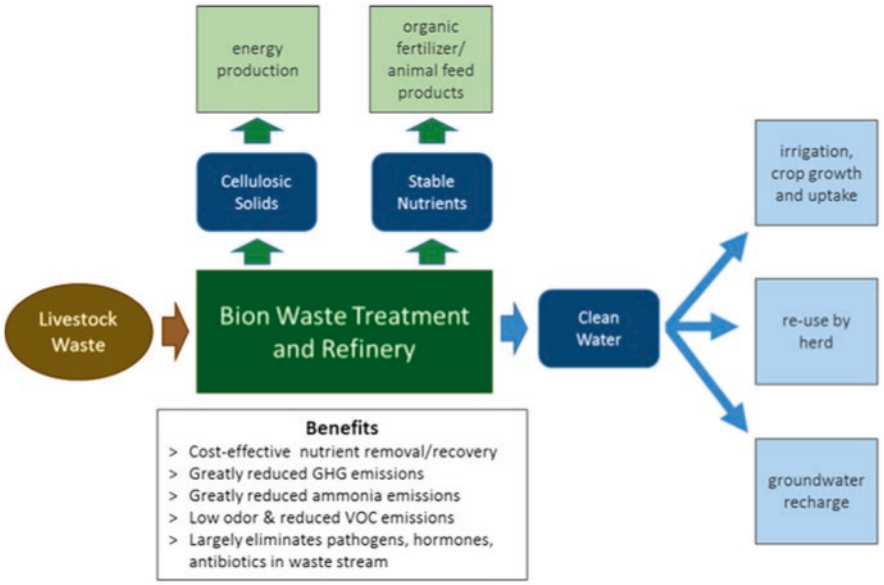


**Fig. 1.25** Flow diagram of Bion's livestock waste treatment technology

## 1.11 Conclusion

This chapter provides a plethora of information concerning livestock waste management from treatment, handling, and storage. While this not an all-encompassing manual for all given conditions, it can be used as a catalyst for research and exploration in how to properly maintain and manage livestock waste for a given industry. The readers are referred to the literature [131–135] for additional technical information on treating the livestock's biosolids, concentrated liquid waste stream, or diluted liquid waste stream.

## Glossary of Livestock Waste Management

**Anaerobic digestion** is the fermentation of organic waste by hydrolytic microorganisms into fatty acid chains, carbon dioxide ($CO_2$), and hydrogen ($H_2$). Short fatty acids are then converted into acetic acid ($CH_3COOH$), $H_2$, $CO_2$, and microorganisms.

**Biogas** is a product from anaerobic digestion containing gases such as methane ($CH_4$), $CO_2$, and trace elements. Biogas can be used as a source of energy.

**Chemical oxygen demand (COD)** is a wastewater quality index that determines the amount of oxygen consumed by wastes.

**Concentration animal feeding operations (CAFO)** raises livestock within a restricted space. It is also known as feedlot.

**Constructed wetland** is a treatment method that uses plants (most commonly water hyacinth and duckweed) to degrade organic material.

**Denitrification** converts nitrate into atmospheric nitrogen using microorganisms known as dentrifiers.

**Eutrophication** is the condition of a water body (particularly a lake) where molecular oxygen levels have been depleted. Eutrophication most commonly occurs when nutrient levels are high within the water body, forming the presence of algal blooms. When eutrophication occurs, all organisms rely on molecular oxygen to survive.

**Five-day biochemical oxygen demand ($BOD_5$)** is a wastewater quality index that determines the amount of oxygen required for microorganisms to degrade a given substance within a 5-day period.

**Lagoon** is a basin that treats wastewater and stores waste. There are three major types of lagoons—anaerobic, aerobic, and facultative.

**Liquid manure** contains dry matter less than 5%.

**Mesophilic** is a state in an anaerobic digester or composting when the temperature remains between 35 and 40 °C.

**National Pollution Discharge Elimination System (NDPES)** regulates the quantity of waste entering navigable waters and point sources. It was first introduced by the USEPA in the Clean Water Act of 1977. Livestock waste operations are required to have NPDES permits to discharge. State legislation defines the operations that require NPDES permit.

**Nitrification** is the process of converting ammonium nitrogen ($NH_4^+$) into nitrate ($NO_3^{2-}$) with an intermediate step of producing nitrite ($NO_2^-$). Nitrification is converted by nitrogen-fixing bacteria (nitrifiers).

**Psychrophilic** is a state in an anaerobic digester or composting when the temperature remains below 20 °C.

**Semisolid manure** contains 5–10% dry matter.

**Solid manure** contains dry matter greater than 15%.

**Thermophilic** is a state in an anaerobic digester or composting when the temperature remains between 51 and 57 °C.

**Volatilization** is a phase change process that converts constituents into gaseous form. The most common volatilization experienced is ammonia volatilization or the conversion of ammonium-nitrogen to ammonia-nitrogen. This is problematic for livestock operations because plants' nitrogen is lost for plant uptake.

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# Chapter 2
# Waste Treatment in the Pharmaceutical Biotechnology Industry Using Green Environmental Technologies

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## 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 |
| Wi | Mole fraction of organic compound i in the wastewater phase |

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## 2.1 Introduction to Biotechnology

### *2.1.1 Pharmaceutical Industry and Biotechnology Terminologies*

Pharmaceutical industry is an industry responsible for manufacturing of drugs, vaccines, antibiotics, etc. using chemical reactors, biological systems, or organisms. The chemical synthesis-based pharmaceutical industry is a part of chemical industry using chemical technology and chemical reactors, while the fermentation process-based pharmaceutical industry is a part of biotechnology industry using biological systems or organisms in biochemical reactors.

Biotechnology is an engineering science field involving the use of biological systems found in organisms or the use of the living organisms themselves to make scientific advances and adapt those knowledge to various application branches, such as medical biotechnology, agricultural biotechnology, industrial biotechnology, environmental biotechnology, computational biotechnology, and military biotechnology.

Medical biotechnology (including pharmaceutical biotechnology) involves the use of living cells and other cell materials to find cures for preventing diseases and bettering the health of humans; development of vaccines and antibiotics is a typical example. Specific pharmaceutical biotechnology related to medicine and veterinary products (vaccines, antibiotics, molecular diagnostics techniques, genetic engineering techniques, etc.) is also termed red biotechnology.

Agricultural biotechnology focuses on developing genetically modified plants to increase crop yields or introduce characteristics to those plants that provide them with an advantage growing in regions that place some kind of stress factor on the plant, namely, weather and pests. Development of pest-resistant crops and improvement of plant and animal breeding are typical examples. Green biotechnology refers to specific agricultural biotechnology that creates new plant varieties of agricultural interest, biopesticides, biofertilizers, etc. This area of agricultural biotechnology is based on transgenics (genetic modification), i.e., an extra gene or genes inserted into their DNA. The additional gene may come from the same species or a different species.

Industrial biotechnology (including industrial fermentation biotechnology) involves the utilization of cells, such as microorganisms, or components of cells, like enzymes, to generate products in sectors that are industrially useful, such as food and feed, chemicals, detergents, paper and pulp, textiles, biofuels, and biogas, or to create genetically modified organisms (GMOs) that enhance the diversity of applications and the economic viability of industrial biotechnology. Development of biocatalysts (such as enzymes, to synthesize chemicals), improvement of fermentation process, and production of new plastics/textiles, biofuels, etc. are typical examples. Specific industrial biotechnology related to production of wine, cheese, and beer by fermentation is also termed yellow biotechnology. Designing more energy-efficient, less polluting, and low resource-consuming processes and products that can beat traditional ones is also termed white biotechnology.

Environmental biotechnology is an interdisciplinary branch of biotechnology using biological systems and/or organisms for conservation of environment, resources, and energy and for protection of humans, animals, and plants on Earth and beyond. It can be of green biotechnology, gray biotechnology, blue biotechnology, gold biotechnology, or white biotechnology, depending on the applications or achievement goals. Modern green environmental biotechnology has a symbol of "green cross" that involves the construction of resource recovery facilities (RRF), bioreactor landfills, in-vessel or in-bin composting reactors, bioremediation sites, wildlife sanctuary areas, environmental protection parks, global warming control facilities, salmon ladders, etc. using the best available technologies (BAT) for reclamation of water, air, land, nutrients, methane gas, animals, plants, etc. and production of biofuels, bioplastics, waste-converted animal foods, etc. in turn, achieving environmental conservation, resource sustainability, biodiversity, climate control, ozone layer protection, etc. Gray biotechnology refers to an old traditional environmental biotechnology applications to maintain biodiversity and the partial removal of certain pollutants or contaminants using microorganisms and plants to isolate and dispose of many kinds of substances such as heavy metals and hydrocarbons, but without sustainability of natural resources. Typical examples are the old biological secondary wastewater treatment plants (WWTP) and old sanitary landfills. Modern environmental biotechnology is considered to be a green biotechnology. Blue biotechnology is based on the use of marine resources to produce goods, generate energy, or reduce pollution.

Computational biotechnology can be defined as "conceptualizing biotechnology" to address biotechnology problems using computational techniques and makes the rapid organization as well as analysis of biotechnological data possible. It can also be termed gold biotechnology or bioinformatics.

Military biotechnology is also termed dark biotechnology because it is associated with bioterrorism or biological weapons and bio-warfare using microorganisms and toxins to cause diseases and death in humans, domestic animals, and crops.

Biotechnology itself is an academic field of engineering science, while any other academic field dealing with the law and ethical and philosophical issues around the engineering science biotechnology is liberal art biotechnology or violet biotechnology. This publication emphasizes environmental biotechnology to be applied to environmental control of medical (pharmaceutical) and industrial biotechnology industry.

### *2.1.2 Historical Development of Biotechnology Industry*

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 century-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 2000 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 2.1 shows the distribution of research activities and biotechnology firms in the USA.

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

**Table 2.1** 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 |

**Table 2.2** 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 |
| Specially 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 |

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 2.2 and 2.3 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 [5–108].

**Table 2.3** 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 |

### *2.1.3 Core Technologies*

The core technique of biotechnology is elegant in its simplicity. The cell is a miniature factory, containing a 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.

### *2.1.4 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 the following [1]:

1. *Cytokines.* Hormone-like proteins that stimulate the growth or regulate the function of various cell types. They include such agents as erythropoietin, which stimulates the production of red blood cells and can be used to treat severe ane-

mia associated with renal disease, and 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, which help regulate and target the body's immune response and can be used to treat certain cancers and selected viral infections.
2. *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.
3. *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.
4. *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.
5. *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.
6. *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.

### *2.1.5 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]:

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.
2. *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.
3. *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.

### *2.1.6 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.

### *2.1.7 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 the following [1]:

1. *Diagnostics.* Tests that use biotechnology materials to detect the presence or risk of disease or pollution of a cell or material.
2. *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.
3. *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.

### 2.1.8 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 freshwater 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.

### 2.1.9 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 (USEPA'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, the USEPA's responsibilities for overseeing the cleanup of polluted sites give it the power to create important new markets for the industry.

The USEPA'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 the use of bioremediation technologies. The USEPA 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.

## 2.2 General Industrial Description and Classification

### *2.2.1 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 US Department of Commerce (US 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 USEPA 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 USEPA'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 USEPA, the following sections present those SIC codes for the pharmaceutical manufacturing quoted by the USEPA [11–15].

### 2.2.2 *Biotechnology Industry's Pharmaceutical SIC Subcategory Under the USEPA's Guidelines*

According to the USEPA'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 US 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 1987 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 [15–108].

## 2.3 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 to better understand how to control and treat the manufacturing wastes. The five common processes used in the manufacture of pharmaceutical products are as follows:

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 USEPA'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 USEPA as the subcategories of pharmaceutical manufacturing and will be used throughout this chapter, instead of using the SIC subcategories.

The USEPA [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.

### 2.3.1 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 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 2.1 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.

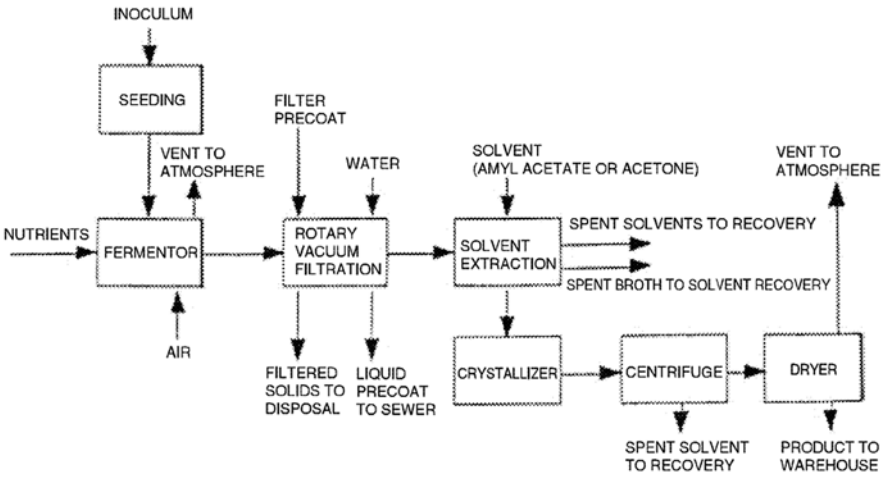


**Fig. 2.1** Fermentation process diagram [16]

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, 1,1-dichloroethylene, and 1,2-transdichloroethylene [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 and 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]:

1. Group A: spent fermentation mash
2. Group B: wastes containing acids, bases, and solvents (used in the purification of the product)
3. Group C: condensate from barometric condensers in evaporation and drying
4. 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 4000–13,000 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 2500 mg/L, and for aureomycin, it is in the range of 4000–7000 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.

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.

Group C waste consists of condensates from barometric condensers which are only slightly polluted. Those wastes from the manufacturer of aureomycin, however, have a 5-day BOD of 60–120 mg/L.

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 1500 mg/L. But in penicillin production, the washing wastewater contains alkaline, due to the use of basic substances for removing unwanted matter from equipment tanks and fermentors.

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 wastewater (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 washwater 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-metalbearing 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.

### *2.3.2 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. The material is transported in portable containers through the plant in batches of 75–100 gallons (283.9–378.SL). 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.

### 2.3.3 *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 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 2.2 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 3000-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 (3028-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.

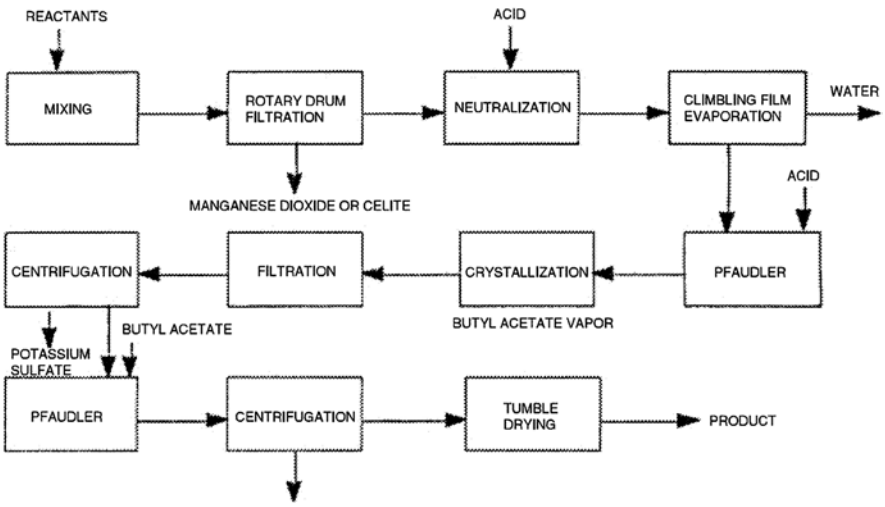


**Fig. 2.2** Process flow diagram of chemical synthesis for an anti-convulsive drug plant [16]

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 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.

### *2.3.4 Formulation, Mixing, and 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 and 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, with near neutral pH (6.0–8.0).

Air emissions may result from the use of volatile solvents in the formulation processes.

### *2.3.5 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 (1514 L) of acetonitrile and 990 gal (3747 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 (1703 L). In addition, a large quantity of sulfuric acid is used in glassware washing at an annual acid consumption of approximately 1080 gal (4088 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 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 2.1 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.

## 2.4 Waste Characterization and Options for Waste Disposal

### *2.4.1 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 1500 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, 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 2.2 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 2.3 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.

### *2.4.2 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, or 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 2.4.

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

**Table 2.4** 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 (liber, 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 | Miters, tubing, diatomaceous earth |
| Used chemical reagents | R & D operations | Miscellaneous chemicals |
| Natural gas combustion products | Steam boilers | Carbon compounds, oxides of nitrogen and sulfur |

discharge. Lane [25] 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 and (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 "puddling" will result in odors that will most likely render the entire operation a public nuisance.

## 2.5 Environmental Regulations on Pharmaceutical Wastewater Discharges

Wastes generated from pharmaceutical manufacturing could exert various impacts on the environment, such as the following:

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 pollution load of wastewaters generated by the pharmaceutical manufacturing industry in the USA was reported by the USEPA [13] as shown in Table 2.5.

### *2.5.1 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 Pollutant Discharge Elimination System (NPDES) permit and/or a State Pollutant Discharge Elimination System (SPDES) permit. The NPDES permit or the SPDES permit is granted on a case-by-case basis.

The USEPA [11, 12, 15] regulation applies to facilities organized into five subcategories for this pharmaceutical industry (40 CFR Part 439): (a) subcategory A (fermentation products), (b) subcategory B (extraction products), (c) subcategory C (chemical synthesis products), (d) subcategory D (mixing/compounding and formulation), and (e) subcategory E (research).

The USEPA has regulated what is known as the Best Practicable Control Technology Currently Available (BPT). The direct discharge limitations are presented in Table 2.6.

The regulation for cyanide is the same in the Best Available Control 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 USEPA tends to deregulate the effluent discharge from R & D, because only an

**Table 2.5** 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 | 1668 | 3452 | 1023 | 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 MGD = 3.784 $m^3$/day)

**Table 2.6** Organic priority pollutants from pharmaceutical manufacturing

| Organic compounds | Concentration (μg/L) | |
|---|---|---|
| | 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 | 0–17,000 |
| 4. Halogenatcd 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 |
| 1,1,2,2-Tetrachloroethane | 2 | 0–10 |
| Chloroform | 300 | 0–1600 |
| 1,1-dichloroethylene | 8.9 | 0–95 |
| Methylene chloride | 2600 | 0–20,000 |
| 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 | 0–360 |
| Di-n-butyl phthalate | 10 | 0–90 |
| Diethyl phthalate | 8 | 0–31 |

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 level or they are infrequent for occurrence, or their presence amount is too small to be effectively reduced by the current technology.

#### 2.5.1.1 Best Practicable Control Technology Currently Available (BPT)

The USEPA is revising the BPT effluent limitation guidelines for chemical oxygen demand (COD) for subcategories A, B, C, and D. Appendix 1 presents these final limitations, which are based on the application of advanced biological treatment. The existing BPT effluent limitation guidelines for pH, $BOD_5$, and TSS are being maintained for all subcategories. The existing BPT effluent limitation guidelines for cyanide are being refined; the compliance monitoring requirements for these limitations have been clarified. Limitations on cyanide for B and D subcategories are being withdrawn.

#### 2.5.1.2 Best Available Control Technology Economically Achievable (BAT)

The EPA is revising the BAT effluent limitation guidelines for subcategories A and C. For subcategories A and C, the EPA is adding BAT effluent limitations for ammonia as nitrogen (N), COD, and 30 priority and nonconventional organic pollutants. For subcategories B and D, the EPA is setting a BAT effluent limitation for COD that is equivalent to the BPT limitation. No additional BAT effluent limitations are being set for subcategories B and D. However, EPA is withdrawing the current BAT effluent limitations for cyanide for subcategories B and D. Appendixes 2 and 3 present these final effluent limitation guidelines, which are based on the following: end-of-pipe advanced biological treatment with nitrification for subcategories A and C and end-of-pipe advanced biological treatment for subcategories B and D.

#### 2.5.1.3 New Source Performance Standards (NSPS)

The USEPA is setting NSPS for priority and nonconventional pollutants for subcategories A and C. The NSPS for subcategories A and C include ammonia (as N) and 30 priority and nonconventional organic pollutants, based on advanced biological treatment with nitrification.

The USEPA is also revising the NSPS controlling discharges of $BOD_5$, COD, and TSS for subcategories A, B, C, and D based on advanced biological treatment. The USEPA is withdrawing cyanide standards for subcategories B and D. Final NSPS for subcategories A and C are presented in Appendix 4. Final NSPS for subcategories B and D are presented in Appendix 5.

### 2.5.2 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 USEPA 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 2.7.

The PSES and PSNS regulate an indirect discharge limitation for cyanide.

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.

#### 2.5.2.1 Pretreatment Standards for Existing Sources (PSES)

The USEPA is revising PSES for priority and nonconventional pollutants for subcategories A, B, C, and D. For subcategories A and C, the USEPA is setting PSES for ammonia (as N) and 23 priority and nonconventional organic pollutants based on steam stripping. For subcategories B and D, the USEPA is setting PSES for five priority and nonconventional organic pollutants based on steam stripping. Revised PSES for subcategories A, B, C, and D are presented in Appendixes 6 and 7.

**Table 2.7** 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 MGD = 3.784 $m^3$/day)

#### 2.5.2.2 Pretreatment Standards for New Sources (PSNS)

The USEPA is revising PSNS for priority and nonconventional pollutants for subcategories A, B, C, and D equal to PSES. Revised PSNS for subcategories A, B, C, and D are presented in Appendixes 8 and 9.

### *2.5.3 Historical View on Regulations*

To protect the environment, the USEPA has regulated the BPT, which is basically identical to those shown in Table 2.6. As mentioned earlier, the wastewaters from fermentation and 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 2600 to 5200 mg/L to meet 1976 BPT [26]. In November 1982, the USEPA 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 USEPA-proposed technology. In 1983 and modified in 1998, the USEPA 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 2.6 are basically the 1976 version and finalized in 1983. However, the USEPA 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, the USEPA 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, the 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].

### 2.5.4 Regulations for Managing Pharmaceutical Wastes

*Managing Pharmaceutical Waste A 10-Step Blueprint for Healthcare Facilities In the United States* [108] was published by the USEPA in 2008, and therefore, does not cover the most recent federal and state regulations for hazardous waste pharmaceuticals. On February 22, 2019, the USEPA published *Management Standards for Hazardous Waste Pharmaceuticals and Amendment to the P075 Listing for Nicotine* (referred to as the "Final Rule") [109]. The Final Rule became effective in the US federally managed states and territories on August 21, 2019. All states in the USA are expected to adopt the Final Rule by the deadline of July 1, 2022. One aspect of this rule, prohibiting the disposal of hazardous waste pharmaceuticals into sewers, took effect in all states and territories on August 21, 2019, under the Hazardous and Solid Waste Amendments (HSWA) regulations.

In 2021, the USEPA work was initiated to update the 10-Step Blueprint to reflect the 2019 regulatory changes. It is anticipated that this work will be completed by March 2022 and the new document will be available on the Healthcare Environmental Resource Center (HERC). Although the 2008 document is not current with regard to federal regulation of hazardous waste pharmaceuticals, it does contain valuable non-regulatory waste management information that remains valid today.

A "10-Step Blueprint for Managing Pharmaceutical Waste of Healthcare Facilities In the United States" [108, 109] is introduced in this section. The steps in this blueprint do not necessarily have to be taken consecutively. Some steps will occur in parallel, and other steps will probably be referenced throughout the development of your pharmaceutical waste management program. The following is a summary of the ten steps and how each can be used to develop and implement your pharmaceutical waste management program:

1. Step l begins with some action items that you can begin immediately.
2. Step 2 is an overview of how the federal Resource Conservation and Recovery Act (RCRA) regulations apply to pharmaceutical waste management.
3. Step 3 begins where the regulations leave off providing guidance on how to manage non-regulated hazardous pharmaceutical waste.
4. Step 4 walks you through the steps necessary to perform a drug inventory review. This step can be very tedious and time consuming.
5. Step 5 alerts you to waste minimization opportunities. It will be helpful to become familiar with the waste minimization opportunities before assessing your current practices based on the guidance provided in Step 6. Review these opportunities again upon completion of the department reviews.
6. Step 6 discusses performing department reviews and determining your generator status.
7. Step 7, taking on the communication/labeling challenge, is one of the most critical aspects of implementing a pharmaceutical waste management program and possibly the most challenging. How you decide to communicate pharmaceutical disposition information to the people handling the waste will depend

and be dependent upon which of the management options presented in Step 8 you select.
8. Step 8, considering the management options, introduces you to five implementation models that have worked for other hospitals. You may choose one model or a hybrid.
9. Step 9, getting ready for implementation, assists you with vendor selection, satellite and storage accumulation, and pilot program development.
10. Step 10, launching the program, is the culmination of the first nine steps, plus the actual rollout to the entire facility.

After the program is launched, the next steps will be (a) providing additional pharmaceutical waste management assistance to hospitals; (b) clarifying, reconsidering, and expanding RCRA hazardous waste regulations, (c) eliminating drain disposal; (d) making the hazardous waste determination, a communications challenge; (e) broadening national knowledge base of pharmaceutical waste generation; (f) managing waste minimization; and (g) managing routinely wasted drugs.

## 2.6 Waste Management

### *2.6.1 Strategy of Waste Management*

The main objectives of pharmaceutical waste management are to reduce waste generation through improved manufacturing process and enhanced solvent recovery; to 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 USEPA 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, inplant 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.

## *2.6.2 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.

### 2.6.2.1 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 savings of US $15,000 per year in solvent makeup 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 US Food and Drug Administration (USFDA) 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.

#### 2.6.2.2 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 wash-up area, providing better prevention of dumping of hazardous pollutants to the sewer system.

#### 2.6.2.3 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 restricted 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.

#### 2.6.2.4 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. Recycling and reusing renovated wastewater is recommended. It has been estimated that about 1–100 tons (0.9072–90.72 metric tons) of water are used per ton of product. By modifying processing procedures or auxiliary equipment, water usage and wastewater generation may be significantly reduced [21]. Examples are the 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.

#### 2.6.2.5 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 non-polluted 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.

#### 2.6.2.6 Good Operating Practices

Good operating practices, which can help reduce waste generation, material losses, and production cost, include closer supervision, production scheduling, material 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.

#### 2.6.2.7 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 Abbott Laboratories in North Chicago, IL, and at Eli Lilly and 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 fermented 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 preventing secondary pollutant from dust.

#### 2.6.2.8 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. The USEPA [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, alkali salts and other inorganic chemicals, organic chemicals, metal sludge, 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.

### *2.6.3 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.

### 2.6.3.1 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].

#### 2.6.3.1.1 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:

$$Cl_2 + NaCN + 2NaOH = NaOCN + 2NaCl + H_2O \tag{2.1}$$

$$3Cl_2 + 6NaOH + 2NaOCN = 2NaHCO_3 + N_2 + 6NaCl + 2H_2O \tag{2.2}$$

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 (thousand fold reduction) of the waste. Figure 2.3 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

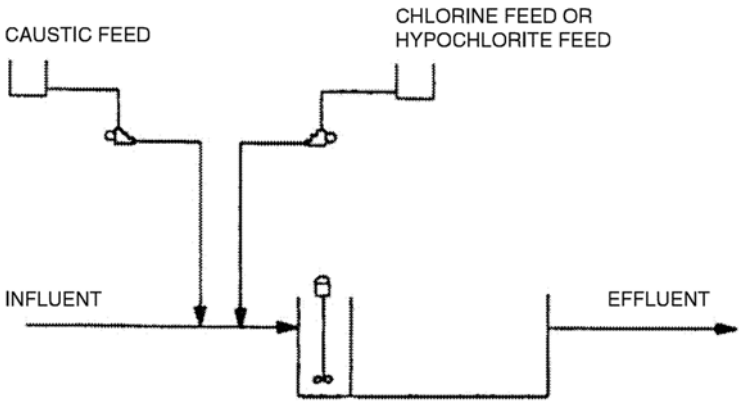


**Fig. 2.3** Chlorination process for a cyanide destruction system [13]

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 chlorine, nearly 3–4 parts of chlorine are needed for oxidizing 1 part cyanide to cyanate, and 8 parts of chlorine are 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, 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.

#### 2.6.3.1.2 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 the following:

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.

#### 2.6.3.1.3 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–ll0 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.

### 2.6.3.2 Metal Removal

Although the USEPA does not promulgate effluent guideline 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.

#### 2.6.3.2.1 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. 2.4. It should be noted that the solid contact clarifier shown in Fig. 2.4 can be either a settling or a dissolved air flotation (DAF) 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.

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 (a) alkaline precipitation is subject to interference when mixed wastes are treated and (b) relatively high quantities of residue can be generated.

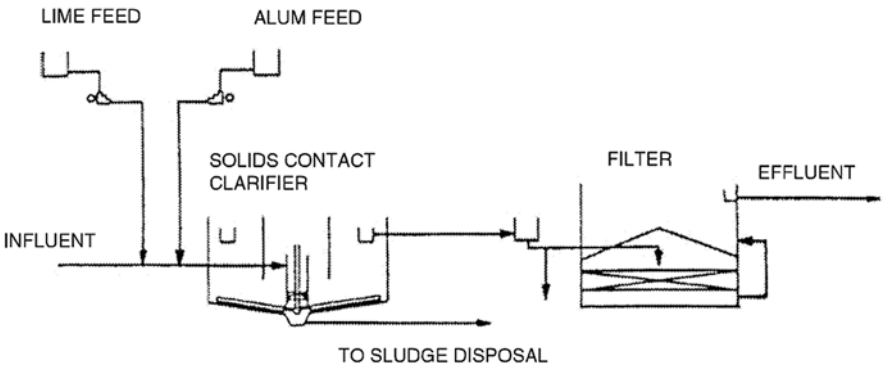


**Fig. 2.4** Alkaline precipitation for a metal removal system [13]

#### 2.6.3.2.2 Sulfide Precipitation

For many heavy metals (such as copper, nickel, and zinc), their sulfides have much lower $_{Ksp}$ than their hydroxides (see Table 2.8). 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].

#### 2.6.3.2.3 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 adnd 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 dissolved air flotation clarification [41, 42].

Some heavy metals are bonded in organic compounds, making their removal more complicated. A typical example is from Merck, one of the largest

**Table 2.8** Annual mass loadings from direct and indirect pharmaceutical wastewater discharges

| Pollutants | Mass loadings for direct dischargers (1000 lb/year | | | | Mass loadings for indirect dischargers (1000 lb/year)+ | | | |
|---|---|---|---|---|---|---|---|---|
| | Subcategories A, B, and C | | Subcategory D | | Subcategories A, B, and C | | Subcategory D | |
| | 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 | 5900 | 4100 | 300 | 169,000 | 169,000 | 5600 | 5600 |
| TSS | 45,000 | 4600 | 1200 | 290 | 64,500 | 64,500 | 3000 | 3000 |
| **Priority pollutants** | | | | | | | | |
| Volatile organics | 2000 | 77 | 240 | 6 | 2400 | 2000 | 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 | 7500 | 800 | 411,000 | 411,000 | 24,000 | 24,000 |
| Volatile organics | 5100 | – | 1000 | – | 7700 | – | 2200 | – |
| Semivolatile organics | 59 | – | 10 | – | 87 | – | 25 | – |
| Pesticides/ Herbicides | 63 | – | II | – | 92 | – | 26 | – |
| **Industry characteristics** | | | | | | | | |
| Number of facilities | 30 | | 21 | | 130 | | 155 | |
| Wastewater flow, MGD | 21.38 | | 3.54 | | 31.1 | | 8.8 | |

– Negligible

pharmaceutical companies. The company used an organomercury compound (thimerosal, RSHgEt) as a slow killing 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 thimerosal to release mercury at cationic state in water with the reaction:

$$\mathrm{Al} \rightarrow \mathrm{A1}^{3+} + 3\mathrm{e}^{-} \tag{2.3}$$

and one of the following reactions:

$$2\mathrm{e}^{-} + \mathrm{RSHgEt} + 2\mathrm{H_2O} \rightarrow \mathrm{RH} + \mathrm{HSHgEt} + 2\mathrm{OH}^{-} \tag{2.4}$$

$$2\mathrm{e}^{-} + \mathrm{RSHgEt} + \mathrm{H_2O} \rightarrow \mathrm{RH} + \mathrm{HOHHgEt} + \mathrm{S}^{2-} \tag{2.5}$$

$$4\mathrm{e}^{-} + \mathrm{RSHgEt} + 2\mathrm{H_2O} \rightarrow \mathrm{RH} + \left[\mathrm{HHgEt}\right] + \mathrm{S}^{2-} + 2\mathrm{OH}^{-} \tag{2.6}$$

(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:

$$4\mathrm{Hg}^{2+} + \mathrm{NaBH_4} + 8\mathrm{OH}^{-} \rightarrow 4\mathrm{Hg} + \mathrm{NaBO_2} + 6\mathrm{H_2O} \tag{2.7}$$

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:

$$\mathrm{BH_4^{-}} + \mathrm{H_2O} \rightarrow \mathrm{B}\left(\mathrm{OH}\right)_3 + \mathrm{OH}^{-} + 4\mathrm{H_2} \tag{2.8}$$

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 l0 ppb in the effluent). Mercury can be reclaimed from the filter cake of the ultrafiltration process.

#### 2.6.3.3 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.

2.6.3.3.1 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:

$$K_i = V_i / W_i \tag{2.9}$$

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

$$K_i = r_i\left(P_i / P\right) \tag{2.10}$$

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, and $P$ is the total pressure.

Equations (2.9) and (2.10) 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 2.5 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

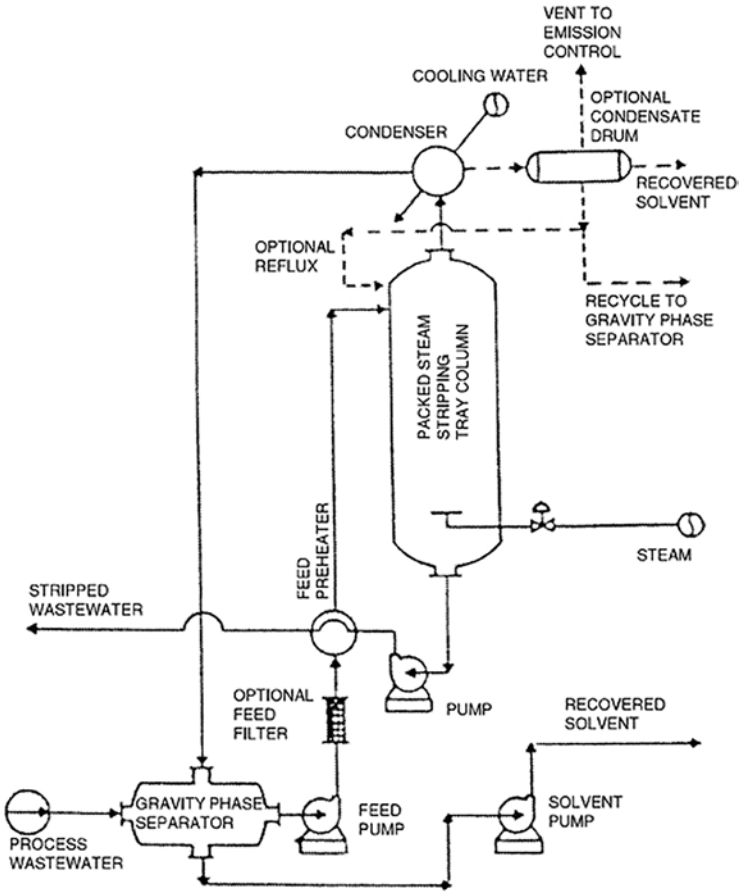


**Fig. 2.5** Equipment for stream stripping solvents from wastewater [13]

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, with the highest for the bottom temperature and the lowest for the feed temperature (Table 2.9). 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.

**Table 2.9** 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 diluent from cyanide destruction unit. Applies only when all cyanide-bearing wastes are diverted to a cyanide destruction unit and subsequently arc 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

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.

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 wastewater processes in another pretreatment system for further end-of-pipe treatment, or further combined with sanitary wastewater and then discharged to the POTW.

#### 2.6.3.3.2 Air Stripping

Air stripping is also used to recover volatile organic compounds, such as benzene, chloroform, 1,1,-I-trichloroethane, 1,2-dichloroethane, ethylbenzene, methyl chloride 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.

#### 2.6.3.3.3 Advanced Physocochemical Treatment Processes

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.

Advanced physicochemical treatment processes available for treating the pharmaceutical wastewater include coagulation and clarification, dissolved air flotation (DAF), flotation-filtration (DAFF; filtration can be either sand filtration or GAC filtration), granular activated carbon (GAC) adsorption, powdered activated carbon (PAC) adsorption, wet air oxidation (WAO), supercritical water oxidation (SCWO), Fenton oxidation, UV photocatalytic oxidation, ultrasound oxidation, air stripping, distillation, electrochemical oxidation, ozonation, membrane filtration (MF, UF, RO, ED, MBR), or other advanced oxidation processes (AOP), combined oxidation-reduction process, etc. Evaluation of these processes is presented in Sects. 2.7.7.1 and 2.7.7.2. Of these advanced treatment processes, DAF, DAFF, GAC, air stripping, distillation, and membrane processes are suitable for recycling and reusing of chemical compounds and/or water. In view of the pollution load reduction and chemical cost saving, it is necessary to recover chemical compounds or raw materials as much as possible. In view of the scarcity of water resources, it is necessary to understand and develop methodologies for the treatment of pharmaceutical wastewater as part of water management. While most of the advanced treatment processes are technically feasible for treating the pharmaceutical wastewater, their economical feasibility needs to be carefully evaluated before any implementation.

### *2.6.4 End-of-Pipe Treatment Technologies*

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.

#### 2.6.4.1 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.

##### 2.6.4.1.1 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 as follows:

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 2.2.

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, nutrient 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].

#### 2.6.4.1.2 Screening and Clarification

All waste flows should be passed through screens to remove large suspended matter and through clarification (sedimentation or flotation) tanks to remove suspended solids. Rectangular gravity clarifiers are usually used for primary sedimentation, although circular gravity tanks or dissolved air flotation tanks are equally efficient.

Chemical coagulation and flocculation can also be combined with primary treatment to increase TSS removals. Primary treatment is an important pretreatment for the subsequent secondary biological waste treatment, which may remove 20–50% of 5-day BOD.

#### 2.6.4.1.3 Primary Flotation Clarification and Secondary Flotation Clarification

When conventional sedimentation cannot effectively remove suspended solids or oil and grease, primary flotation may be used instead of primary sedimentation before secondary biological waste treatment [46, 47].

In dissolved air flotation (DAF), 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.

DAF can also be used as a secondary clarifier.

### 2.6.4.2 Secondary Biological Treatment

#### 2.6.4.2.1 Activated Sludge

Activated sludge is the most widely used secondary biological process for treating pharmaceutical wastewater [50–56]. 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 [57]. 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 2.6 shows the flow diagrams of a few selected activated sludge

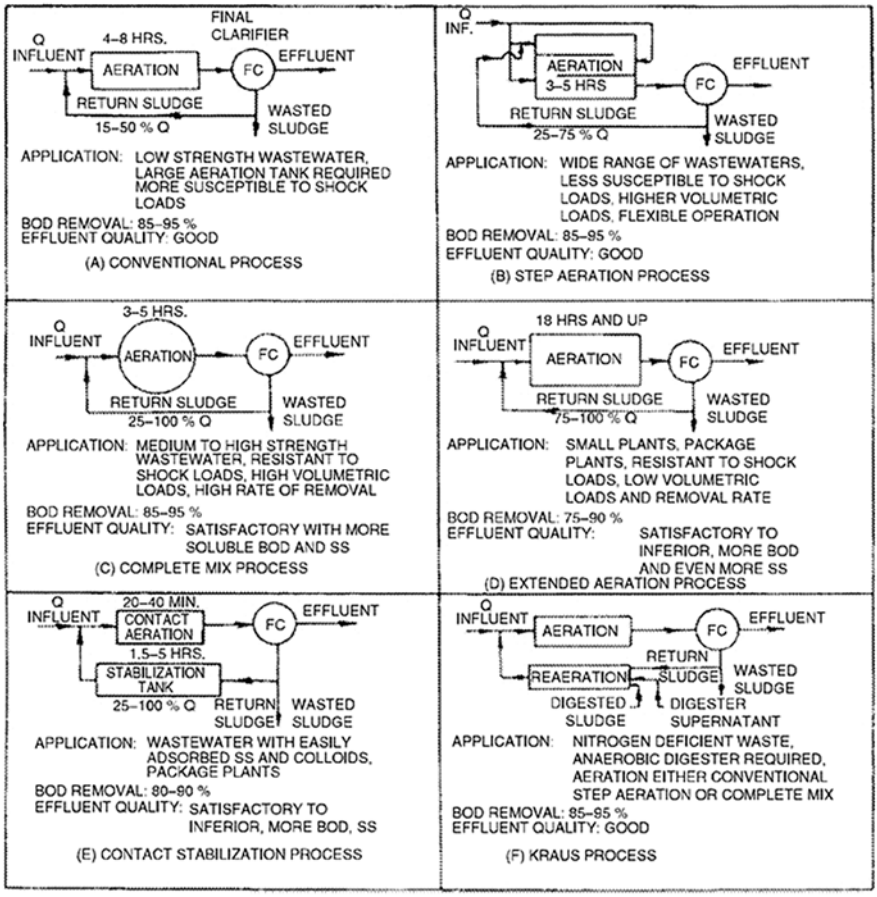


**Fig. 2.6** Flow diagrams and applications of major activated sludge processes

processes. The treatment mode is selected according to the characterization of the wastes and the goal of treatment [58–60].

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 of 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 [61, 62].

Some activated sludge treatment systems experience severe filamentous microorganisms buildup accompanied with very poor settling. A pilot-scale experiment was conducted to 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, 57, 63].

According to Mayabhate et al. [64], 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, postaeration, and chlorination. The effluent was disposed of by irrigation, while the biosolids were dried and utilized as fertilizer.

Schumann [65] described a treatment system for high-strength pharmaceutical wastewater, which included neutralization and aerobic activated sludge treatment with aerobic sludge stabilization [29].

#### 2.6.4.2.2 Aerated Lagoon

Aerated lagoons are usually rectangular in shape, with a length-to-width ratio of 2:1. 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 are 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 [66].

Complete mixing and adequate aeration are essential. Sufficient oxygen should be furnished to maintain dissolved oxygen throughout the entire 8–12-ft depth (Fig. 2.7). 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

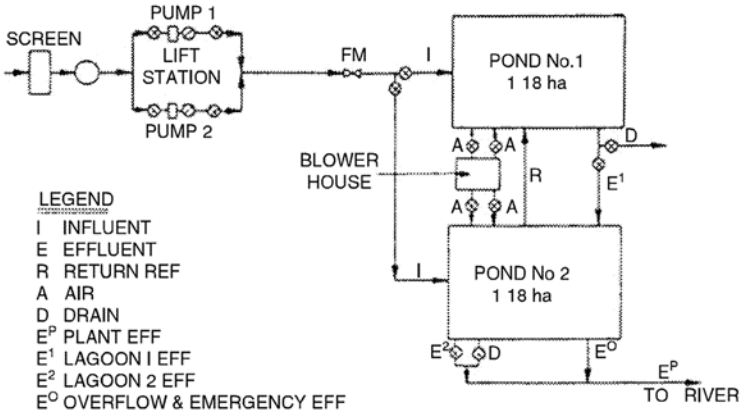


**Fig. 2.7** Aerated lagoon system

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.

### 2.6.4.2.3 Trickling Filter

Trickling filters are fixed film reactors using a biological process for wastewater treatment [67]. 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. 2.8). 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

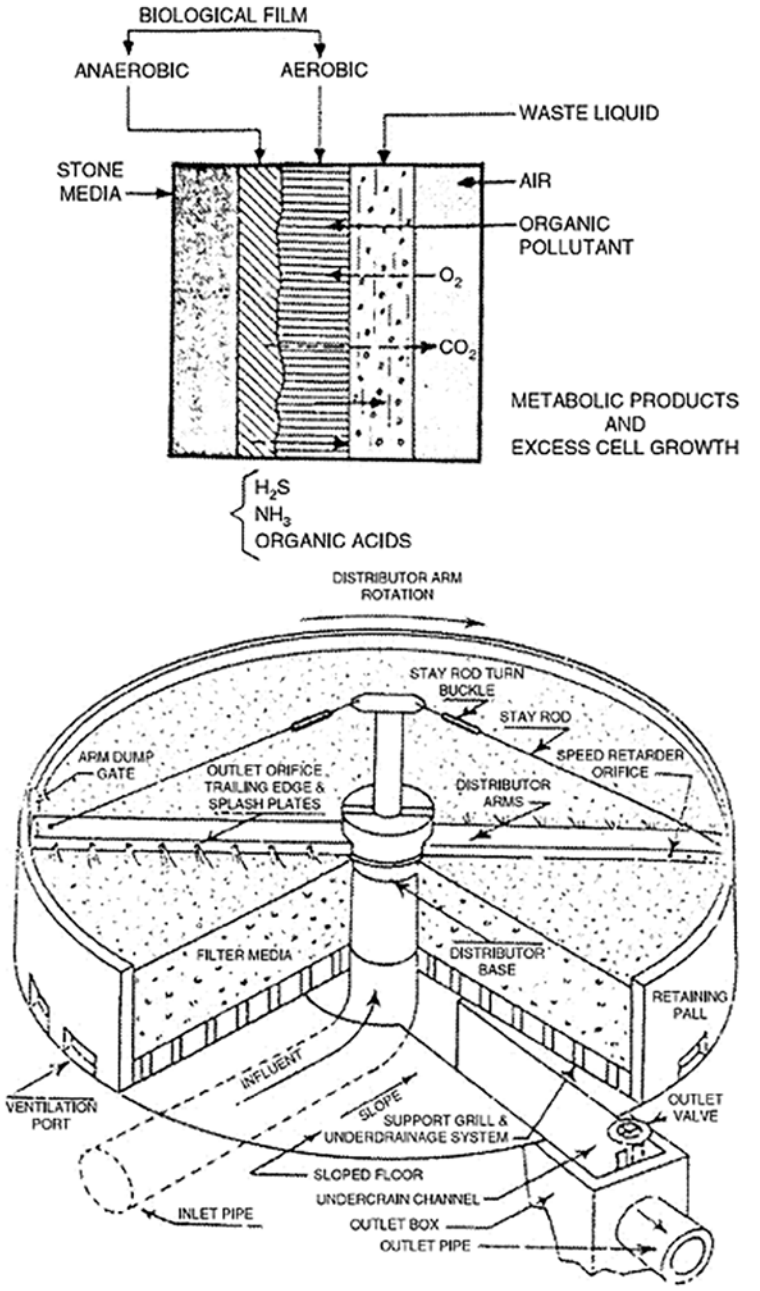


**Fig. 2.8** Trickling filter

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 [67–69]. 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 [67, 70, 71].

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 [63].

#### 2.6.4.2.4 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, 72].

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 considerably less than those in the activated sludge process or the trickling filter process. Figure 2.9 illustrates several anaerobic processes that have been used in the treatment of pharmaceutical wastewater [73–76].

The conventional anaerobic treatment process provides a continuous or intermittent feeding without solid separation. The detention time is usually 10–30 days and the minimum time is 3–5 days.

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 [77].

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 [78] treated pharmaceutical wastewater and achieved a 97% removal of COD at a loading of 3.5 kg COD/$m^3$/day at 37 °C. Sachs et al. [79] used an anaerobic filter to treat biological or chemically synthesized pharmaceutical wastewater.

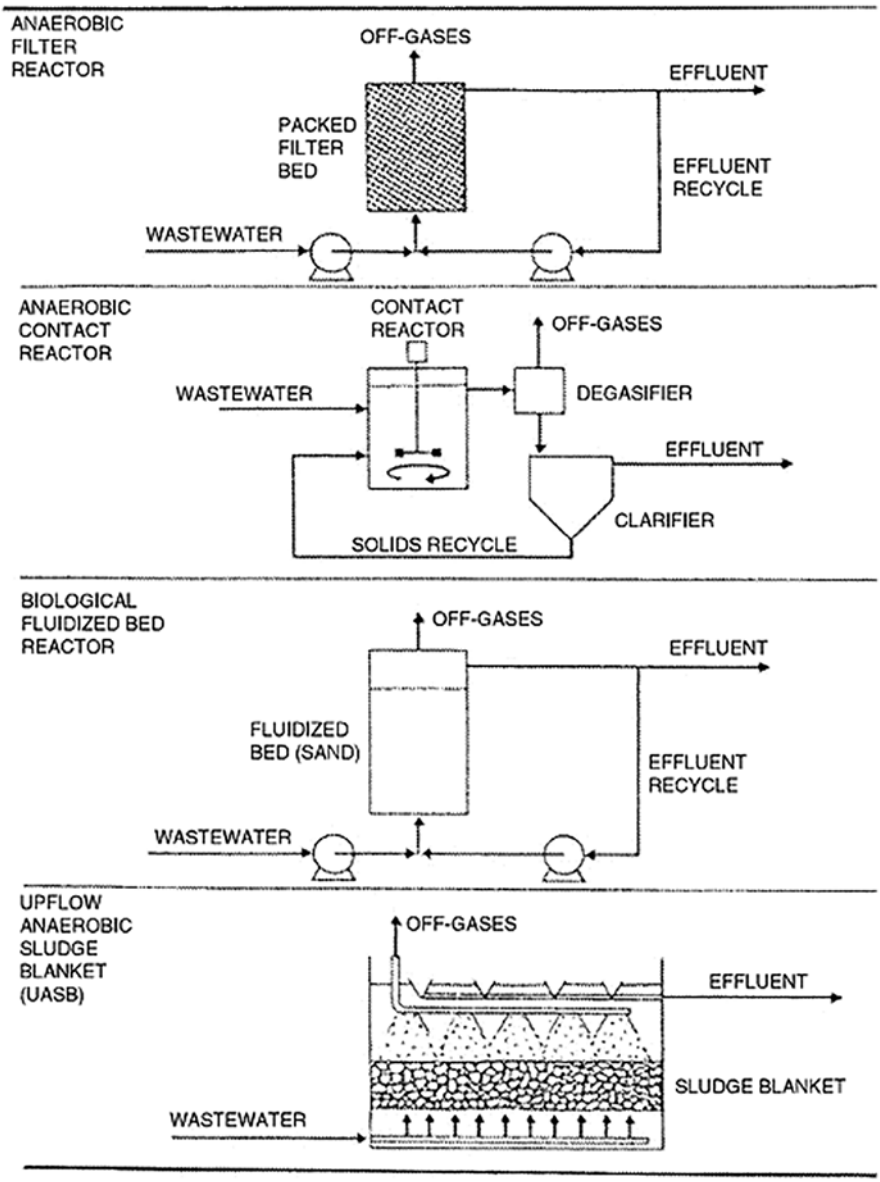


**Fig. 2.9** Anaerobic wastewater treatment processes

With a loading of 0.56 kg COD/$m^3$/day at 35 °C and 36 h hydraulic retention time, they 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. [80] 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 methylformamidecontaining 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 acid production. The feed flow had a COD concentration of 2500 mg/L, which 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 [81] 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 [82] 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 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 healthcare product 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 3500 lb/day, respectively (11,340, 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. 2.10. The low-rate anaerobic reactor performance operating at a temperature 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.

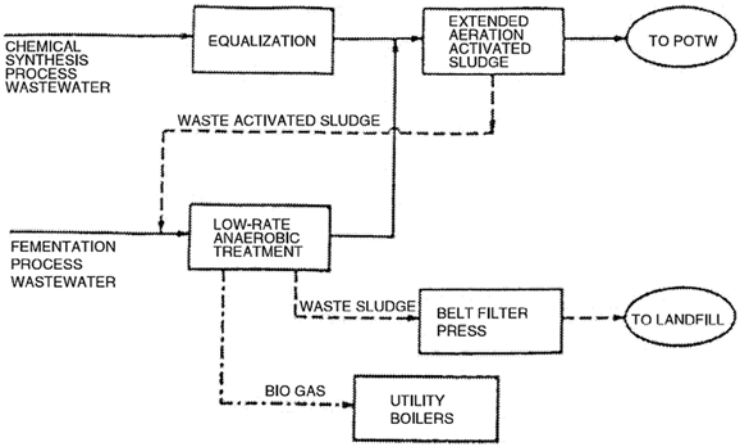


**Fig. 2.10** Aerobic-anaerobic treatment of chemical synthesis and fermentation wastewater effluents

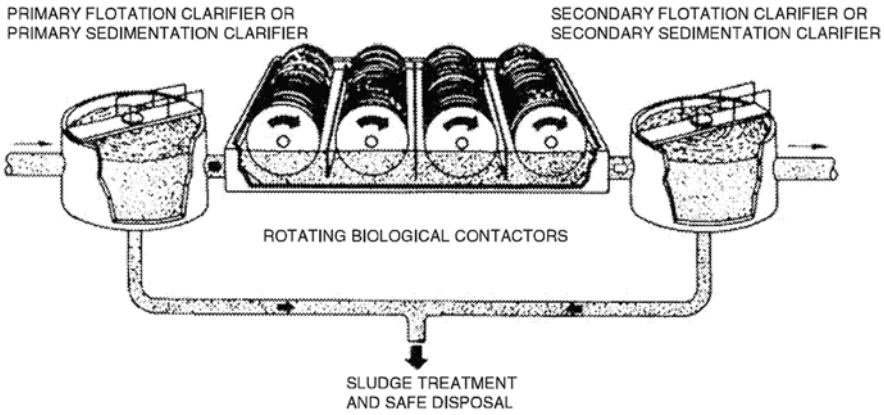


**Fig. 2.11** Schematic diagram of rotating biological contactors

#### 2.6.4.2.5 Advanced Biological Treatment Methods

Other biological treatment methods utilized in pharmaceutical wastewater are waste stabilization ponds [66], rotating biological contactors (RBC) [83–85] (see Fig. 2.11), polishing ponds, sequencing batch reactors (SBR) [86], membrane bioreactor (MBR), and sequencing batch biofilters [87]. For detailed description of these processes, the readers are referred to the books *Biological Treatment Processes* [51], *Advanced Biological Processes* [72], *Membrane and Desalination Technologies* [123], *and Environmental Flotation Engineering* [124]. Additional biological treatment processes are introduced in other chapters of this book [175–176].

#### 2.6.4.3 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, 88].

##### 2.6.4.3.1 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 [164, 169].

Carbon adsorption uses activated carbon which has a great specific surface area (surface area per unit volume) to effectively adsorb pollutants [40, 88]. 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 1600 and 1800 °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 [89] 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 effluents. Bauer et al. [90] 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 [91–99].

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 5850 to 8830 mg/L as the PAC dosage to the influent was increased from 208 to 1520 mg/L. The 0.7 mg/mg PAC dosage resulted in 50% additional removal of COD.

#### 2.6.4.3.2 Coagulation, Flocculation, and Clarification

Coagulation is a process used for the removal of colloidal and fine suspended particles [100, 101]. Kharlamova et al. [102] 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, 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 [64].

PAC can also be applied to a coagulation/flocculation/clarification system for removal of toxic substances [63]. Clarification can be either a sedimentation clarification or a flotation clarification.

#### 2.6.4.3.3 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 2.10 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.

### 2.6.4.4 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 byproducts 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,

**Table 2.10** 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 | 1350 |
| 1,1-dichloroethylene | 2 | 10 |
| 1,2-trans-dichloroethylene | 1 | 550 |
| Ethylbenzene | 3 | 21 |
| Methylene chloride | 9 | 890.000 |
| Bromoform | 1 | 12 |
| Tetrachloroelhylene | 1 | 2 |
| Toluene | 6 | 1050 |
| Trichloroethylene | 1 | 7 |

and reuse. In the latter two cases, sludge stabilization and disinfection will be needed [29, 65].

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 [103] 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 [104, 105] 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 solid 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 [106]. 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.

## 2.7 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.

### *2.7.1 Factory Profiles*

Ansa, a plant at Izmit, Turkey, produces antibiotic 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/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 [107].

### *2.7.2 Raw Materials and Production Process*

The production used different raw materials from agricultural sources and used various chemicals (Table 2.11).

Figure 2.12 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

**Table 2. 11** Solubility Products ($K_{sp}$) for Insoluble Metal Salts [13]

| Compound | $K_{sp}$ | Metal Ion Conc. (μg/L) |
|---|---|---|
| CuS | $6 \times 10^{-36}$ | $1 \times 10^{-10}$ |
| NiS | $2 \times 10^{-25}$ | $8 \times 10^{-6}$ |
| ZnS | $1.6 \times 10^{-25}$ | $2 \times 10^{-5}$ |
| $Cu(OH)_2$ | $3.5 \times 10^{-19}$ | 25 |
| $Ni(OH)_2$ | $1.5 \times 10^{-15}$ | 400 |
| $Zn(OH)_2$ | $1.8 \times 10^{-14}$ | $1 \times 10^{-3}$ |

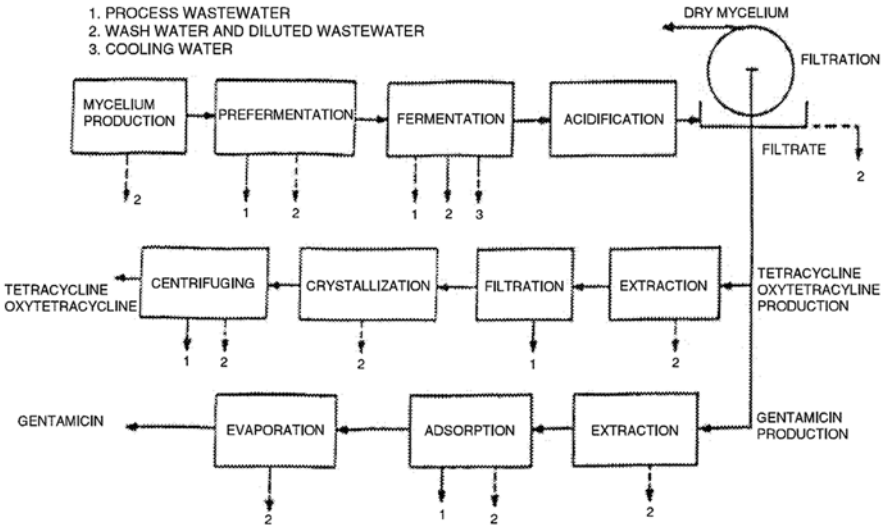


**Fig. 2.12** Antibiotic production process system [107]

product. For gentamicin production, the filtrates were treated by extraction, chromatographic resin adsorption, evaporation, filtration, crystallization, or spray drying to yield the final product.

### *2.7.3 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)

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 the cooling water. The strong process wastes were from fermentation process, extraction and purification processes, and recovery process. The diluted wastes were 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 as follows:

1. Strong process wastes: $Q = 120\ m^3/day$
2. Diluted wastes: $Q = 160\ m^3/day$
3. Cooling water: $Q = 1000\ m^3/day$

Table 2.12 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 1680 kg/day of 5-day BOD and 4180 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 8400 and 50 mg/L, respectively, and the flow rates at 200 and 800 $m^3/day$,

**Table 2.12** 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°

respectively, as shown in Table 2.12. Combining the waste streams yielded a total flow of 1000 $m^3$/day and 5-day BOD of 1720 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.

## *2.7.4 End-of-Pipe Treatment Case Histories and Green Environmental Technologies*

### 2.7.4.1 Case Histories of Current Technologies

Table 2.13 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 were possibly due to high sulfate levels, frequent changes in products, and fluctuation in wastewater characteristics.

An activated sludge treatment system shown in Fig. 2.13 was selected and designed for the pharmaceutical plant [107]. Tables 2.14 and 2.15 introduce the raw material consumption and the wastewater characteristics, respectively, of the antibiotic production plant [107]. It basically involved a separate equalization of waste streams, pH adjustment, aeration, activated sludge system, secondary clarification, and biosolid treatment.

The strong and diluted wastes (flow rates of 200 and 800 $m^3$/day and with 5-day BOD at 8400 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 1720 mg/L and a flow rate of 1000 $m^3$/day.

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

**Table 2.13** 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 dotation | 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 treatment | 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 |

level as attested by a high half saturation constant (Ks). 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 4200 mg/L. The designed treatment plant was capable to achieve 90% removal for 5-day BOD and 80% removal for COD.

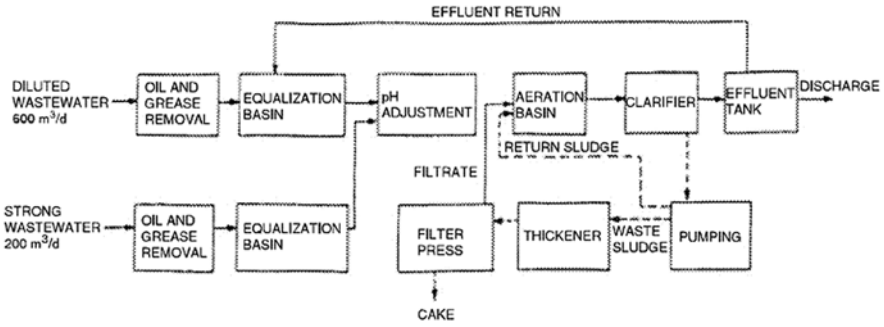


**Fig. 2.13** Wastewater treatment system: a case study [107]

**Table 2.14** Raw materials consumption for antibiotic production—case study [107]

| Raw materials | Usage (tone per year) |
|---|---|
| Carbohydrate sources: Starch, dextrin, sugars, vegetable oils | 1500 |
| Protein sources: Soy meal, soy flour, com. Steep liquor gluten | 300–100 |
| 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, HCl, $H_2SO_4$, 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

### 2.7.4.2 Green Environmental Technologies Developed by the Lenox Institute of Water Technology (LIWT)

The Lenox Institute of Water Technology (LIWT) has developed many modern dissolved air flotation (DAF) clarifiers, dissolved air flotation-filtration (DAFF) package plants, and DAF-DAFF package plants. The filtration portion can be either sand filtration or GAC filtration. Through Krofta Engineering Corporation (KEC) and its partners, over 3000 DAF, DAFF, and combined DAF-DAFF water and wastewater treatment plants have been installed around the world. The LIWT has also developed several multistage hybrid green environmental technologies which are both technically feasible and economically feasible for treating the pharmaceutical and other industrial wastewater depending on the characteristics of original industrial effluent: (a) DAF-DAFF-UV photocatalytic oxidation (one of AOP) for pretreatment, (b) DAF-aerobic biological-DAFF-UV photocatalytic oxidation for treating low-concentration wastewater, (c) DAF-anaerobic-aerobic biological-DAFF-UV photocatalytic oxidation for treating medium- to high-concentration wastewater, and (d) DAF-anaerobic-aerobic biological-DAFF-UF-UV photocatalytic oxidation

**Table 2.15** Characteristics of wastewater streams—case study [107]

| 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 | 13,500 | 400 |
| COD, mg/L | 34,000 | 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 |

for treating extremely high-concentration wastewater. The researchers and PhD students are invited to study the LIWT systems further. Adopting a hybrid green environmental technology consisting of both biological system (aerobic alone or anaerobic-aerobic depending on the organic concentration of the wastewater) and UV-photocatalytic oxidation will be a feasible solution to treating the pharmaceutical wastewater or similar.

## *2.7.5 Pharmaceutical Waste Minimization Case Study of Hennepin County Medical Center*

### 2.7.5.1 Company Overview [108]

Hennepin County Medical Center (HCMC), a public teaching hospital in Minneapolis, Minnesota, is a nationally recognized level one trauma center and the third largest hospital in the Twin Cities. HCMC has over 356,000 patient visits annually.

### 2.7.5.2 Waste Reduction Project [108]

In 2006, HCMC returned over 900 different outdated pharmaceuticals, most in multiple quantities, through the reverse distribution process. The total cost to purchase was $146,411. Of this amount, only 202 items were credited for a total of $75,657. Therefore, a waste reduction project was conducted at HCMC that focused on reducing pharmaceutical waste from the reverse distribution process at the inpatient pharmacy. Waste reduction resulted in over $80,000 in cost savings and 378 lbs of pharmaceutical waste.

#### 2.7.5.2.1 Crash Boxes

Crash boxes, similar to crash carts, were found to be a significant source of waste. These boxes contain emergency medicine needed to revive someone in the event of a cardiac event. Waste occurs when boxes contain drugs that are not used by their expiration date. When this occurred in the past, the pharmacy exchanged the box and updated all the drugs so they are good for about 1 year. Outdated and nearly outdated drugs were sent for reverse distribution.

In investigating the crash boxes, it was determined that many of the drugs found in the boxes are regularly used in other locations in the hospital. It was recommended the pharmacy bring back the crash boxes 3 months prior to expiration and move the drugs to locations where they are used more frequently, potentially using them prior to expiration.

Other recommendations for the crash box drugs included the following:

(a) Replacing the specialty epinephrine intracardiac syringe that was rarely used. It expired and was returned 98% of the time. The use of a more commonly used epinephrine syringe and an 18-gauge, 3-inch needle banded to the epinephrine box was recommended.

(b) Changing the dosage of glutose gel from the 45 g dosage type, much of which was wasted. In most cases, a 30 g dose of glucose is used. A recommendation was made to lower the dosage carried from 45 g to 15 g for the drug to be used in more applications.

(c) Lowering the size of the nitroglycerin bottle from 100 count to 25 count and switching to a generic form from a brand name.

#### 2.7.5.2.2 Other Reverse Distribution Drugs

Review of reverse distribution manifests helped identify the most common and costly drugs returned. HCMC also found they were returning 4% of their inventory, which is 2% above the average as determined by the American Society of Health-System Pharmacists. The top ten of these were (a) crash box epinephrine, (b) epinephrine, (c) glucagon, (d) glutose gel, (e) Nitrostat, (f) hydralazine, (g) lidocaine, (h) amiodarone, (i) adenosine, and (j) naloxone.

It was recommended that HCMC review par usage reports for the top ten returns and adjust inventory quantities accordingly. Doing so would save at least $80,000 and eliminate 210 pounds of pharmaceutical waste.

### 2.7.5.3 Results

HCMC implemented all the recommendations. It is estimated they are saving $80,000 annually and have eliminated 378 pounds of drug waste.

### *2.7.6 Pharmaceutical Waste Minimization Case Study of Falls Memorial Hospital*

#### 2.7.6.1 Company Overview [108]

Falls Memorial Hospital (FMH), a 25-bed facility, is a charitable, not-for-profit Critical Access Hospital located in International Falls, Minnesota. It has planned to reduce its drug inventory and pharmaceutical waste.

#### 2.7.6.2 Inventory Reduction Project [108]

Prior to undergoing the inventory reduction project, FMH was checking for outdated drugs every other month, stock was not rotated regularly, and par usage reports were not available. In 2006, a staff pharmacist noticed many drugs on site were outdated and the facility was stocking too many extra medications. Because par usage reports had not been used previously, FMH, at that point, did not know how many drugs were required for the facility. Due to these factors, the facility began to look at ways to reduce inventory, save money, and decrease pharmaceutical waste.

##### 2.7.6.2.1 Chemotherapy Drugs [108, 109]

Looking closely into quantities ordered and costs, FMH realized that chemotherapy drugs were the largest expense for the facility. They were being ordered monthly, and in December 2007, the facility spent over $90,000. Because of the long holding time for some of the chemotherapeutics, they were outdating on the shelf. FMH also realized, through facility-wide research, that some chemotherapy drugs were extremely expensive and came in multiple strengths. FMH changed their ordering for chemotherapeutic drugs from once per month to once a week.

##### 2.7.6.2.2 Routine Stock on Floors

FMH utilizes AcuDose, an automated dispensing machine, to supply most of their stock of drugs. AcuDose machines were stationed in the emergency room, medical/surgical area, operating room, and intensive care unit. As part of the inventory reduction project, the pharmacist noted that the AcuDose machine in the intensive care unit was rarely used because most of the pharmaceuticals were special order for the patients and resulted in numerous expired drugs and the inventory not being rotated frequently enough. Therefore, the pharmacist recommended placing the medications only where they are needed and rotating the stock on a more regular basis.

2.7.6.2.3 Therapeutic Substitution

In order to reduce the amount of drugs at the hospital, the pharmacist recommended using therapeutic substitution lists. For example, there are five medications in a class of drugs called proton pump inhibitors, or PPI. Instead of having all five medications on the formulary, FMH chose to carry just two of them. This would ensure that the hospital was not carrying multiple medications in the same category and make it easier to rotate stock. If a patient comes into the hospital on a PPI not on the formulary, they will be automatically switched to an equivalent dose of a PPI that is on the formulary.

Multiple dosage types were also noted. The number of dosage forms has been reduced to those used most often and multiples of those to achieve the strengths for esoteric doses. The pharmacy now also searches out and purchases only from those vendors that have the least packaging.

#### 2.7.6.3 Pollution Prevention Impacts

Due to FMH's inventory reduction project, the facility is ordering and stocking fewer drugs, reducing packaging waste and shipping costs. This project reduced FMH's monthly overhead from $210,000 in January 2006 to $87,000 in October 2007 and dramatically reduced the amount of waste from expiring medications and excess stock.

### *2.7.7 Recent Investigations of Pharmaceutical Wastewater Treatment Technologies*

A detailed review of available technologies for wastewater treatment and water reuse in pharmaceutical industry has been conducted by Gadipelly et al. [110]. In their review, the various sources of wastewaters in the pharmaceutical industry are identified, and the best available technologies (BAT) for removing pollutants from them are critically evaluated. Effluents arising from different sectors of active pharmaceutical ingredients (API), bulk drugs, and related pharmaceutics, which use large quantities of water, are analyzed, and strategies are proposed to recover valuable compounds, and finally the treatment of very dilute but detrimental wastewaters is discussed [110]. It appears that no single technology can completely remove pharmaceuticals and other pollutants from pharmaceutical wastewaters. The use of conventional biological treatment methods along with innovative membrane reactors and advanced posttreatment methods resulting in a combined hybrid wastewater treatment system appears to be the best [110–136]. Appendix I and Appendix II document many researchers' investigations reviewed by Gadipelly et al. [110]. The authors of this publication list the original research sources of many useful

investigations for reference by the readers. The environmental technologies used for treating various pharmaceutical wastes and their process terminologies have been introduced in the previous sections of this book chapter, and they can also be found from the literature [1–136] for further research.

#### 2.7.7.1 Chemical Synthesis-Based Pharmaceutical Wastewater Treatment Technologies

The chemical synthesis-based pharmaceutical wastewater treatment technologies, which have been investigated with various degrees of success, are as follows:

1. Sulfate anion radical oxidation (Fe and Co sulfate salts used with hydrogen peroxide and ozone) [132]
2. Dissolved air precipitation with solvent sublation simulated water: mineral oil layer with organic solvents (toluene, methylene chloride, benzene, chlorobenzene, hexane, butyl acetate) [133]
3. Electrocoagulation (EC) followed by heterogeneous photocatalysis ($TiO_2$; iron electrodes were used as cathode and anode) [130]
4. Upflow anaerobic sludge blanket (UASB) + micro-aerobic hydrolysis acidification reactor (NHAR) + two-stage aerobic process, cyclic activated sludge system (CASS), and biological contact oxidation tank (BCOT) [143]
5. Two-phase anaerobic digestion (TPAD) system and a subsequential membrane bioreactor (MBR). TPAD system comprised of a continuous stirred tank reactor (CSTR) and an upflow anaerobic sludge blanket-anaerobic filter (UASBAF), working as the acidogenic and methanogenic phases [131]
6. Adsorption: granular activated carbon (a series of columns of GAC were used) [164]
7. Electrochemical treatment (boron doped diamond BDD anode for corrosion stability) [168]
8. Continuous heterogeneous catalytic wet peroxide oxidation (CWPO) process using a Fe2O3/SBA-15 nanocomposite catalyst [134]
9. Acidogenic reactor (USAB sludge from an alcohol industry was used with high glucose as initial feed and then varying pharmaceutical wastewater) [135]
10. Hybrid upflow anaerobic sludge blanket reactor [149]
11. Conventional treatment: activated sludge reactor using sequencing batch reactor (SBR) [142]
12. Hybrid upflow anaerobic sludge blanket reactor (ASBR) [153]
13. Catalytic wet air oxidation [136]
14. Membrane bioreactor (MBR) [143]
15. Photo/Fenton followed by lime or sodium hydroxide precipitation/coagulation [160]

#### 2.7.7.2 Fermentation Process-Based Pharmaceutical Wastewater Treatment Technology

The fermentation process-based pharmaceutical wastewater treatment technologies, which have been investigated with various degrees of success, are as follows:

1. Photocatalysis ($TiO_2$) + $H_2O_2$; a single baffled reactor for the process [159].
2. Biodegradation using bacterial strains (*Pseudomonas aeruginosa* and *Pseudomonas pseudomallei*) [165].
3. Photocatalysis (Fenton + photo-Fenton + ozonation) [155].
4. Ozonation (pretreatment) + biological activated sludge reactor combination in series [157].
5. Fenton-biological process: first Fenton coagulation and then biological treatment by activated sludge [161].
6. Chemical oxidation ozonation and ozonation coupled with treatment with hydrogen peroxide [156].
7. Membrane bioreactor technology (hollow fiber membrane) [144].
8. Upflow anaerobic stage reactor (UASR) [148, 153].
9. Upflow anaerobic stage reactor (UASR) [152].
10. Ozonation (pretreatment) + biological activated sludge treatment by synthetic biomass with 30% COD [158].
11. Activated sludge reactor in batch and continuous flow [146].
12. Anaerobic biological treatment using activated sludge reactor [151].
13. Hybrid treatment technology (aerobic biological pretreatment + ozonation + MBR), the biological treatment for reducing the ozone demands. Ozonation reduces almost all of the organic compounds [145, 157].
14. Anaerobic granulation batch/column reactor [150].
15. Catalytic wet air oxidation coupled with anaerobic biological oxidation [154].
16. Aerobic biological treatment with variable temperature study [51].
17. Biological treatment by activated sludge: in seven stages, a pilot plant study [55].
18. Suspended growth photo-bioreactor: non-sulfur photosynthetic bacterium isolated from the soil and fluorescent light reactor [137].
19. Membrane bioreactor (GE ZeeWeed membrane bioreactor technology) [138].
20. Semiconductor photocatalysis Ti/$TiO_2$: $RuO_2$-$IrO_2$ as anode, graphite as cathode, and chloride as electrolyte [163].
21. Penraporation through water-selective membranes [139].
22. Sequencing batch reactor (SBR): an activated sludge reactor [140].
23. Solar photo-Fenton and biological treatment [162].
24. Anaerobic multichamber bed reactor (AMCBR) + AMCBR with continuous stirred tank reactor (CSTR) [166].
25. ANAMMOX (anaerobic ammonium oxidation) process with sequential biocatalyst (ANAM-MOX granules) addition (SBA-ANAMMOX process) [167].
26. Fenton oxidation (pretreatment) by oxidation and coagulation stage followed by aerobic biological degradation in sequencing batch reactor [147].

27. Catalytic wet air oxidation (CWAO) mixtures of waste streams used in autoclave to form polyoxometalates (POMs) as a cocatalyst system [141].

## 2.8 Summary and Conclusions

1. Toxic or hazardous pharmaceutical pollutants are typically produced in batch pharmaceutical manufacturing processes leading to the presence of a wide variety of undesirable pharmaceuticals in wastewaters, air, and soil. Common use of pharmaceutical compounds by human consumption and farming operations is also an input source of undesirable pharmaceuticals in the environment. It is concluded that the presence of pharmaceutical compounds in drinking water, livestock, and human body comes from both of the above two sources: (a) production processes of the pharmaceutical industry and (b) common use of pharmaceutical compounds resulting in their presence in urban and farm wastewaters.
2. The pharmaceutical wastewaters generated in different processes in the manufacture of pharmaceuticals and drugs contain a wide variety of chemical compounds. Some pharmaceutical pollutants are biodegradable; some are not biodegradable or toxic to microorganisms. Conventional cost-effective biological waste treatment technologies (i.e., gray environmental technologies), such as activated sludge, trickling filters, lagoons, sequencing batch reactor, membrane bioreactor, composting, sanitary landfill, etc., alone cannot properly treat the liquid and solid wastes. An integrated approach must be taken to manage all wastes within a pharmaceutical manufacturing plant. A "10-Step Blueprint for Managing Pharmaceutical Waste of Healthcare Facilities In the United States" has been developed by the US Environmental Protection Agency (USEPA), and this blueprint must be examined and followed in order to reduce the hazardous pharmaceuticals; in turn, a sustainable green biotechnology, bioreactor landfill, can be used to treat the nonhazardous pharmaceutical solid waste, generate methane gas as biofuel, and protect groundwater.
3. Pharmaceutical industry manufactures drugs, vaccines, antibiotics, products with therapeutic value, etc. using chemical reactors, biological systems or organisms, and many different raw materials. Pharmaceutical products are produced by chemical synthesis, fermentation, extraction from naturally occurring plant or animal substances, or by refining a technical grade product. The USEPA regulation applies to pharmaceutical industrial facilities which are organized into five subcategories: (a) subcategory A (fermentation products), (b) subcategory B (extraction products), (c) subcategory C (chemical synthesis products), (d) subcategory D (mixing, compounding, and formulation), and (e) subcategory E (research organizations).
4. Fermentation process of pharmaceutical plants produces most antibiotics and steroids using three basic steps: inoculum and seed preparation, fermentation, and product recovery. Fermentation is conventionally a large-scale batch process. The fermentation step begins with a wash water and steam sterilization of

the fermenter vessel. Sterilized nutrient raw materials in water are then charged to the fermenter. The process wastewater from fermentation plants is characterized by high BOD, COD, and TSS concentrations, relatively large flows, and a pH range of approximately 4.0–8.0.

5. Biological and natural extraction operations of pharmaceutical plants use many materials as pharmaceuticals are derived from such natural sources as the roots and leaves of plants, animal glands, and parasitic fungi. These products have numerous and diverse pharmaceutical applications, ranging from tranquilizers and allergy-relief medications to insulin, morphine, plasma, and its derivatives. The extraction process consists of a series of operating steps beginning with the processing of a large quantity of natural or biological material containing the desired active ingredient. Residual wastes from an extraction plant essentially will be equal to the weight of raw material. Solid wastes are the greatest source of the pollutant load; however, solvents used in the processing steps can cause both air and water pollution. The principal sources of wastewater from biological/natural extraction operations are (a) spent raw materials, (b) floor and equipment wash water, (c) chemical wastes (e.g., spent solvents), and (d) cleanup of spills. Wastewater from extraction plants is generally characterized by low BOD, COD, and TSS concentrations, small flows, and pH values of approximately 6.0–8.0.
6. Chemical synthesis operations of pharmaceutical plants manufacture most of the active ingredients marketed and sold as drugs using organic and inorganic chemical reactions. The conventional batch reaction vessel is the major piece of equipment used on the process line. The reaction vessel is one of the most standardized equipment designs in the industry. Chemical synthesis effluent generally has a high BOD and COD waste load. The pollutants in chemical synthesis wastewater vary with respect to toxicity and biodegradability. Chemical synthesis wastewater may be incompatible with biological treatment systems because it is too concentrated or too toxic for the biomass in the treatment system. Thus, it may be necessary to equalize and/or chemically pretreat some chemical synthesis wastewater prior to biological treatment. Primary sources of wastewater from chemical synthesis operations are (a) process wastes such as spent solvents, filtrates, and concentrates; (b) floor and equipment wash water, (c) pump seal water, (d) wet scrubber wastewater, and (e) spills. Wastewater from chemical synthesis plants can be characterized as having high BOD, COD, and TSS concentrations, large flows, and extremely variable pH values, ranging from 1.0 to 11.0.
7. Mixing, compounding, or formulating operations of pharmaceutical plants produce pharmaceutically active ingredients batch processes in bulk form and convert them to dosage form such as tablets, capsules, liquids, and ointments, for consumer use. In addition, active ingredients can also be incorporated into patches and time release capsules. Wastewater sources from mixing, compounding, or formulating operations are (a) floor and equipment wash water, (b) wet scrubbers, and (c) spills. The use of water to clean out mixing tanks can periodically flush dilute wastewaters of unusual composition into the plant

sewer system. In general, this wastewater is readily treatable by biological treatment systems. The wastewater from mixing, compounding, or formulating plants normally has low BOD5, COD, and TSS concentrations, relatively small flows, and pH values of 6.0–8.0.

8. The USEPA pharmaceutical industry effluent limitations (pretreatment or end-of-the pipe treatment) are provided in this publication for the US readers. The readers in other countries must contact their own country for the effluent limitation details. Knowing both the government effluent limitations and the wastewater characteristics will help select an integrated waste management plan and a feasible wastewater treatment system.
9. Advanced treatment processes available for treating the pharmaceutical wastewater include coagulation and clarification, dissolved air flotation (DAF), flotation-filtration (DAFF; filtration can be either sand filtration or GAC filtration), granular activated carbon (GAC) adsorption, powdered activated carbon (PAC) adsorption, wet air oxidation (WAO), supercritical water oxidation (SCWO), Fenton oxidation, UV photocatalytic oxidation, ultrasound oxidation, air stripping, distillation, electrochemical oxidation, ozonation, membrane filtration (MF, UF, RO, ED, MBR), or other advanced oxidation processes (AOP), combined oxidation-reduction process, etc. Of these advanced treatment processes, DAF, DAFF, GAC, air stripping, distillation, and membrane processes are suitable for recycling and reusing chemical compounds, and/or water. In view of the pollution load reduction and chemical cost saving, it is necessary to recover chemical compounds or raw materials as much as possible. In view of the scarcity of water resources, it is necessary to understand and develop methodologies for treatment of pharmaceutical wastewater as part of water management. While most of the advanced treatment processes are technically feasible for treating the pharmaceutical wastewater, their economical feasibility needs to be carefully evaluated before any implementation.
10. The Lenox Institute of Water Technology (LIWT), a nonprofit educational institute, has developed several multistage hybrid green environmental technologies which are both technically feasible and economically feasible for treating the pharmaceutical and other industrial wastewater depending on the characteristics of original industrial effluent: (a) DAF-DAFF-UV photocatalytic oxidation (one of AOP) for pretreatment, (b) DAF-aerobic biological-DAFF-UV photocatalytic oxidation for treating low-concentration wastewater, (c) DAF-anaerobic-aerobic biological-DAFF-UV photocatalytic oxidation for treating medium- to high-concentration wastewater, and (d) DAF-anaerobic-aerobic biological-DAFF-UF-UV photocatalytic oxidation for treating extremely high-concentration wastewater. The researchers are invited to study the LIWT systems further. Adopting a hybrid green environmental technology consisting of both biological system (aerobic alone or anaerobic-aerobic depending on the organic concentration of the wastewater) and UV photocatalytic oxidation will be a feasible solution to treating the pharmaceutical wastewater or similar.

## Glossary of Biotechnology and Pharmaceutical Industry

**Agricultural Biotechnology** (a) It focuses on developing genetically modified plants to increase crop yields or introduce characteristics to those plants that provide them with an advantage growing in regions that place some kind of stress factor on the plant, namely, weather and pests; (b) development of pest-resistant crops and improvement of plant and animal breeding are typical examples; (c) green biotechnology refers to specific agricultural biotechnology that creates new plant varieties of agricultural interest, biopesticides, biofertilizers, etc. This area of agricultural biotechnology is based on transgenics (genetic modification), i.e., an extra gene or genes inserted into their DNA. The additional gene may come from the same species or a different species.

**Biological and Natural Extraction (Pharmaceutical)** Many materials used as pharmaceuticals are derived from such natural sources as the roots and leaves of plants, animal glands, and parasitic fungi. These products have numerous and diverse pharmaceutical applications, ranging from tranquilizers and allergy-relief medications to insulin and morphine. Also included in this group is blood fractionation, which involves the production of plasma and its derivatives. The extraction process consists of a series of operating steps beginning with the processing of a large quantity of natural or biological material containing the desired active ingredient. After almost every step, the volume of material being handled is reduced significantly. Neither continuous processing methods nor conventional batch methods are suitable for extraction processing. Residual wastes from an extraction plant essentially will be equal to the weight of raw material, since the active ingredients extracted are generally present in the raw materials at very low levels. Solid wastes are the greatest source of the pollutant load; however, solvents used in the processing steps can cause both air and water pollution. Detergents and disinfectants used in equipment cleaning operations are normally found in the wastewater. Priority pollutants, including methylene chloride, toluene, chloroform, 1,2-dichloroethane, and phenol, were identified as being used in the manufacturing of extractive pharmaceuticals. The principal sources of wastewater from biological/natural extraction operations are (a) spent raw materials (e.g., waste plasma fractions, spent media broth, plant residues), (b) floor and equipment wash water, (c) chemical wastes (e.g., spent solvents), and (d) cleanup of spills. Wastewater from extraction plants is generally characterized by low BOD, COD, and TSS concentrations, small flows, and pH values of approximately 6.0–8.0.

**Biotechnology** It is an engineering science field involving the use of biological systems found in organisms or the use of the living organisms themselves to make scientific advances and adapt those knowledge to various application branches, such as (a) medical biotechnology (including pharmaceutical biotechnology), (b) agricultural biotechnology, (c) industrial biotechnology (including industrial fermentation biotechnology), (d) environmental biotechnology, (e) computational biotechnology, and (f), military biotechnology:

**Blue Biotechnology** It is a specific environmental biotechnology which is based on the use of marine resources to create products, energy, or pollution control.

**Chemical Synthesis (Pharmaceutical)** Most of the active ingredients marketed and sold as drugs are manufactured by chemical synthesis. Chemical synthesis is the process of manufacturing pharmaceuticals using organic and inorganic chemical reactions. The conventional batch reaction vessel is the major piece of equipment used on the process line. The reaction vessel is one of the most standardized equipment designs in the industry. By using heating or refrigeration devices, the chemicals may be boiled or chilled in them, according to process needs. By adding reflux condensation equipment, the vessel may perform complete reflux operations (i.e., recycling of condensed vapors). The vessels can also become evaporators if vacuum is applied. The reactors may also be used to perform solvent extraction operations, and, by operating the agitator at a slow speed, the vessels can serve as crystallizers. Synthetic pharmaceutical manufacture consists of using one or more of these reactor vessels to perform, in a step-by-step fashion, the various operations necessary to make the product. Chemical synthesis effluent generally has a high BOD and COD waste load. The pollutants in chemical synthesis wastewater vary with respect to toxicity and biodegradability. The production steps may generate acids, bases, cyanides, metals, and other pollutants, while the waste process solutions and vessel wash water may contain residual organic solvents. Occasionally, chemical synthesis wastewater is incompatible with biological treatment systems because it is too concentrated or too toxic for the biomass in the treatment system. Thus, it may be necessary to equalize and/or chemically pretreat some chemical synthesis wastewater prior to biological treatment. Primary sources of wastewater from chemical synthesis operations are (a) process wastes such as spent solvents, filtrates, and concentrates, (b) floor and equipment wash water, (c) pump seal water, (d) wet scrubber wastewater, and 5) spills. Wastewater from chemical synthesis plants can be characterized as having high BOD, COD, and TSS concentrations, large flows, and extremely variable pH values, ranging from 1.0 to 11.0.

**Computational Biotechnology** (a) It can be defined as "conceptualizing biotechnology" to address biotechnology problems using computational techniques and makes the rapid organization as well as analysis of biotechnological data possible; (b) it can also be termed gold biotechnology or bioinformatics.

**Dark Biotechnology** It means the military biotechnology that is associated with bioterrorism or biological weapons and bio-warfare using microorganisms and toxins to cause diseases and death in humans, domestic animals, and crops.

**Environmental Biotechnology** (a) It is an interdisciplinary branch of biotechnology using biological systems and/or organisms for conservation of environment, resources, and energy and for protection of humans, animals, and plants on Earth and beyond; it can be of green biotechnology, gray biotechnology, blue biotechnology, gold biotechnology, or white biotechnology; (b) modern green environmental biotechnology has a symbol of "green cross" that involves the construction of resource recovery facilities (RRF), bioreactor landfills, in-vessel or in-bin composting reactors, bioremediation sites, wildlife sanctuary areas,

environmental protection parks, global warming control facilities, salmon ladders, etc. using the best available technologies (BAT) for reclamation of water, air, land, nutrients, methane gas, animals, plants, etc. and production of biofuels, bio-plastics, waste-converted animal foods, etc., in turn achieving environmental conservation, resource sustainability, biodiversity, climate control, ozone layer protection, etc. (c) Gray biotechnology refers to an old traditional environmental biotechnology applications to maintain biodiversity and the partial removal of certain pollutants or contaminants using microorganisms and plants to isolate and dispose of many kinds of substances such as heavy metals and hydrocarbons, but without sustainability of natural resources. Typical examples are the old biological secondary wastewater treatment plants (WWTP) and old sanitary landfills. Modern environmental biotechnology is considered to be a green biotechnology. (d) Blue biotechnology is based on the use of marine resources to create products, energy, or pollution control.

**Fermentation (Pharmaceutical)** Most antibiotics and steroids are produced by the fermentation process, which involves three basic steps: inoculum and seed preparation, fermentation, and product recovery. Fermentation is conventionally a large-scale batch process. The fermentation step begins with a wash water and steam sterilization of the fermenter vessel. Sterilized nutrient raw materials in water are then charged to the fermenter. Microorganisms grown from seed to aid in the fermentation process are transferred to the fermenter from the seed tank and fermentation begins. During fermentation, air is sparged into the batch, and temperature is carefully controlled. After a period that may last from 12 h to 1 week, the fermenter batch whole broth is ready for filtration. Filtration removes mycelia (i.e., remains of the microorganisms), leaving the filtered aqueous broth containing product and residual nutrients that are ready to enter the product recovery phase. There are three common methods of product recovery: solvent extraction, direct precipitation, and ion exchange or adsorption. Fermentation broth contributes pollutants to wastewater from the food materials contained in the broth, such as sugars, starches, protein, nitrogen, phosphate, and other nutrients. Fermentation wastes are very amenable to biological treatment. The spent broth can be satisfactorily handled by biological treatment systems in a concentrated form. Equalizing the broth prior to treatment helps avoid system upsets that may occur if the biota receive too high feed concentrations at one time. The process wastewater from fermentation plants is characterized by high BOD, COD, and TSS concentrations, relatively large flows, and a pH range of approximately 4.0–8.0.

**Gold Biotechnology** It is equivalent to bioinformatics, or computational biotechnology, that addresses biotechnology problems using computational techniques and makes the rapid organization as well as analysis of biotechnological data possible.

**Gray Biotechnology** It refers to an old traditional environmental biotechnology applications to maintain biodiversity and the partial removal of certain pollut-

ants or contaminants using microorganisms and plants to isolate and dispose of many kinds of substances such as heavy metals and hydrocarbons, but without sustainability of natural resources. Typical examples are the old biological secondary wastewater treatment plants (WWTP) and old sanitary landfills. Modern environmental biotechnology is considered to be a green biotechnology.

**Green Biotechnology** (a) It is modern environmental biotechnology that achieves environmental conservation and resource sustainability, or a specific agricultural biotechnology that creates new plant varieties of agricultural interest, biopesticides, biofertilizers, etc. (b) Modern green environmental biotechnology has a symbol of "green cross" that involves the construction of resource recovery facilities (RRF), bioreactor landfills, in-vessel or in-bin composting reactors, bioremediation sites, wildlife sanctuary areas, environmental protection parks, global warming control facilities, salmon ladders, etc. using the best available technologies (BAT) for reclamation of water, air, land, nutrients, methane gas, animals, plants, etc., in turn achieving environmental conservation, resource sustainability, biodiversity, climate control, ozone layer protection, etc.; (c) the area of green agricultural biotechnology is based on transgenics (genetic modification), i.e., an extra gene or genes inserted into their DNA. The additional gene may come from the same species or a different species.

**Industrial Biotechnology (including industrial fermentation biotechnology)** (a) It is the utilization of cells, such as microorganisms, or components of cells like enzymes, to generate products in sectors that are industrially useful, such as food and feed, chemicals, detergents, paper and pulp, textiles, biofuels, and biogas, or to create genetically modified organisms (GMOs) that enhance the diversity of applications and the economic viability of industrial biotechnology; (b) development of biocatalysts (such as enzymes, to synthesize chemicals), improvement of fermentation process, and production of new plastics/textiles, biofuels, etc. are typical examples; (c) a specific industrial biotechnology related to production of wine, cheese, and beer by fermentation is also termed yellow biotechnology; (d) designing more energy-efficient, less polluting, and low resource-consuming processes and products that can beat traditional ones is also termed white biotechnology.

**Medical biotechnology (including pharmaceutical biotechnology)** (a) It has a symbol of "red cross" and involves the use of living cells and other cell materials to find cures for preventing diseases and bettering the health of humans; (b) development of vaccines and antibiotics is a typical example; (c) a specific pharmaceutical biotechnology related to medicine and veterinary products (vaccines, antibiotics, molecular diagnostics techniques, genetic engineering techniques, etc.) is also termed red biotechnology.

**Military Biotechnology** It is also termed dark biotechnology because it is associated with bioterrorism or biological weapons and bio-warfare using microorganisms and toxins to cause diseases and death in humans, domestic animals, and crops.

**Mixing, Compounding, or Formulating (Pharmaceutical)** Pharmaceutically active ingredients are generally produced by batch processes in bulk form and must be converted to dosage form for consumer use. Common dosage forms for the consumer market are tablets, capsules, liquids, and ointments. In addition, active ingredients can also be incorporated into patches and time release capsules. Wastewater sources from mixing, compounding, or formulating operations are (a) floor and equipment wash water, (b) wet scrubbers, and (c) spills. The use of water to clean out mixing tanks can periodically flush dilute wastewaters of unusual composition into the plant sewer system. In general, this wastewater is readily treatable by biological treatment systems. The wastewater from mixing, compounding, or formulating plants normally has low BOD5, COD, and TSS concentrations, relatively small flows, and pH values of 6.0–8.0.

**Pharmaceutical Biotechnology** It is a part of medical biotechnology (or a part of red biotechnology) related to manufacturing of drugs, vaccines, antibiotics, etc. using biological systems or organisms.

**Pharmaceutical Industry** Pharmaceutical industry manufactures drugs, vaccines, antibiotics, products with therapeutic value, etc. using chemical reactors, biological systems or organisms, and many different raw materials Pharmaceutical products are produced by chemical synthesis, fermentation, extraction from naturally occurring plant or animal substances, or refining a technical grade product. The USEPA regulation applies to pharmaceutical industrial facilities are organized into five subcategories: (a) subcategory A (fermentation products), (b) subcategory B (extraction products), (c) subcategory C (chemical synthesis products), (d) subcategory D (mixing, compounding, and formulation), and (e) subcategory E (research organizations).

**Red Biotechnology** It is a specific medical (including pharmaceutical) biotechnology related to medicine and veterinary products (vaccines, antibiotics, molecular diagnostics techniques, genetic engineering techniques, etc.).

**White Biotechnology** It is a specific industrial biotechnology involving white biotechnology designing more energy-efficient, less polluting, and low resource-consuming processes and products that can beat traditional ones.

**Yellow Biotechnology** It is a specific industrial biotechnology related to production of wine, cheese, and beer by fermentation.

## Appendix 1: BPT effluent limitations for subcategory A (fermentation operations), subcategory B (biological and natural extraction operations), subcategory C (chemical synthesis operations), and subcategory D (mixing, compounding, or formulating operations)

| Subcategory | Pollutant or pollutant property | BPT effluent limitation for end-of-pipe monitoring points | |
|---|---|---|---|
| | | Maximum for any one day (mg/L) | Monthly average (mg/L) |
| A: Fermentation operations | COD | 1675 | 856 |
| B: Biological and natural extraction operations | COD | 228 | 86 |
| C: Chemical synthesis operations | COD | 1675 | 856 |
| D: Mixing, compounding, or formulating operations | COD | 228 | 86 |

## Appendix 2: BAT effluent limitations for subcategory A (fermentation operations) and subcategory C (chemical synthesis operations)

| Pollutant or pollutant property | BAT effluent limitations for end-of-pipe monitoring points | |
|---|---|---|
| | Maximum for any 1 day mg/L | Monthly average mg/L |
| Acetone | 0.5 | 0.2 |
| Acetonitrile | 25.0 | 10.2 |
| Ammonia as N | 84.1 | 29.4 |
| n-Amyl acetate | 1.3 | 0.5 |
| Amyl alcohol | 10.0 | 4.1 |
| Benzene | 0.05 | 0.02 |
| n-Butyl acetate | 1.3 | 0.5 |
| Chemical oxygen demand (COD) | 1675 | 856 |
| Chlorobenzene | 0.15 | 0.06 |
| Chloroform | 0.02 | 0.01 |
| o-Dichlorobenzene | 0.15 | 0.06 |
| 1,2-Dichloroethane | 0.4 | 0.1 |
| Diethylamine | 250.0 | 102.0 |
| Dimethyl sulfoxide | 91.5 | 37.5 |

| Pollutant or pollutant property | BAT effluent limitations for end-of-pipe monitoring points | |
|---|---|---|
| | Maximum for any 1 day mg/L | Monthly average mg/L |
| Ethanol | 10.0 | 4.1 |
| Ethyl acetate | 1.3 | 0.5 |
| n-Heptane | 0.05 | 0.02 |
| n-Hexane | 0.03 | 0.02 |
| Isobutyraldehyde | 1.2 | 0.5 |
| Isopropanol | 3.9 | 1.6 |
| Isopropyl acetate | 1.3 | 0.5 |
| Isopropyl ether | 8.4 | 2.6 |
| Methanol | 10.0 | 4.1 |
| Methyl cellosolve | 100.0 | 40.6 |
| Methylene chloride | 0.9 | 0.3 |
| Methyl formate | 1.3 | 0.5 |
| MIBK | 0.5 | 0.2 |
| Phenol | 0.05 | 0.02 |
| Tetrahydrofuran | 8.4 | 2.6 |
| Toluene | 0.06 | 0.02 |
| Triethylamine | 250.0 | 102.0 |
| Xylenes | 0.03 | 0.01 |

## Appendix 3: BAT effluent limitations for subcategory B (biological and natural extraction operations) and subcategory D (mixing, compounding, or formulating operations)

| Pollutant or pollutant property | BAT effluent limitations for end-of-pipe monitoring points | |
|---|---|---|
| | Maximum for any 1 day mg/L | Monthly average mg/L |
| Chemical oxygen demand (COD) | 228 | 86 |

## Appendix 4: NSPS for subcategory A (fermentation operations) and subcategory C (chemical synthesis operations)

| Pollutant or pollutant property | NSPS for end-of-pipe monitoring points | |
|---|---|---|
| | Maximum for any 1 day mg/L | Monthly average mg/L |
| Acetone | 0.5 | 0.2 |
| Acetonitrile | 25.0 | 10.2 |

| Pollutant or pollutant property | NSPS for end-of-pipe monitoring points | |
|---|---|---|
| | Maximum for any 1 day mg/L | Monthly average mg/L |
| Ammonia as N | 84.1 | 29.4 |
| n-Amyl acetate | 1.3 | 0.5 |
| Amyl alcohol | 10.0 | 4.1 |
| Benzene | 0.05 | 0.02 |
| n-Butyl acetate | 1.3 | 0.5 |
| Chlorobenzene | 0.15 | 0.06 |
| Chloroform | 0.02 | 0.01 |
| o-Dichlorobenzene | 0.15 | 0.06 |
| 1,2-Dichloroethane | 0.4 | 0.1 |
| Diethylamine | 250.0 | 102.0 |
| Dimethyl sulfoxide | 91.5 | 37.5 |
| Ethanol | 10.0 | 4.1 |
| Ethyl acetate | 1.3 | 0.5 |
| n-Heptane | 0.05 | 0.02 |
| n-Hexane | 0.03 | 0.02 |
| Isobutyraldehyde | 1.2 | 0.5 |
| Isopropanol | 3.9 | 1.6 |
| Isopropyl acetate | 1.3 | 0.5 |
| Isopropyl ether | 8.4 | 2.6 |
| Methanol | 10.0 | 4.1 |
| Methyl cellosolve | 100.0 | 40.6 |
| Methylene chloride | 0.9 | 0.3 |
| Methyl formate | 1.3 | 0.5 |
| MIBK | 0.5 | 0.2 |
| Phenol | 0.05 | 0.02 |
| Tetrahydrofuran | 8.4 | 2.6 |
| Toluene | 0.06 | 0.02 |
| Triethylamine | 250.0 | 102.0 |
| Xylenes | 0.03 | 0.01 |
| $BOD_5$ | 267 | 111 |
| COD | 1675 | 856 |
| TSS | 472 | 166 |

## Appendix 5: NSPS for subcategory B (biological and natural extraction operations) and subcategory D (mixing, compounding, or formulating operations)

| Pollutant or pollutant property | NSPS for end-of-pipe monitoring points | |
|---|---|---|
| | Maximum for any 1 day mg/L | Monthly average mg/L |
| $BOD_5$ | 35 | 18 |
| COD | 228 | 86 |
| TSS | 58 | 31 |

## Appendix 6: PSES for subcategory A (fermentation operations) and subcategory C (chemical synthesis operations)

| Pollutant or pollutant property | PSES for end-of-pipe monitoring points | |
|---|---|---|
| | Maximum for any 1 day mg/L | Monthly average mg/L |
| Acetone | 20.7 | 8.2 |
| Ammonia as N | 84.1 | 29.4 |
| n-Amyl acetate | 20.7 | 8.2 |
| Benzene | 3.0 | 0.6 |
| n-Butyl acetate | 20.7 | 8.2 |
| Chlorobenzene | 3.0 | 0.7 |
| Chloroform | 0.1 | 0.03 |
| o-Dichlorobenzene | 20.7 | 8.2 |
| 1,2-Dichloroethane | 20.7 | 8.2 |
| Diethylamine | 255.0 | 100.0 |
| Ethyl acetate | 20.7 | 8.2 |
| n-Heptane | 3.0 | 0.7 |
| n-Hexane | 3.0 | 0.7 |
| Isobutyraldehyde | 20.7 | 8.2 |
| Isopropyl acetate | 20.7 | 8.2 |
| Isopropyl ether | 20.7 | 8.2 |
| Methyl cellosolve | 275.0 | 59.7 |
| Methylene chloride | 3.0 | 0.7 |
| Methyl formate | 20.7 | 8.2 |
| MIBK | 20.7 | 8.2 |
| Tetrahydrofuran | 9.2 | 3.4 |
| Toluene | 0.3 | 0.1 |
| Triethylamine | 255.0 | 100.0 |
| Xylenes | 3.0 | 0.7 |

## Appendix 7: PSES for subcategory B (biological and natural extraction operations) and subcategory D (mixing, compounding, or formulating operations)

| Pollutant or pollutant property | PSES for end-of-pipe monitoring points | |
|---|---|---|
| | Maximum for any 1 day mg/L | Monthly average mg/L |
| Acetone | 20.7 | 8.2 |
| n-Amyl acetate | 20.7 | 8.2 |
| Ethyl acetate | 20.7 | 8.2 |
| Isopropyl acetate | 20.7 | 8.2 |
| Methylene chloride | 3.0 | 0.7 |

## Appendix 8: PSNS for subcategory A (fermentation operations) and subcategory C (chemical synthesis operations)

| Pollutant or pollutant property | PSNS for end-of-pipe monitoring points | |
|---|---|---|
| | Maximum for any 1 day mg/L | Monthly average mg/L |
| Acetone | 20.7 | 8.2 |
| Ammonia as N | 84.1 | 29.4 |
| n-Amyl Acetate | 20.7 | 8.2 |
| Benzene | 3.0 | 0.6 |
| n-Butyl acetate | 20.7 | 8.2 |
| Chlorobenzene | 3.0 | 0.7 |
| Chloroform | 0.1 | 0.03 |
| o-Dichlorobenzene | 20.7 | 8.2 |
| 1,2-Dichloroethane | 20.7 | 8.2 |
| Diethylamine | 255.0 | 100.0 |
| Ethyl acetate | 20.7 | 8.2 |
| n-Heptane | 3.0 | 0.7 |
| n-Hexane | 3.0 | 0.7 |
| Isobutyraldehyde | 20.7 | 8.2 |
| Isopropyl acetate | 20.7 | 8.2 |
| Isopropyl ether | 20.7 | 8.2 |
| Methyl cellosolve | 275.0 | 59.7 |
| Methylene chloride | 3.0 | 0.7 |
| Methyl formate | 20.7 | 8.2 |
| MIBK | 20.7 | 8.2 |
| Tetrahydrofuran | 9.2 | 3.4 |
| Toluene | 0.3 | 0.1 |

| Pollutant or pollutant property | PSNS for end-of-pipe monitoring points | |
|---|---|---|
| | Maximum for any 1 day mg/L | Monthly average mg/L |
| Triethylamine | 255.0 | 100.0 |
| Xylenes | 3.0 | 0.7 |

## Appendix 9: PSNS for subcategory B (biological and natural extraction operations) and subcategory D (mixing, compounding, or formulating operations)

| Pollutant or pollutant psroperty | PSNS for end-of-pipe monitoring points | |
|---|---|---|
| | Maximum for any 1 day mg/L | Monthly average mg/L |
| Acetone | 20.7 | 8.2 |
| n-Amyl Acetate | 20.7 | 8.2 |
| Ethyl acetate | 20.7 | 8.2 |
| Isopropyl acetate | 20.7 | 8.2 |
| Methylene chloride | 3.0 | 0.7 |

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# Chapter 3
# Vermicomposting Process for Treating Agricultural and Food Wastes


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## Acronyms and Nomenclature

| | |
|---|---|
| B | Biochar |
| C | Carbon |
| C:N | Carbon-to-nitrogen ratio |
| CEC | Cation exchange capacity |
| CPMV | Cowpea mosaic virus |

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| | |
|---|---|
| DEH | Dehydrogenase |
| EC | Electric conductivity |
| ELISA | Enzyme-linked immunosorbent assay |
| FTIR | Fourier transform infrared |
| GC-MS | Gas chromatography-mass spectrometry |
| GW | Green waste |
| HA | Humic acid |
| K | Potassium |
| MSW | Municipal solid waste |
| N | Nitrogen |
| P | Phosphorus |
| PFRP | Process to Further Reduce Pathogens |
| SC | Skin coffee |
| TCLP | Toxicity characteristic leaching procedure |
| TMV | Tobacco mosaic virus |
| TOC | Total organic carbon |
| US EPA | US Environmental Protection Agency |
| WSC | Water-soluble carbon |
| WSP | Water-soluble phosphorus |

## 3.1 Introduction

### *3.1.1 Summary*

Vermicomposting is a novel municipal/agricultural sludge and solid waste treatment process that uses earthworms (oligochaete annelids) for the biodegradation of the sludge and/or organic solid wastes, such as agricultural and food wastes. This novel biological system is alternately called earthworm conversion, vermicomposting, vermistabilization, worm composting, or annelidic consumption. The worms maintain aerobic conditions in the organic substances while accelerating and enhancing the biological decomposition of the organic substances. The main product of the vermicomposting (earthworm conversion) process is the worm's castings. In some process arrangements, there may be a net earthworm production. The excess earthworms may then be sold for fish bait or animal protein supplement. Earthworm marketing is a complex problem; for municipal sludge applications, surplus earthworms may be considered a by-product, while the principal product is the castings, which can be a resource, called vermicompost, compost, soil conditioner, or compost fertilizer.

This chapter presents the following: (a) an introduction and review of the vermicomposting process; (b) technology development, technical problems, legal problems, and technology breakthrough of the process; (c) current status and resources; (d) vermicomposting process design considerations; (e) process applications with special emphasis on agricultural and food waste treatment; and (f) future

development and directions of the process. Recent advances in vermicomposting process research and new process applications are reported.

### 3.1.2 Process Description

Vermicomposting differs from the conventional composting of wastewater treatment plant sludge. In the vermicomposting process, worms are used to develop an optimum environment for consuming or metabolizing the sludge and produce feces or castings. These castings may be used as a soil conditioner [1–38, 41–63]. In the conventional composting process, microorganisms are used for the degradation of sludge and other putrescible organic solid materials under an aerobic metabolism environment. Conventional composting is also suitable for converting undigested primary sludge, secondary sludge, and certain solid wastes into an end product amenable to resource recovery with a minimum capital investment and relatively small operating commitment [39–40, 45].

Figure 3.1 (Source: US EPA) shows a basic simple vermicomposting process [60, 62] that requires worm beds and an ample supply of worms. Generally, digested and dewatered sludge is put into the beds, although experiments are underway, where raw liquid sludge is placed in beds. If anaerobic digestion is used prior to earthworm conversion, additional pretreatment may be needed. A bulking agent such as wood chips may be useful in some cases for keeping the bed porous and

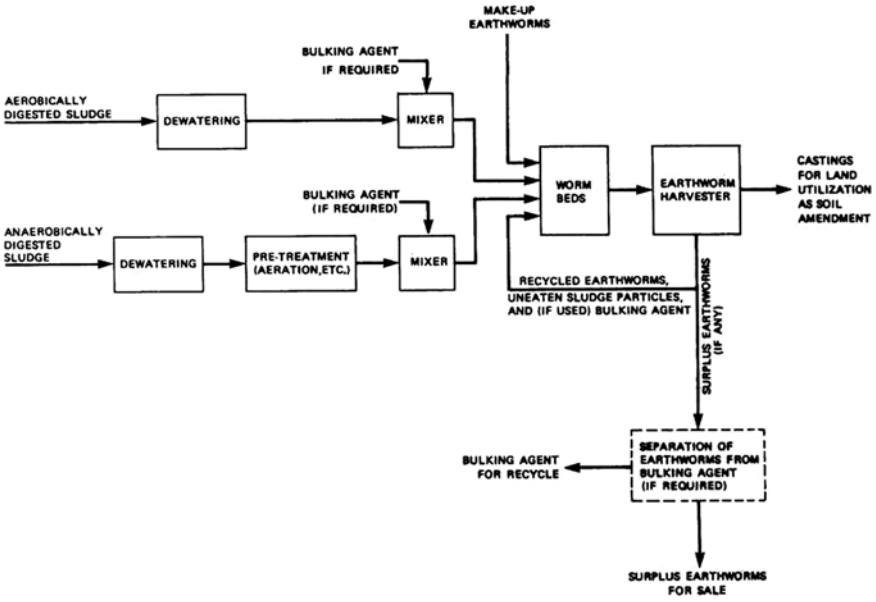


**Fig. 3.1** Diagram of an earthworm conversion process

aerobic, especially if moisture is high. Sludge is, however, generally applied without any bulking agent. A worm bed may take the form of a simple tray. Windrows similar to those for composting may also be used. After the worms have consumed the sludge, they must be separated from the castings. This may be done with an earthworm harvester, a drum screen that rotates on a nearly horizontal axis. Castings fall through the screen openings, while worms tumble through the length of the drum. Section 3.6 contains some critical operational parameters for the earthworm conversion process.

## 3.2 Technology Development

Conversion of sludges (or biosolids) into topsoil by earthworms was initially attempted by Mitchell et al. of the State University of New York at Syracuse, College of Environmental Science and Forestry in 1977 [1]. Later, Mitchell et al. investigated the potential role of the earthworm, *Eisenia foetida*, on the decomposition of sewage sludge in drying beds and reported the results in 1980 [2]. Specifically, Mitchell et al. sought to determine the decomposition rates of biosolids in drying beds as indexed by consumption of oxygen and evolution of carbon dioxide and methane, to ascertain whether *E. foetida* can alter the form and rate of decomposition, and to ascertain the relationship among specific biotic and abiotic components in decomposition. At two facilities tested, the aerobic and anaerobic bacteria were abundant, and the dominant bacteria were not enteric. A computer simulation model regarding the role of macroinvertebrates in decomposition was used to analyze the effects of the earthworm.

In August 1980, Camp, Dresser and McKee, Inc., of Boston, MA, USA, completed a technical report [3] which assessed the technical and economic feasibility of vermicomposting or vermistabilization process based on several pilot-scale studies conducted by private entrepreneurs. The assessment was based on examining facilities and costs for a municipal operation serving (a) a community of 50,000 persons and (b) a community of about 500,000 persons. Vermicomposting was compared to three other methods of solid waste management: sanitary landfill, windrow composting, and combustion. In 1980, vermicomposting was estimated to cost about $24–32 per ton of waste processed (note: 1 ton = 2000 pounds; 1 pound = 0.454 kg).

In 1981, Hornor and Mitchell [4] studied the effect of the earthworm, *Eisenia foetida*, on fluxes of volatile carbon and sulfur compounds from sewage sludges. Hartenstein [5] suggested the potential use of earthworms as a solution to sludge management. In Hartenstein's study at the State University of New York at Syracuse [5], the feasibility of using earthworms in the management of municipal sludges was examined in detail. Results of tests performed by Hartenstein on two earthworm species—*E. eugeniae* and *E. foetida*—were reported. The following observations were made: (a) the toxicity of worm casts to the earthworms signifies the need to retain *E. foetida* in its source of food (biosolids) as long as, or slightly longer

than, the time required to convert the sludge into castings; (b) knowledge of the quantity of material passing through the earthworm gut per unit of time, for a particular ingestible sludge, permits prediction of sludge quantity manageable per unit time; and (c) *E. foetida* fails to gain weight rapidly, if at all, on unlimited supplies of certain organic materials.

Also in 1981, Collier and Livingstone [6] completed research sponsored by the National Science Foundation. They used earthworms of the redworm (*E. foetida*) species to accomplish vermicomposting or vermistabilization of biosolids from the San Jose and Santa Clara Wastewater Treatment Plants in California, USA. Ninety tons of earthworm manure were produced from the sludge over a 5-year period. Different size windrows were populated with different densities of earthworms, and castings were harvested by passing windrow contents through a rotating screen which separated the worms from the castings for reuse. Plants in castings outgrew plants in topsoil by a factor of 4 to 1. Their 1981 cost analysis showed the system to be cost effective at a cost of $29.45 per dry ton in a 10 ton per day facility and to return a profit of $3.34 per dry ton if castings were produced at the rate of 50 tons per day.

In 1982, Hartenstein [8] reported (a) the metabolic parameters of the earthworm *Eisenia foetida* in relation to temperature and (b) the potential use for manure management and as a source of protein biomass. In 1983, Chosson and Dupuy [9] demonstrated their improvement of the cellulolytic activity of a natural population of aerobic bacteria (enrichment culture) and presented their isolation and characterization of worm gut and compost cellulolytic strains. In 1984, Hartenstein et al. [10] attempted to use earthworms in trickling filters for wastewater treatment.

In March 1984, Loehr et al. [11] presented the results of an investigation of the vermistabilization process using stabilized and unstabilized wastewater treatment sludges. Four earthworm species were evaluated: *E. foetida*, *E. eugeniae*, *P. hawayana*, and *P. excavatus*. *E. foetida* was found to have the greatest overall reproductive capacity. The best growth of *E. foetida* in terms of total biomass weight gain occurred in media that had a total solids content, wet basis, of between 9 and 17%. The best growth and cocoon production for this earthworm species was shown to occur at temperatures of 20–25 °C. With both dewatered and liquid sludges, vermistabilization units functioned successfully for long periods of time—up to 1 year for dewatered sludge and at least 6 months for the liquid sludges. Cost estimates indicated that the capital and annual costs of liquid vermistabilization were competitive with those for other sludge management systems.

In 1985, Loehr et al. of Cornell University [12] evaluated several fundamental factors that affect the performance of the vermistabilization process such as temperature, moisture content of the waste material, and the combined use of several earthworm species (polyculture). The earthworms *Dendrobaena veneta*, *Eisenia foetida*, *Eudrilus eugeniae*, *Perionyx excavatus*, and *Pheretima hawayana* were used in one or more of the studies. The best growth and reproduction of these species occurred at temperatures of 20–25 °C. The growth of all five species was reduced at 30 °C and death occurred at 35 °C. Of the five species, *Eisenia foetida* produced the largest number of young in a 20-week study. The growth of *Eisenia*

*foetida* occurred optimally in media with a total solids content, wet basis, of between 9 and 16%. Polyculture did not exhibit any obvious advantages over monoculture.

Stabilization of liquid sludge, or biosolids, by the vermistabilization process was also reported by Loehr et al. of the University of Texas at Austin, TX, USA [13]. The investigators conducted basic studies to identify fundamental factors that affect the performance of the vermistabilization process and applied studies to determine design and management relationships. As earthworms are a key component of the liquid sludge vermistabilization (LSVS) process, control reactors that did not contain worms failed in a much shorter period of time than did the reactors with the worms. LSVS reactors that were not overloaded functioned successfully for 140–198 days and were stopped only because the project ended. Oxidized nitrogen (nitrates) in the drainage from the LSVS reactors indicated that aerobic conditions were being maintained. Liquid primary sludge and liquid waste activated sludge (biosolids) can be stabilized by the LSVS process.

LSVS reactors were not adversely affected by short-term, large variations in loading rates. Liquid primary sludge was stabilized to about the same degree as liquid aerobically digested sludge in the LSVS process. Moisture balances indicated an overall moisture loss of 4–20%. Loading rates of about 21,000 g/m$^2$/week volatile solids or less resulted in satisfactory operation of LSVS reactors stabilizing liquid primary and liquid waste activated sludge. Loading rates greater than 1200 g/m$^2$/week volatile solids could be used for LSVS reactors stabilizing liquid aerobically digested sludge. With LSVS reactors, the disposal of residual stabilized solids occurs at long intervals. The total solids content of the stabilized residual solids in the LSVS reactors was from 14 to 24%, a considerable increase from the 0.6 to 1.3% that was added. LSVS proved to be a successful process for both dewatering and stabilization. The stabilized residual solids had approximately the same characteristics regardless of the type of liquid sludge added to the reactors. Size and cost estimates indicated that LSVS might be an economically feasible sludge management process.

Reviews of the literature on sludge characteristics, solids concentration and conditioning, stabilization and inactivation, incineration, and ultimate disposal and utilization were conducted by Hasit of Weston, Inc., West Chester, PA, USA, in 1985 [14] and 1986 [15]. Vermistabilization was one of the sludge management technologies reviewed and assessed.

In 1986, Stafford and Edwards [16] of Rothamsted Experimental Station, Harpenden, England, used earthworms in the field to indicate levels of soil pollution and in the laboratory for the ecotoxicological testing of industrial chemicals. An earthworm bioassay procedure developed at the Waterways Experiment Station in Vicksburg, Mississippi, USA, was modified and evaluated as a method of providing information on heavy metal bioavailability in contaminated soils and sediments from Europe. Eight soils/sediments containing elevated levels of at least one of the elements Zn, Cu, Cd, and Pb were selected, as well as a control and a reference soil. Six earthworm species, including the WES bioassay earthworm *E. foetida*, and five field species were grown in the soil for periods of 15, 28, or 56 days. Concentrations of the elements Zn, Cu, Cd, Ni, Cr, and Pb present in the earthworm samples

(corrected for the presence of soil-derived metals within the earthworm gut) were compared between earthworm species from the same soil and for each earthworm species from a range of metal-contaminated soils/sediments.

A US Patent No. 4971616, entitled "Process for Preparing Organic Compost from Municipal Refuse," was awarded to Mark E. Glogowski on November 20, 1990 [17]. The patent involved the use of earthworms for treatment and disposal of shredded cellulose refuse.

The earthworm *Eisenia foetida* is known to contain bactericidal enzymes. In 1990, Amaravadi et al. tested the earthworm for virucidal activity using cowpea mosaic virus (CPMV) and tobacco mosaic virus (TMV) as model agents [18]. Earthworms were fed cellulose saturated with a virus suspension, and their excreted castings were analyzed for structurally intact virus protein using enzyme-linked immunosorbent assay (ELISA) and virus infectivity by local lesion assays. Observations of the feeding experiments indicated a considerable reduction in the infectivity of both viruses. Virucidal activity was also observed when virus suspensions were incubated with the earthworm enzyme extract and analyzed by local lesion assay. The observed reductions in the infectivity of both viruses suggested that *E. foetida* might possess a virucidal enzyme system and, accordingly, might contribute to the inactivation of pathogenic viruses potentially associated with land application of sewage sludges and livestock manure.

Another US Patent No. 5055402, entitled "Removal of Metal Ions with Immobilized Metal Ion-Binding Microorganisms," was awarded to Greene et al. on October 8, 1991 [19]. The inventors cited the use of earthworms.

## 3.3 Problems and Technology Breakthrough

### *3.3.1 Introduction*

While vermicomposting has demonstrated its benefits, the process faces obstacles in meeting US regulatory requirements. This section presents the problems and progress made in vermicomposting, i.e., new technologies that have been developed to overcome the technical and legal problems.

### *3.3.2 Problems*

Scientific interest in earthworms is on the rise worldwide [20–26]. At the Fifth International Symposium on Earthworm Ecology in 1994, 183 presentations were given at the 1994 International Symposium that were divided into two general categories: using earthworms directly in horticulture and agriculture to enhance crop growth and using earthworms to turn various residuals into beneficial composts for

reuse. Despite the increasing number of studies, however, financial support for vermicomposting research has been cut by the funding agencies in the USA since 1990.

Another problem is the process's failure to meet regulatory requirements. The US Environmental Protection Agency's "Process to Further Reduce Pathogens (PFRP) Requirements" for in-vessel or aerated static pile composting of biosolids requires maintaining a temperature of 55 °C or higher in composting for 3 days. Worms can survive in thermophilic composting windrows, but they tend to stick to the edges of the pile. Temperatures above 35 °C, which is the heat generated by thermophilic composting, are too high for earthworms and will kill them. In vermicomposting, temperatures are generally kept below 30 °C. While organic substances can be effectively processed by worms at low temperature range, the US EPA's PFRP requirements cannot be met. Progress in vermicomposting of organic substances proceeded slowly due to the above technical and legal problems.

There has been continuous debate in the State of California, USA, regarding the classification and potential regulation of composting facilities. A draft of regulations released in August 1994 by the California Integrated Waste Management Board (CIWMB) excludes vermicomposting operations from the notification and permitting that would be required of most larger facilities using conventional thermophilic composting to process yard trimmings, manure, biosolids, and other organic substances [24]. Under current California ruling, vermicomposting may be considered an agricultural operation, in which vermiculture uses organics as a feedstock for raising worms in a worm farm. The advantage is that the owners and operators of the vermicomposting facilities have free rein in process control and management and are not subject to the state inspections. The disadvantage is that as long as vermicomposting is not recognized as solid waste disposal process, the progress for its technology development and application will be slow.

Noting the US federal requirements on PFRP, vermiculturists now precompost the organic substances in the thermophilic temperature range for pretreatment and disinfection. Worms are added to compost windrows for a subsequent vermiphilic decomposition after the heat of initial thermophilic decomposition subsides. In comparison with conventional thermophilic composting as a process, the modified vermicomposting process has a shorter processing time. With conventional thermophilic composting alone, it is difficult to produce high-quality products under 6 months, while with the modified vermicomposting (i.e., thermophilic composting pretreatment plus vermicomposting posttreatment), it is possible to create a marketable end product in one-sixth of the operating time. Compared to the conventional thermophilic compost end product, vermicompost contains smaller particles and worm cocoons (meaning a free workforce for the future) and has lower odor and enhanced microbial activity. According to commercial estimates, consumers would be willing to pay up to three times more for the vermicompost, or worm castings, than they would pay for most normal thermophilic compost. Many commercial-scale breakthroughs in vermicomposting technology have been noted and are introduced below [23–25].

The Resource Conversion Corporation (7825 Fay Avenue, Suite 380, La Jolla, CA 92037, USA) has developed a proprietary "Vermiconversion System" which

significantly modifies traditional vermiculture windrow methods. Variations include sloped plastic liner beneath the windrow, reclaim water, aeration piping, and a sprinkler to maintain proper temperature levels. In July 1994, Resource Conversion Corporation and Sanifill, a national landfill company, together opened Canyon Recycling outside of San Diego, which is a 6-acre (note: 1 acre = 4047 $m^2$ = 0.4046 ha) facility currently processing around 100 tons per day of brush, green material, and wood from construction and demolition operations and manure from the San Diego Zoo. After grinding and screening, some woody materials are marketed "as is." Leafy greens, wood fines, and manures proceed through a blending plant, then "cured" via thermophilic composting to neutralize pathogens. After curing, the pre-processed material is applied to the vermiculture windrows in thin layers. The rows are carefully segregated and check for biological reactions to new feedstock. Two to four inches of material are applied every other day continuously. The rows are compartmentalized to prevent possible contamination of the entire facility. The facility adopts both the thermophilic composting pretreatment (for 3–15 days aiming at pathogen reduction and decomposition) and the vermicomposting posttreatment (for additional 15–30 days aiming at final curing and decomposition). Their worm castings product is being sold for $33 per ton on the bulk market. The company is now building a 100-acre facility to manage San Diego's biosolids under a 20-year contract.

The Oregon Soil Corporation (17,810 SSW Bunker Oak Road, Aloha, OR, USA) has developed a technology to reduce the space requirements for a vermiculture operation using a "continuous flow system." The newly developed continuous flow system utilizes a raised, 120-foot trough (note: 1 foot = 0.3048 meter) that is 2.5 ft deep and 8 ft wide, with a mesh floor. An adapted manure spreader makes a daily pass over the trough, laying down about 3 inches (note: 1 inch = 2.54 cm) of prepared organic materials, or roughly 6 tons per day (note: 1 ton = 2000 pounds; 1 pound = 0.454 kg). As the worms eat up through it, the worm castings sink down and are mechanically scraped off the bottom of the screen and collected. Under the protection of a greenhouse-like structure, the worm reactor can handle about 2500 tons of organic residuals a year. Currently, the Oregon Soil Corporation accepts year trimmings deliveries from local landscapers and picks up food scraps and paper from 15 Fred Meyers grocery stores around Portland. They process around 5 or 6 tons of food scraps, over 2 tons of supplemental yard trimmings or compost, and around half a ton of paper per day. It takes only 21 days to make earthworm castings using the continuous flow system.

The Worm Concern (note: it is The Worm Connection now in California, USA) had grown to a 22-acre spread during its 18 years in business. Around 100 tons per day of brush, leaves, tree limbs, grass clippings, and horse manure are delivered to the site for processing. Incoming material first passes through a grinder and a trammel before being placed in windrows by a front-end loader. The facility adopts both anaerobic windrow preprocessing (in which the piles are not turned at all until material is moved to the worm rows) and vermicomposting posttreatment using worms. At harvest time, worm rows are scooped up with a front-end loader and placed in screen. Castings come out one end and the worms come out the other, unharmed.

Their vermicastings are sold in bulk, blended on site with mulch or other landscape products, and bagged for retail sale.

Finally, the Environmental Earthworm Projects, Inc. (8114 Port Said Street, Orlando, FL 32813, USA) currently operates two sites handling a combined total of 30 tons per month of composted yard trimmings from the Orange County landfill and 20 tons per month of shredded cardboard. They also have conducted earthworm trials with RDF fines from Palm Beach County and other organics.

### *3.3.3 Progress in Vermicomposting Outside the USA*

Engineers and scientists in the countries other than the USA have shown their interest in the theories, principles, and applications of the vermistabilization process since 1992. Practical applications of the vermicomposting process in disposal of biosolids and organic solid wastes have been attempted by many entrepreneurs around the world. The progress in vermicomposting process development and applications outside of the USA is discussed below [20–26].

In November 1992, Concheri et al. of Italy reported humification of organic waste materials during earthworm composting [20]. In March 1993, Anton et al. of Spanish Council for Scientific Research, Madrid, Spain, reported carbofuran acute toxicity to *Eisenia foetida* earthworms [21].

In 1993, Van-Gestel and Ma of the National Institute of Public Health and Environmental Protection, Bilthoven, Netherlands, reported their results on the development of QSARs in soil ecotoxicology [22]. The earthworm toxicity and its soil sorption of chlorophenols, chlorobenzenes, and chloroanilines were documented by the investigators of Netherlands.

Also in 1993, Original Vermitech Systems, Ltd. (2328 Queen Street East, Toronto, Ontario M4E1G9, Canada; Tel. No. 416-693-1027) installed a composting unit with a capacity of up to 600 pounds of organics per day at the Brockville Psychiatric Hospital in Ontario, Canada. It is the largest composter in Canada right now [23]. The system is equipped with panels and temperature sensors for maintaining a tolerable environment for the worms.

At the Fifth International Symposium on Earthworm Ecology, held at Ohio State University in 1994, scientists from the University of Agricultural Sciences in Dharwad, India, told conference attendees that in their experiments, earthworms could turn crop and weed residuals into vermicompost at the rate of 8–10 tons per year from a bed area of 100 $m^2$ [24, 25]. At the same symposium, scientists from the Biosystems Research Group at the Open University, Milton Keynes, in England, reported on their experiments of the modified vermicomposting process [24, 25]. The English scientists added earthworms to compost windrows after the heat of initial decomposition subsided. Their worms worked well in this situation and shortened the time of curing and stabilization of the compost.

Changes in heavy metal extractability and organic matter fractions after vermicomposting of sludges from a paper mill industry and wastewater treatment plant

were reported by Elvira et al. of the University of Vigo, Spain, in 1995 [24–26]. According to the researchers from the Department of Natural Resources, University of Vigo, vermicomposting of paper mill sludge has been proven to be viable in their country.

## 3.4 Pioneers, Current Status, and Resources

The pioneers of the vermistabilization process, as well as its current status and resources, are introduced in this section in detail.

### *3.4.1 Pioneers and Current Status*

Many pioneers of the vermicomposting process deserved to be recognized. Jack E. Collier and Diane Livingstone were the principal investigators of a milestone research project sponsored by the National Science Foundation entitled "Conversion of Municipal Wastewater Treatment Plant Residual Sludges into Earthworm Castings for Use as Topsoil" [7]. Collier and his wife still operate an earthworm farm in California, USA, which provides high-quality earthworms for all types of earthworm research including vermistabilization. The Colliers often serve as consultants on their vermistabilization technology to individuals or organizations. Dr. Mark Buchannon, a soil scientist of the University California at Santa Cruz, USA, recently collaborated with the Colliers to complete his PhD research in a similar field.

Dr. Raymond C. Loehr of the University of Texas at Austin, Department of Civil Engineering, Austin, TX, USA, is another legend in vermistabilization technology development [11–13]. Dr. Loehr, too, consults on vermistabilization research and applications, if requested.

Dr. Clive Edwards, Professor of Entomology at Ohio State University, has also been instrumental as the founder of the International Symposium on Earthworm Ecology and has conducted several key vermicomposting projects leading to commercialization of the process.

Practicing vermicomposting technologists who can provide assistance in vermicomposting facility installation and process operation include Frank Stevenson of the Environmental Earthworm Projects, Inc.; Dan Holcombe of Oregon Soil Corporation; Albert Eggen of Original Vermitech Systems, Ltd.; Joseph Roberts of Resource Conversion Corporation; Tim Morhar of The Worm Connection; and Sandra Kandracs of Enviro-Ganics.

Writers/reporters Gene Logsdon, David Riggle, and Hannah Holmes discussed the progress of vermicomposting technology in two articles for *BioCycle* [24, 25], a trade journal that documents and reports the scientific knowledge and commercial news involving worms.

Steven Zorba Frankel and Stephen White of the Edible City Resource Center have published a 32-page quarterly newspaper, *Worm Digest* [27–39], which promotes vermicomposting technology as well as other technologies involving the use of earthworms. Today, *Worm Digest* reports on the subjects of worms and worm composting for organic waste conversion and soil enrichment. The newspaper generally features a wide variety of interesting and practical information to help promote awareness of vermiculture eco-technology on all levels. Columns such as the following appear intermittently in each issue [27–29]: Worm Shorts x New Products x International Worm News x The Industrious Worm (large-scale projects) x Hands-On x Worm Workers x Kids' Corner/Page x Questions & Answers x Eco-Logic x Worm Stories x Cyber-Worm x Advertisements & Resource Listings x Calendar of Events.

At the request of environmental engineers in Ukraine, the authors conducted an investigation on the current status and future direction of the vermistabilization process. It was discovered that the vermistabilization (vermicomposting) operations/research in sites such as Syracuse, NY; Ithaca, NY; West Chester, PA; San Jose, CA; and Austin, TX, in the USA was terminated due to minor technical and legal problems and a lack of financial and public support. It is encouraging to learn, however, that several companies in the USA and Canada have seriously conducted their research for modification and optimization of the vermicomposting (or vermistabilization) process despite the lack of proper funding. Now the process has been improved and commercialized, and many large-scale vermicomposting or vermiculture projects in Florida, California, Oregon, and Ontario are in progress.

Earthworm research is still being widely conducted by soil scientists and environmental scientists around the world. Earthworms are tested as the organisms for organic waste disposal, the toxicity indicators of the ecological system, or as the topsoil producers. As mentioned, there is even an annual International Symposium on Earthworm Ecology.

Interest in the vermistabilization process for sludge management has quickly spread from the USA to European and Asian countries [20–105], indicating that there will always be ample room for additional research on process improvement.

To explore or establish any international cooperative programs in the field of environmental engineering, readers are encouraged to contact the authors and the experts listed in Sect. 3.4.2 for technical or managerial assistance.

### *3.4.2 Resources*

Important resources of the vermicomposting process around the world are introduced in this section. It should be noted that the first letter of each resource defines its nature in accordance with the following *key: Associations (A), Publications (P), Retail Businesses (R), Consultants (C), Distributors (D).*

P 1. Edible City Resource Center, *Worm Digest*, PO Box 544, Eugene, OR 97440, U.S. Tel. No./Fax No. (call first) 541-485-0456.
R 2. The Worm Factory, RR # 3, Perth, Ontario, Canada K7H 3C5. Tel. No. 613-267-5540.
A 3. The Composting Council of Canada, Canada. Tel. No. 416-535-0240; Fax No. 416-536-9892. e-mail address: ccc@compost.org.
A 4. Association of Oregon Recyclers, PO Box 15279, Portland, OR 97210, U.S. Tel. No. 503-661-4475.
P 5. BioCycle, Journal of Composting & Recycling (monthly), 419 State Avenue, Emmaus, PA 18049, U.S. Tel. No. 800-661-4905; 610-967-4135.
W 6. Lake County Worm Farm, PO Box 1332, Kelseyville, CA 95451, U.S. Tel. No. 800-399-9464; Fax No. 707-279-8031.
P 7. Australian Worm Growers Association, PO Box 318, Ferntree Gully, VIC 3156, Australia.
R 8. Arlan & Sons (bookseller), 11881 Arroyo, Santa Ana, CA 92705, U.S. Tel. No. 714-838-8539; Fax No. 714-838-4950. e-mail address: arlan@neptune.net.
R 9. Avant Garden Vermicomposting Systems (worm bins), PO Box 1047, Point Reyes Station, CA 94956, U.S. Tel. No. 415-663-1975; Fax No. 415-663-1975.
C 10. Vermitechnology Unlimited, Inc., PO Box 130, Orange Lake, FL 32681, U.S.
A 11. International Worm Growers Association, PO Box 887, Littlerock, CA 93543 U.S. Tel. No. 805-9442994, Fax No. 805-944-3965.
R 12. WormWide Books, 20 Forest Avenue, Kingston Park, South Australia 5049, Australia. Tel. No. 610412-112285; Fax No. 61-08-377-2668.
W 13. Rainbow Worm Farm, 24700 County Road, No. 95, Davis, CA 95616, U.S. Tel. No. 916-758-9906; Fax No. 916-756-0414.
C 14. Oregon Soil Corporation, 1324 Beaver Lane, Oregon City, OR 97045, U.S. Tel Nos. 503-557-9742, 503-629-5933.
R 15. Flowerfield Enterprises, 10332 Shaver Road, Kalamazoo, MI 49002, U.S. Tel. No. 616-327-0108.
C 16. Roberta Trombley, 3030 Marshall, Cincinnati, OH 45220, U.S. Tel. No. 513-683-2340.
D 17. Viscor Distribution Inc. (Worm Bins), 12165 Cherrywood Drive, Maple Ridge, BC, Canada V2X OB7. Tel. No. 800-609-1223; Fax No. 604-467-9661.
R 18. Worms & Worm Boxes, 968 Valencia Street, San Francisco, CA 94110, U.S. Tel. No. 415-282- WORM.
W 19. Willingham Worm Farm, Rt. # 1, Box 241, Butler, GA 31006, U.S. Tel. No. 912-862-5545.
W 20. Manchester Worm Farm, 1131-0 Tolland Turnpike, Manchester, CT 06040, U.S. Tel. No. 203-647-8067.
C 21. Environmental Recycling Systems, PO Box 904, Alpine, CA 91903, U.S. Tel. No. 619-445-1873; Fax No. 619-445-6057.
C 22. Vermiculture Services International, U.S. Tel. No. 800-399-9464; Fax No. 707-279-8031.
D 23. Recycle-It Corporation, U.S. (distributor of worm bins, curbside recycling bins, and backyard composting bins) Tel. No. 800-769-1044.

W 24. Olympic Worm Casting Farm, McCleary, WA, U.S. Tel. No. 206-495-3762.
C 25. Casting a New Future, Portland, OR, U.S. Tel. No. 503-246-7382.
D 26. RPM, 2829 152nd Ave. NE, Redmond WA 98052, U.S. Tel. No. 800-867-3201.
27. Sound Resource Management Group, Inc., 119 Pine Street, Seattle, WA 98101, U.S. Tel. No. 206-622-9454; Fax No. 206-622-9569.
R 28. Worm World, 26 Ihnat Lane, Avella, PA 15312, U.S. Tel. No. 412-356-2397.
C 29. Resource Conversion Corporation, 7825 Fay Avenue, Suite 380, La Jolla, CA 92037, U.S. Tel. No. 619551-4800.
C 30. Environmental Earthworm Projects, Inc., 8114 Port Said Street, Orlando, FL 32813, U.S. Tel. No. 407678-6454.
C 31. Original Vermitech Systems, Ltd., 2328 Queen Street East, Toronto, Ontario, M4E1G9, Canada. Tel. No. 416-693-1027.
C 32. The Worm Connection, 581 Camino Manzanas, Thousand Oaks, CA 91360, U.S. Tel. No. 805-496-2872, Tel/Fax No. 805-376-9918.

## 3.5 Process Design Considerations

### *3.5.1 Process Adoption and Advantages*

Earthworm castings are essentially odorless when dry; when damp, they have a mild odor like a good quality topsoil. Also, castings have a favorable appearance. When sifted and dry, they are granular, about 0.02–0.1 inches (0.5–3 mm) in maximum dimension (with some fines); color is brownish gray. In a study where municipal sludge was applied to a wheat crop, it was found that when earthworms were added to the sludge, the germination rate of the wheat improved [52]. The odor, appearance, and soil supplementation advantages of the earthworm conversion process may help in the acceptance of sludge by farmers and householders.

Earthworm conversion affects several other sludge characteristics. The oxygen uptake rate increases [48]; the acid-extractable fraction of various nutrients increases [52]. The volatile content of the solids drops slightly, and humic acid concentrations fluctuate [48]. While these effects may be beneficial, there are no data to show how the results affect design or operation of earthworm conversion installations.

The earthworm conversion process would appear to be low in cost, although this cannot be said with certainty, since no cost data are available for full-scale operations on sludge. The process does not require chemicals, high temperatures, or large amounts of electricity. Only a small amount of low-speed mechanical equipment is needed. Significant expenditures may be required to offset the potential operating difficulties discussed below.

### 3.5.2 *Process Operation and Troubleshooting*

A number of potential operating difficulties and their solutions exist in the earthworm conversion process. None of these difficulties are insurmountable, however. Probably the most difficult problem is to economically pretreat anaerobically digested sludge so that it is nontoxic to the worms [61, 63]. Other problems that must be considered include:

- Worm drowning: Worms must be protected from flooding.
- Worm loss due to migration from the process: Caused by flooding, toxic sludge, unpalatable sludge, adjoining areas attractive to worms, lack of artificial lighting on rainy nights.
- Toxicity of sludge to worms: Significant for anaerobically digested sludge. However, toxicity is eliminated by exposing the sludge to air for 2 months [48] or wetting sun-dried sludge daily for 14 days [52]. Stabilization by lime or chlorine is not recommended for sludge that will be fed to earthworms. Toxicants such as copper salts might also cause problems. Aerobic digestion is best suited for sludge to be converted by earthworms.
- Toxicity or unpalatable nature of dewatering chemicals: Avoided at Hagerstown, Md., by use of food-grade polymer [50]. Drying beds may be used; drying beds do not usually require chemicals.
- Worm shortage in the process, so that worm additions are required: Worms reproduce via egg capsules, which may be lost from the process in the castings. Also, toxic conditions, drowning, and other problems will cause worm populations to drop. At Hagerstown, Md., a worm-raising operation has been proposed to supply the necessary makeup worms to the sludge conversion process [50].
- Shortage of worms for initial inventory or restart: To begin operation, a large worm inventory may be needed, but local worm suppliers may be unable to meet this demand. Gradual start-up is therefore desirable, especially for large plants. Also, earthworm exchanges may become available nationwide so that sludge operations can draw on larger numbers of earthworm suppliers.
- Temperature extremes: Worm feed most rapidly at 15–20 °C; about 5 °C, feeding is quite slow [48]. Freezing will kill worms. High temperatures can also cause problems. It may be necessary to stockpile sludge during the winter or provide a heated building for the conversion process.
- Shortage of enzymes: Not a problem, despite claims by marketers of enzyme preparations that these preparations are valuable to the process [54].
- Exposure to light: Worms avoid bright light. Some sort of cover or shade should be provided so that worms will convert the top layer of the sludge.
- Dehydration: There is a minimum moisture content for the worm bed [54].
- Salinity in castings: Under some conditions, castings may have sufficient dissolved salts to inhibit plant growth. This problem may be eliminated by leaching or by mixing the castings with other materials with lower dissolved salts [55, 56].

- Contamination of castings by heavy metals, motor oil, rags, and similar materials: Source control may be used where feasible, as for other processes aimed at reuse of sludge as a soil conditioner.
- Odors: The most likely source is raw or aerobically digested sludge, which has been stockpiled to await earthworm conversion.

### *3.5.3 Process Limitations*

Limitations of the earthworm conversion process include, but are not limited to, the following [60, 62]:

- Earthworm conversion decreases the total nitrogen values in the sludge, as ammonia nitrogen will be lost to the atmosphere.
- Costs are unpredictable.
- Two common ions in municipal wastewater sludge, ammonium and copper, may be toxic to worms. Studies have found that these ions were lethal at additions equivalent to 180 mg NH4-N and 2500 mg Cu per kg of wet substrate [57, 58]. Safe limits for these elements are not known.
- Cadmium accumulates in the worm *Eisenia foetida*. Zinc apparently does not accumulate in *Eisenia foetida* but does accumulate in other species [58, 59]. If the worms are to be used as animal feed, the system must be operated such that cadmium and zinc concentrations in the worms do not exceed recommended levels for animal consumption.
- Space requirements may rule out earthworm conversion at some treatment plants.
- The earthworm business has been afflicted with unsound investments and excessive claims. For example, it has been claimed that earthworm processing is able to reduce concentrations of heavy metals [60]. Any such reduction could only be caused by simple dilution with uncontaminated waste or by concentration of the contaminants in the earthworms.
- If a particular sludge is suitable for earthworm conversion, that sludge should also be suitable for reuse as a soil conditioner without being processed by earthworms. However, earthworm conversion reduces odor, improves texture, and may increase germination rate.

These limitations may seem significant but are not overwhelming. Considerable research and development is underway, and it appears that earthworm conversion may soon have a role in municipal wastewater treatment plant sludge processing.

### *3.5.4 Process Design Criteria*

Design criteria have been generated by the operators and researchers in the field [48–51, 61, 63, 69–90, 102] for the vermicomposting process.

Species of worm being tested were *Eisenia foetida* (redworm, hybrid redworm, tiger worm, dung worm) [48, 51], *Lumbricus rubellus* (red manure worm, red wiggler worm) [49], and *Lumbricus terrestris* (night crawler) [48]. The following are the compiled design criteria:

Detention time of sludge in worm beds = 2–32 days [49, 50].
Worm reproductive cycle = 1–2 months.
Rate of worm feeding (15 °C) = 0.17–1.7 g dry sludge per gram dry worm weight per day [48].
Optimum temperature = 15–20 °C.
Dry matter content of worms = 20–25% (*Eisenia foetida*) [51].

Minimum solids content of the worm bed mixture = 20%: Actual minimum solids content depends on such factors as porosity, type of sludge, and ability to keep aerobic. Experiments are being conducted to better define these parameters.

## 3.6 Process Application Examples

The Wright-Patterson Air Force Base in Dayton, Ohio, USA [45], launched a vermicomposting program in July 2002, using earthworms to consume a daily average of 500 pounds of solid waste. The worms digest vegetable matter and old newspapers, saving the base about $25 per day on transporting and disposing of waste. As the number of worms grows, so does the amount of waste they consume. The base acquired 250,000 worms and their climate-controlled home (at a constant 70 degree F) for the environmental project. At the base, which produces fruit and vegetable waste from its commissary, the earthworms have flourished, now numbering more than 300,000. Their numbers eventually could top one million. The worm casings replace chemical fertilizer at the base's golf course, which saves additional money. More successful stories can be found in the literature [42–61].

Vermicomposting has gained popularity in schools and municipalities, according to Stuckey and Hudak [62]. In Boston, Massachusetts, USA, Josiah Quincy Elementary School received a grant to build a rooftop organic garden. The students maintain garbage-eating red wiggler worms to break down fruits and vegetables. Once processed in the bin, the compost is applied to the garden. In Orange County, Florida, USA, a revolutionary worm-use concept has been promoted where worms stabilize biosolids to a "Class A pathogen standard" substance.

Mudrunka et al. [92] attempted to eliminate microbial pollution of domestic animal excrements using vermicomposting. Their investigations were carried out in a three-chamber domestic wooden vermicomposter, in which aerobic degradation of three types of animal excrements (cow, pig, dog) using the earthworm *Eisenia andrei*. Before laying the individual excrements to the compost batch, the appropriate input samples were taken for the microbiological examination of the biopathogens. After 6 months, final samples of the final substrate were taken to determine whether proper compost sanitization took place during the vermicomposting

process. According to valid legislation, the bacteria *Escherichia coli*, *Enterococcus* sp., and *Salmonella* sp. were identified as indicator microorganisms. After the evaluation of the performed laboratory analyses, it was proved that the use of earthworm bioactivity resulted in elimination or at least significant reduction of the concentrations of these bacterial strains in the final vermicompost samples [92].

Agricultural wastes include mainly the organic wastes generated from various operations in the agricultural and forestry industries, such as crop residues, weeds, leaf litter, sawdust, forest wastes, livestock waste, biosolids, fruit pomace, animal dung, cardboard compost, rice straw, paper mill sludge and fibers, vegetable plant debris, fruit plant debris, etc. Theoretically, all organic agricultural and food wastes can be properly treated by the vermicomposting process for solid waste volume reduction, pathogen reduction, and vermicompost recycle.

Among the various agricultural wastes, livestock waste is always a preferred choice for researchers as feedstock for earthworms and as bulking substrate for vermicomposting [90, 105]. Livestock waste is considered as the suitable organic amendment to enhance the process of vermicomposting because of its low cost, easy availability, sufficient nutrient content, and ideal C/N ratio [71]. The chemical composition of livestock waste depends on the type of feed given to the animal, bedding material, and fresh or dried including the manner how excreta is collected, stored, and handled prior to vermicomposting [74]. Hence, differences in physicochemical characteristics of livestock waste have effects on the life cycle of earthworm species [71].

Many researchers [69–89] have conducted vermicomposting studies on the use of various agricultural wastes as feedstock. Sharma and Garg [90] have compiled their research data together as shown in Table 3.1.

An extensive vermicomposting research involving the use of a skin coffee (SC) amended with green waste (GW) and biochar (B) has been conducted by Zulhipri et al. [102]. Skin coffee is a food manufacturing waste from Cibulao coffee farm, Bogor, Indonesia. Green wastes are mainly the branch cuttings and leaves from the garden of the Universitas Negeri Jakarta. Biochar is a carbon-rich product made from the rice husk pyrolysis process at 450 °C. Biochar was mixed by the researchers [102] with SC and GW in different proportions, i.e., 6%, 8%, and 10%, along with control and allowed to pass through earthworm guts for 2 months' processing. The 8% biochar addition rate achieved maturity of vermicomposting and resulted in the highest-quality vermicompost based on parameters such as organic C, C:N ratio, total N, P, K content, and pH in comparison with a control vermicompost. They further used the produced vermicompost for cultivating the growth of coffee plant. Physiological parameters and morphology of coffee plant growth such as number of leaves, height, plant diameter, and shoot dried weight were recorded. Their important research data are presented in Tables 3.2, 3.3, and 3.4 [102].

The analytical results on the characteristics of earthworm media are shown in Table 3.2 [102]. The high nutrient content in biochar is expected to increase the nutrient content in vermicompost. Table 3.3 shows that the earthworm media significantly affected the chemical content and macro- and microelements in vermicompost. There is no significant increase in nitrogen content with an increase in

**Table 3.1** Vermicomposting of various agricultural wastes. Credit: K Sharma and VK Garg [90]

| No. | Type of waste (bulking material) | Earthworm | Duration | Results | References |
|---|---|---|---|---|---|
| 1 | Buffalo waste, sheep waste, goat waste, cow waste | *Eisenia foetida* | 90 days | Maximum earthworm growth rate was achieved in the various combinations of buffalo dung and minimum growth rate in sheep waste. TOC content and C/N ratio decreased during vermicomposting, whereas total nutrient content increased | Sharma and Garg [71] |
| 2 | Rice straw + paper waste + cow dung | *E. foetida* | 105 days | Paper waste and rice straw effectively convert into nutrient-rich vermicompost. Vermicompost is more fragmented than parent feedstocks. Use of rice straw in higher ratio was not recommended | Sharma and Garg [72] |
| 3 | *Salvinia molesta* | *E. foetida* | 45 days | Chemical compounds responsible for weed allelopathic effects destroyed completely. The C/N ratio of *Salvinia* was reduced sharply from 53.9 to 9.35 | Hussain et al. [79] |
| 4 | Sewage sludge (cattle dung) | *E. foetida* | 80 days | Vermicomposting modifies the structure of bacterial community in the waste and reduces the pathogenic human bacteria population | Lv et al. [74] |
| 5 | Pig manure and rice straw | *E. foetida* | 40 days | Vermicompost has higher pH, P, K, Zn, and CEC but lower available N and Cu than the parent substrate. Increment in aromatic compounds indicated high humification during vermicomposting. Earthworm tissues accumulated $^{13}C$ | Zhu et al. [82] |
| 6 | Crop/tree residues | *Eudrilus* sp. | | Earthworm growth and conversion efficiency vary with waste. In all the crop residues, pH, EC, and N and P levels increased, whereas C/N and C/P ratios decreased | Thomas et al. [69] |

(continued)

**Table 3.1** (continued)

| No. | Type of waste (bulking material) | Earthworm | Duration | Results | References |
|---|---|---|---|---|---|
| 7 | Horse manure, apple pomace, grape pomace, and digestate (manure slurry, corn silage, haylage) | *Eisenia andrei* | 240 days | Study evaluated vermicompost characteristics based on 120-day-old layer and 240-day-old layer in vermireactor. Maximum biomass of earthworms was in 120-day-old layer. After 240 days, microbial biomass activity decreased due to a decrease in the earthworm activity, indicating a high degree of stabilization. Enzyme activities differ according to the age of the layers and the type of waste. Germination index increased after vermicomposting and was higher with apple pomace and digestate than that with horse manure and grape pomace | Sanchez et al. [70] |
| 8 | Cow manure and wheat residues | *E. foetida* | 60 days | Urease activity is a suitable indicator of vermicompost maturity and waste stabilization during the process of vermicomposting. Urease activity was highly correlated with the time of vermicomposting resulting in $r = 0.97$ for cattle manure and $r = 0.99$ for wheat waste. Urease activity showed significant correlations with the C/N ratio | Sudkolai and Nourbakhsh [83] |
| 9 | Wheat straw, pig dung, poultry dung, rabbit dung, cattle dung, sheep dung, and vegetal compost | *E. foetida* | 90 days | Highest worm production and growth rate were obtained with cow dung followed by pig dung; however, earthworm growth decreased in vegetable compost. Maximum earthworm growth rate was found on the 90th day. Growth and worm production depend on the biochemical quality of the feedstocks | Vodounnou et al. [75] |

(continued)

**Table 3.1** (continued)

| No. | Type of waste (bulking material) | Earthworm | Duration | Results | References |
|---|---|---|---|---|---|
| 10 | Sawdust, boxwood leaves, and cardboard compost (MSW) | *E. foetida* | 100 days | MSW and carbonaceous materials in different proportions, viz., 50:50, 70:30, 85:15, and 100:0, were vermicomposted for 100 days. Vermicomposting for 75 days is sufficient for vermicompost maturity in terms of EC, WSC, DEH, and C/N ratio. Phosphorus, nitrogen, and pH levels were higher in the vermicompost | Alidadi et al. [73] |
| 11 | *Salvinia natans* (cattle manure and sawdust) | *E. foetida* | 45 days | Total concentration of heavy metals (Zn, Cu, Mn, Fe, Cr, Pb, Cd, Ni) increased; however, concentration of water-soluble and plant-available heavy metals was reduced in the final vermicompost. TCLP tests confirmed the suitability of vermicompost for agriculture | Singh and Kalamdhad [84] |
| 12 | Leaf litter (horse dung, sheep dung) | *Perionyx excavatus* | 60 days | Cashew leaf litter mixed with cow dung at 2:2 ratio was found to best in terms of vermicompost properties. The vermicompost produced had lower pH, organic carbon content, C/N ratio, C/P ratio, and lignin, cellulose, hemicellulose, and phenol content but higher NPK, DEH, and HA content than the waste and compost. Reduction in the lignocellulose and phenol content is due to the combined action of the gut lignocellulolytic microflora and earthworms during the vermicomposting process | Parthasarathi et al. [85] |
| 13 | *Ipomoea* | *E. foetida* | 30 days | Total carbon contents decreased from 527.3 to 282.8 g/kg and total nitrogen contents increased from 20.2 to 28.5 g/kg. C/N ratio of *Ipomoea* vermicompost was 9.9. Spectroscopic analysis revealed transformation of weed into potent organic fertilizer | Hussain et al. [76] |

(continued)

**Table 3.1** (continued)

| No. | Type of waste (bulking material) | Earthworm | Duration | Results | References |
|---|---|---|---|---|---|
| 14 | Coconut husk poultry manure, pig slurry | *Eudrilus eugeniae* | 21 days | Highest recovery of relative N (1.6) and K (1.3) was in 20% feedstock substitution by pig slurry, and highest P recovery (2.4) was with poultry manure substitution. Vermicompost contains higher pH, microbial biomass carbon, and macro- and micronutrients than the initial waste | Swarnam et al. [86] |
| 15 | Cow dung, poultry manure | *E. foetida* | | After vermicomposting, pH, TOC content, and C/N ratio were reduced but EC and HA were increased. Heavy metals stabilized | Lv et al. [74] |
| 16 | Decanter cake + rice straw | *E. eugeniae* | 2 weeks | Four treatments with different ratios of decanter cake and rice straw (2:1, 1:1, 1:2, 1:3) were prepared. Two parts decanter cake and one part rice straw (w/w) was found to best among all the treatments | Lim et al. [87] |
| 17 | Crop residue (rice, wheat, corn, sugarcane) | *E. eugeniae* | 90 days | Highest earthworm weight and vermicomposted matter were achieved in wheat and lowest with corn residue | Aynehband et al. [85] |
| 18 | *Lantana* | *E. foetida* | – | C/N ratio reduced from 22.7 to 8.1; humification index from 8.38 to 2.03. FTIR spectra revealed complete degradation of phenols and sesquiterpene lactones and formation of simple compounds. GC-MS analysis revealed transformation of 24–86 constituents | Hussain et al. [78] |
| 19 | *Parthenium* | *E. foetida* | – | Chemicals responsible for the allelopathic effect of parthenium weed are destroyed. Scanning electron microscopy shows marked disaggregation of the material in the vermicompost as compared with the well-formed matrix of *Salvinia* leaves | Hussain et al. [77] |

(continued)

**Table 3.1** (continued)

| No. | Type of waste (bulking material) | Earthworm | Duration | Results | References |
|---|---|---|---|---|---|
| 20 | Tomato plant debris + paper mill sludge | *E. foetida* | 6 months | Characterize HA isolated from different waste mixtures before and after vermicomposting. HA content increased by 15.9–16.2%. Vermicompost produced from tomato debris/paper mill sludge (2:1) recorded higher C content and C/N ratio. HA from tomato debris/paper mill sludge (1:1) vermicompost showed a higher O content and O/C ratio | Fernandez Gomez et al. [81] |
| 21 | Filter cake (cattle manure) | *E. foetida* | 30 days | Positive correlation of phosphatase activities with TOC content, pH, and WSP but negative correlation with HA content. Nanopore volume found to be negatively correlated with phosphatase activities for filter cake but not for cattle manure. HA content of filter cake vermicompost was higher than that of cattle manure vermicompost | Busato et al. [89] |

Note: *CEC* cation exchange capacity; *DEH* dehydrogenase; *EC* electric conductivity; *FTIR* Fourier transform infrared; *GC-MS* gas chromatography-mass spectrometry; *HA* humic acid; *MSW* municipal solid waste; *TCLP* toxicity characteristic leaching procedure; *TOC* total organic carbon; *WSC* water-soluble carbon; *WSP* water-soluble phosphorus

**Table 3.2** Chemical content of the worming media. Credit: Zulhipri et al. [102]

| No | Parameter | Skin coffee | Cow dung | Biochar |
|---|---|---|---|---|
| 1 | pH | 6.21 | 7.74 | 7.82 |
| 2 | C organic (%) | | 34.48 | 48 |
| 3 | N (%) | 1.27 | 1.05 | 2.26 |
| 4 | P (ppm) | 29 | 84.62 | 97.80 |
| 5 | K (ppm) | 2.46 | 8.08 | 12.04 |
| 6 | C/N ratio | | 32.84 | 21.23 |

%B. The pH and organic C, however, increase with an increase in biochar addition. By increasing C, the C/N ratio also increases. After organic waste becomes vermicompost, the mineral content of vermicompost increases with increasing biochar content. This is because biochar contains porous organic carbon with high surface area which is able to adsorb metallic metals from earthworm media. It was observed in the experiment [102] that the media containing 8% biochar produced more vermicompost than media that contained 4% biochar or did not contain biochar. The

**Table 3.3** Vermicompost characteristic of various media. Credit: Zulhipri et al. [102]

| No | Chemicals | Compost +0%B | Compost +6%B | Compost +8%B |
|---|---|---|---|---|
| 1 | pH | 5.36 | 5.48 | 5.60 |
| 2 | Organic | 53.20 | 61.65 | 65.80 |
| 3 | Carbon organic | 26.30 | 34.50 | 36.40 |
| 4 | Nitrogen | 2.70 | 2.50 | 2.76 |
| 5 | Sodium | 1.73 | 1.29 | 1.32 |
| 6 | Potassium | 3.48 | 3.62 | 3.84 |
| 7 | Calcium | 51.46 | 52.70 | 56.00 |
| 8 | Magnesium | 6.22 | 6.24 | 6.85 |
| 9 | Phosphorus | 28.42 | 28.90 | 32.74 |
| 10 | Zinc | 2245 | 2264 | 2676 |
| 11 | Manganese | 5.24 | 5.18 | 5.42 |

**Table 3.4** Effect of vermicompost on coffee plant. Credit: Vanderholm et al. [105]

| No | Sample | Composition | Height | Leaf |
|---|---|---|---|---|
| 1 | EC | Soil + compost | 12.00 | 4 |
| 2 | EV5 | Soil + 5% vermicompost | 13.14 | 7 |
| 3 | EV10 | Soil + 10% vermicompost | 15.24 | 11 |
| 4 | EV15 | Soil + 15% vermicompost | 17.22 | 13 |
| 5 | EV20 | Soil + 20% vermicompost | 18.86 | 15 |

composting time also has a significant effect on the weight of the vermicompost produced. The composting time of 2 weeks gives the highest vermicompost results, namely, 469 g. This is due to the fact that in the second week the number of young worms increases, and with increasing time, the mother worms decrease, so that less vermicompost is produced. Table 3.4 shows the effect of vermicompost on the growth of coffee plant. The highest height of coffee seedlings was in the EV20, but it was not significantly different from the EV15 treatment. The lowest plant height was in EC treatment and significantly different from other treatments as shown in Table 3.4. EV20 treatment, soil containing 20% vermicompost, gave the best results against all variables observed. This is because in this composition there is more soil containing vermicompost fertilizer. Vermicompost can fertilize the soil through its influence on soil physical, chemical, and biological properties. Physically, vermicompost can (a) affect soil texture, (b) improve soil structure, (c) improve soil consistency, (d) improve soil drainage, (e) improve soil pores, (f) increase soil maturity, (g) increase plant growth power, and (h) loose the soil so that the space for the roots will increase. Chemically, vermicompost fertilizer will (a) contribute macro- and micronutrients, (b) increase soil reaction (soil pH), (c) improve soil colloids (mineral matter), (d) increase ion exchange capacity, and (e) gain base saturation so that the availability of nutrients is getting better. Biologically, vermicompost can increase the population of soil microorganisms so that the soil becomes more fertile.

Vermicompost also functions as a biological control tool in suppressing plant diseases, namely, by inhibiting disease growth through natural processes by increasing competitive activity and antibiotics in the inoculum. The best media composition is P5 treatment (one part of subsoil mixed with four parts of vermicompost from coffee husk waste). The use of vermicompost fertilizer from coffee husk waste can substitute NPK inorganic fertilizer for coffee nurseries in the main nursery.

## 3.7 Future Development and Direction

Vermicomposting (or vermistabilization) should be encouraged by governments in the field of environmental engineering as promising processes for disposal of biosolids and other organic solid wastes. Special efforts should be made in the near future to obtain recognition for the process, and funding sources should be explored at all levels for economical analysis and optimization of the process. At the global level, international agencies should encourage and fund the transfer of vermicomposting technology between the USA and all other countries.

## Glossary

**Agricultural wastes** They include mainly the organic wastes generated from various operations in the agricultural and forestry industries, such as crop residues, weeds, leaf litter, sawdust, forest wastes, livestock waste, biosolids, fruit pomace, animal dung, cardboard compost, rice straw, paper mill sludge and fibers, vegetable plant debris, fruit plant debris, etc.

**Biochar** It is a carbon-rich product made from the rice husk pyrolysis process at 450 °C.

**Green waste** They are mainly the branch cuttings and leaves from the gardens.

**Vermicompost** The end product of a vermicomposting process which is an efficient growth promoter for plants, as it contains plant-available nutrients, rich microbial population, humic substances, growth hormones, and enzymes. It is an organic fertilizer that improves crop growth and yield.

**Vermicomposting** It is a bio-oxidative natural decomposition process that occurs under mesophilic conditions further aided by the biochemical action of microorganisms, such as different earthworm species. The mutual action of worms and microbes converts organic waste, such as agricultural wastes, food wastes, biosolids, etc., into fine, homogenized, odor-free, nutrient-rich, and humus-rich manure that is called vermicompost. Vermicomposting helps achieve a circular bioeconomy by converting waste into useful products that are necessary for the overall sustainable development of a country.

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# Chapter 4
# The Impacts of Climate Change on Agricultural, Food, and Public Utility Industries


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## Acronyms and Nomenclature

| | |
|---|---|
| BC | Black carbon |
| CCS | Carbon capture and sequestration |
| CFC | Chlorofluorocarbon |
| $CH_4$ | Methane |
| $CO_2$ | Carbon dioxide |
| ENSO | El Niño-Southern Oscillation |
| ERF | Effective radiative forcing |
| GHG | Greenhouse gas |

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| | |
|---|---|
| GWP | Global warming potential |
| HBFC | Hydrobromofluorocarbon |
| HC | Hydrocarbon |
| HCFCs | Halogenated fluorocarbons |
| HFCs | Hydrofluorocarbons |
| IPCC | Intergovernmental Panel on Climate Change |
| LFG | Landfill gas |
| MCL | Maximum contaminant level |
| $N_2O$ | Nitrous oxide |
| NPDWR | National Primary Drinking Water Regulations |
| ODS | Ozone-depleting substance |
| $O_3$ | Ozone |
| PFCs | Perfluorinated carbons |
| PM | Particulate matter |
| RF | Radiative forcing |
| USEPA | US Environmental Protection Agency |
| USGS | US Geological Survey |
| WMO | World Meteorological Organization |
| W $m^{-2}$ | Watts per square meter |

## 4.1 Introduction

Global climate change, also referred to as global warming, is a serious threat to our environment. This chapter will present a summary of scientific facts about climate change and discuss its impacts in particular on water resources and planning. The underlying science of climate change is undisputable. Climate change has become a contentious political issue, which is unfortunate if only because it distracts society and our policymakers from necessary discussions and decisions about how to respond to the impacts of climate change on our communities.

### *4.1.1 Weather, Climate, and Climate Change*

The climate of a region can be thought of as the "average" of that region's weather. Climate is predictable, or at least enough so that we rely on it for planning. We speak of the sunny, temperate climate of the Mediterranean, or the harsh, cold climate of Siberia (even though there are both cold winter storms in Italy and warm summer days on the taiga), and vacations and population growth projections are adjusted accordingly. In simple modeling terms, if an input that was previously thought to be constant—climate—is found to be variable, or to have become variable, then the model output will also necessarily change from what was previously expected. If the weather we are used to expecting is no longer what can be expected in the future, then what should current and future infrastructure planning be based

on? Land use planners and water and wastewater utility operators and regulators need to understand what the impacts of climate change will be on water resources and how to prepare for these changes.

It is well known that the Earth has gone through multiple ice ages in periods of dramatic cooling and dramatic warming. Among the many ice ages the Earth has undergone, we are currently going through a warming trend. Questions we would ask are: "Why is the Earth warming?" "How does the source warm the Earth?" "What are the implications on our water resources, animals, and agriculture?" There are more questions than answers [1–38]. But before we discuss how the Earth's climate is changing, there are some technical terminologies presented below as well as in the "Glossary" section of this chapter [26–30].

Stratospheric ozone plays a decisive role in the stratospheric radiative balance. Since ozone absorbs a band of ultraviolet radiation called UVB that is particularly harmful to living organisms, the ozone layer prevents most UVB from reaching the ground. Depletion of stratospheric ozone, due to chemical reactions that may be enhanced by climate change, results in an increased ground-level flux of ultraviolet (UV) B radiation.

Ozone "Hole" is a large area of the stratosphere with extremely low amounts of ozone.

Ozone-depleting substance (ODS) is a family of man-made compounds that includes, but is not limited to, chlorofluorocarbons (CFCs), bromofluorocarbons (halons), methyl chloroform, carbon tetrachloride, methyl bromide, and hydrochlorofluorocarbons (HCFCs). These compounds have been shown to deplete stratospheric ozone and therefore are typically referred to as ODSs.

Ozone layer depletion means chemical destruction of ozone molecules in the ozone layer. Depletion of this ozone layer by ozone-depleting substances will lead to higher UVB levels (a band of ultraviolet radiation), which in turn will cause increased skin cancers and cataracts and potential damage to some marine organisms, plants, and plastics.

Particulate matter (PM) are very small pieces of solid or liquid matter such as particles of soot, dust, fumes, mists, or aerosols. The physical characteristics of particles, and how they combine with other particles, are part of the feedback mechanisms of the atmosphere.

Photosynthesis is a process by which plants take $CO_2$ from the air (or bicarbonate in water) to build carbohydrates, releasing $O_2$ in the process. There are several pathways of photosynthesis with different responses to atmospheric $CO_2$ concentrations.

Phytoplankton are microscopic plants that live in salt and freshwater environments.

Precession means the wobble over thousands of years of the tilt of the Earth's axis with respect to the plane of the solar system.

Precipitation includes rain, hail, mist, sleet, snow, or any other moisture that falls to the Earth.

Radiation is a type of energy transfer in the form of electromagnetic waves or particles that release energy when absorbed by an object.

Radiative forcing means (a) a change in the balance between incoming solar radiation and outgoing infrared radiation and (b) a measure of the influence of a particular factor (e.g., greenhouse gas (GHG), aerosol, or land use change) on the net change in the Earth's energy balance.

Soil is a complex mixture of inorganic minerals (i.e., mostly clay, silt, and sand), decaying organic matter, water, air, and living organisms.

Soil carbon is a major component of the terrestrial biosphere pool in the carbon cycle. The amount of carbon in the soil is a function of the historical vegetative cover and productivity, which in turn is dependent in part upon climatic variables.

Solar energy is also called solar radiation, energy from the Sun, and also referred to as shortwave radiation. Of importance to the climate system, solar radiation includes ultraviolet radiation, visible radiation, and infrared radiation. It also includes indirect forms of energy such as wind falling or flowing water's hydropower, ocean thermal gradients, and biomass, which are produced when direct solar energy interacts with the Earth.

Solar radiation is a radiation emitted by the Sun. It is also referred to as shortwave radiation. Solar radiation has a distinctive range of wavelengths (spectrum) determined by the temperature of the Sun.

Source means any process or activity that releases a greenhouse gas, an aerosol, or a precursor of greenhouse gas into the atmosphere.

Stratosphere is the region of the atmosphere above the troposphere and between the troposphere and the mesosphere. The stratosphere extends from about 8–50 km (6–31 miles) in altitude. Specifically, it has a lower boundary of approximately 8 km at the poles to 15 km at the equator and an upper boundary of approximately 50 km. Depending upon latitude and season, the temperature in the lower stratosphere can increase, be isothermal, or even decrease with altitude, but the temperature in the upper stratosphere generally increases with height due to absorption of solar radiation by ozone. So the stratosphere gets warmer at higher altitudes. In fact, this warming is caused by ozone absorbing ultraviolet radiation. Warm air remains in the upper stratosphere, and cool air remains lower, so there is much less vertical mixing in this region than in the troposphere. Commercial airlines fly in the lower stratosphere.

Terrestrial radiation means the total infrared radiation emitted by the Earth and its atmosphere in the temperature range of approximately 200–300 Kelvin. Terrestrial radiation provides a major part of the potential energy changes necessary to drive the atmospheric wind system and is responsible for maintaining the surface air temperature within limits of livability.

Troposphere is (a) the region of the atmosphere closest to the Earth. The troposphere extends from the surface up to about 10 km (6 miles) in altitude, although this height varies with latitude. Almost all weather takes place in the troposphere. Mt. Everest, the highest mountain on Earth, is only 8.8 km (5.5 miles) high. Temperatures decrease with altitude in the troposphere. As warm air rises, it cools, falling back to Earth. This process, known as convection, means there are huge air movements that mix the troposphere very efficiently; or (b) the lowest part of the atmosphere from the surface to about 10 km in altitude in midlatitudes (ranging

from 9 km in high latitudes to 16 km in the tropics on average) where clouds and "weather" phenomena occur. In the troposphere, temperatures generally decrease with height. All weather processes take place in the troposphere. Ozone that is formed in the troposphere plays a significant role in both the greenhouse gas effect and urban smog. The troposphere contains about 95% of the mass of air in the Earth's atmosphere.

Weather is the specific condition of the atmosphere at a particular place and time. It is measured in terms of such things as wind, temperature, humidity, atmospheric pressure, cloudiness, and precipitation. In most places, weather can change from hour to hour, day to day, and season to season. Climate in a narrow sense is usually defined as the "average weather" or, more rigorously, as the statistical description in terms of the mean and variability of relevant quantities over a period of time ranging from months to thousands or millions of years. The classical period is 30 years, as defined by the World Meteorological Organization (WMO). These quantities are most often surface variables such as temperature, precipitation, and wind. Climate in a wider sense is the state, including a statistical description, of the climate system. A simple way of remembering the difference is that climate is what you expect (e.g., cold winters) and "weather" is what you get (e.g., a blizzard) [30].

### *4.1.2 Greenhouse Gases, Greenhouse Effect, Global Warming, Global Warming Potential*

A greenhouse gas (GHG) is any gas that absorbs infrared radiation in the atmosphere. Greenhouse gases include water vapor, carbon dioxide ($CO_2$), methane ($CH_4$), nitrous oxide ($N_2O$), halogenated fluorocarbons (HCFCs), ozone ($O_3$), perfluorinated carbons (PFCs), hydrofluorocarbons (HFCs), and sulfur hexafluoride. Gases absorb heat in the atmosphere near the Earth's surface, preventing it from escaping into the space. If the atmospheric concentrations of these gases rise, the average temperature of the lower atmosphere will gradually increase, a phenomenon known as the greenhouse effect.

Specifically, greenhouse effect is produced as greenhouse gases allow incoming solar radiation to pass through the Earth's atmosphere, but prevent most of the outgoing infrared radiation from the surface and lower atmosphere from escaping into the outer space. This process occurs naturally and has kept the Earth's temperature about 60 °F warmer than it would otherwise be. Current life on Earth could not be sustained without the natural greenhouse effect. The greenhouse effect is trapping heat and build-up of heat in the atmosphere (troposphere) near the Earth's surface. Some of the heat flowing back toward the space from the Earth's surface is absorbed by water vapor, carbon dioxide, ozone, and several other gases in the atmosphere and then reradiated back toward the Earth's surface [2, 30].

Global warming is known due to the recent and ongoing global average increase in temperature near the Earth's surface. It is the observed increase in average temperature near the Earth's surface and in the lowest layer of the atmosphere. In

common usage, "global warming" often refers to the warming that has occurred as a result of increased emissions of greenhouse gases from human activities. Global warming is a type of climate change; it can also lead to other changes in climate conditions, such as changes in precipitation patterns.

Global warming potential (GWP) is a measure of the total energy that a gas absorbs over a particular period of time (usually 100 years), compared to carbon dioxide. GWP is a number that refers to the amount of global warming caused by a substance. The GWP is also the ratio of the warming caused by a substance to the warming caused by a similar mass of carbon dioxide ($CO_2$). Thus, the GWP of $CO_2$ is 1.0. Chlorofluorocarbon (CFC)-12 has a GWP of 8500; CFC-11 has a GWP of 5000; hydrochlorofluorocarbons and hydrofluorocarbons have GWPs ranging from 93 to 12,100; and water has a GWP of 0 [2, 30].

## 4.2 Main Contributors to Greenhouse Gases

The main reason for the Earth's warming is due to the greenhouse effect (Figs. 4.1 and 4.2). Before human activity, natural activities such as volcanic activity and natural forest fires would emit greenhouse gases in the atmosphere. As greenhouse gases absorb heat and solar radiation, the concentrations of gases get trapped near the Earth's surface and sustain life [2]. A microcosm of this balance can be seen in the relationship between humans and trees. Humans take in oxygen and release carbon

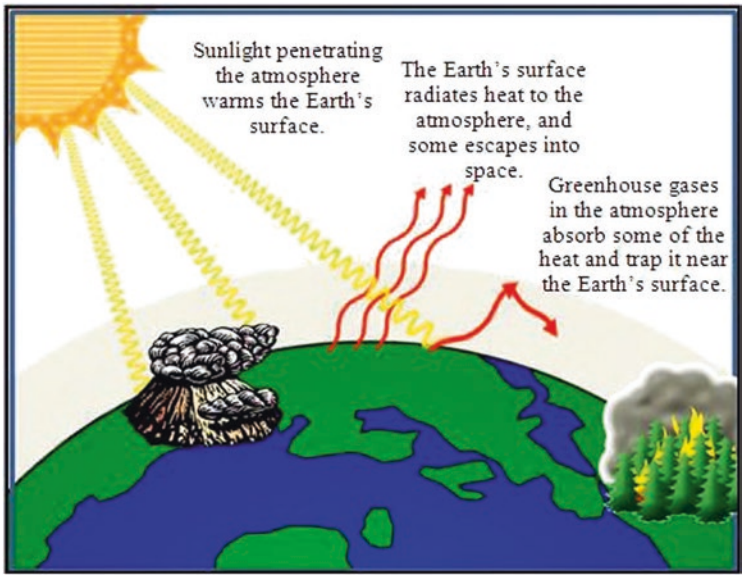



**Fig. 4.1** The natural greenhouse effect before human activity ([2], Permission to use)

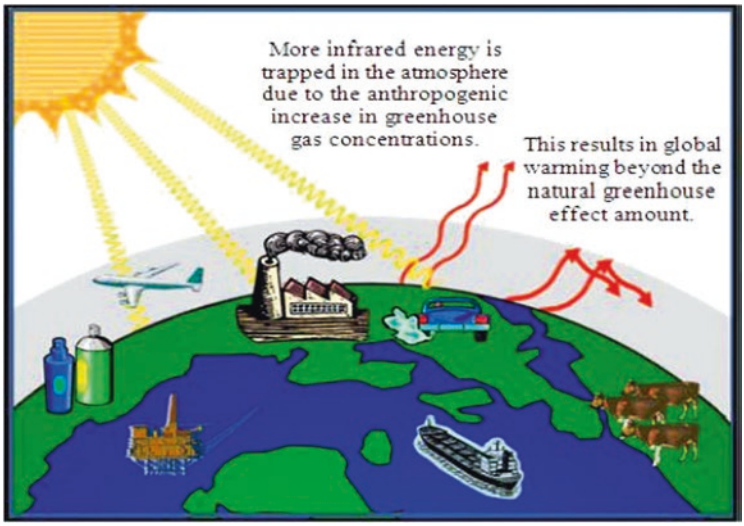



**Fig. 4.2** The greenhouse effect after human activity ([2], Permission to use)

dioxide ($CO_2$), while trees take in carbon dioxide and release oxygen, thereby creating a balance in nature.

However, due to increased human activity (from electricity, transportation, industry, and population increase) [3], large amounts of $CO_2$ and other greenhouse gases have been released to the atmosphere. Also, as trees get cut down and deforested, there are less resources using up the $CO_2$, causing levels to rise beyond natural levels. So, what happens to the additional greenhouse gases floating in the air? The excess $CO_2$ and other greenhouse gases trap the extra radiation near the Earth's surface, causing global temperatures to rise, or global warming [2, 30].

The definitions of greenhouse gases and other technical terms have been introduced in Sect. 4.1. The main contributor to the greenhouse effect is carbon dioxide. Following carbon dioxide are methane gas ($CH_4$) and nitrous oxide ($N_2O$), and the halocarbons as the leading greenhouse gases. Figure 4.3 below shows the major climate changing agents of greenhouse gases and their radiative forcing (W $m^{-2}$), showing their emission ability to retain heat, and their great amounts on the Earth's surface [4]. As seen in Fig. 4.3, carbon dioxide has the greatest amount where it absorbs heat at a radiative forcing above 1.5 W $m^{-2}$ of increased and retained solar radiation at the Earth's surface. Although halocarbons, methane, and nitrous oxide have a greater warming potential than carbon dioxide, the larger quantity of carbon dioxide has a greater impact [28, 30]. Ozone depends on the location. In the troposphere where people live, ozone is a greenhouse gas where it absorbs heat; however, ozone in the stratosphere actually absorbs UV radiation and holds back the radiation from hitting the Earth's surface, thereby keeping the Earth cooler. Water does not affect the warming of the Earth too much since the concentration levels are fairly constant. Land use goes both ways where dark forested areas or black carbon on snow or from diesel engines in the troposphere would absorb heat, whereas planting

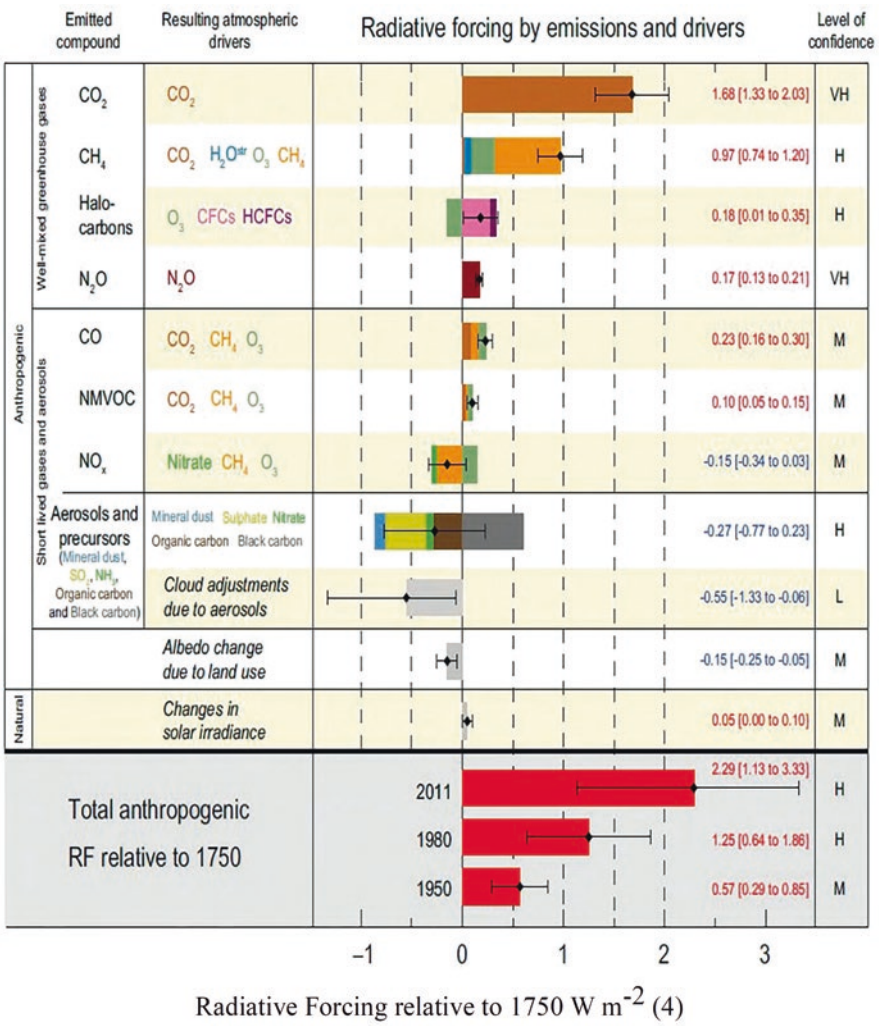



**Fig. 4.3** Radiative forcing estimates in 2011 relative to 1750 and aggregated uncertainties for the main drivers of climate change [4]

lighter colored plants on arid regions where light reflects back to the space would actually cool the Earth. Aerosols' effects are uncertain; the concentration of aerosols can be monitored based on the brightness of clouds; the higher the concentration of aerosols, the brighter the clouds are. The reason is the aerosols feed the water droplets that contribute to the clouds, and the more water droplets there are, the more the droplets reflect light more. Aerosols in the stratosphere from volcanic activity block the radiation to help cool the Earth. There is still much to investigate and discover about aerosols [2, 4].

Figure 4.3 shows the total weighted average of all the climate changing agents; there is a total of 2.29 W $m^{-2}$ increase in the amount of solar energy absorbed at the surface of the Earth [2, 4]. Because carbon dioxide shows the greatest quantity and has the greatest impact, carbon dioxide will be the main focus and should often be a reference point to compare to other greenhouse gases. Values in Fig. 4.3 are global average radiative forcing (RF), partitioned according to the emitted compounds or processes that result in a combination of drivers. The best estimates of the net radiative forcing are shown as black diamonds with corresponding uncertainty intervals; the numerical values are provided on the right of the figure, together with the confidence level in the net forcing (VH, very high; H, high; M, medium; L, low; VL, very low). Albedo forcing due to black carbon on snow and ice is included in the black carbon aerosol bar. Small forcings due to contrails (0.05 W $m^{-2}$, including contrail-induced cirrus) and HFCs, PFCs, and $SF_6$ (total 0.03 W $m^{-2}$) are not shown. Concentration-based RFs for gases can be obtained by summing the like-colored bars. Volcanic forcing is not included as its episodic nature makes it difficult to compare to other forcing mechanisms. Total anthropogenic radiative forcing is provided for three different years relative to 1750.

The strength of drivers in Fig. 4.3 is quantified as radiative forcing (RF) in units watts per square meter (W $m^{-2}$) as in previous IPCC assessments. RF is the change in energy flux caused by a driver and is calculated at the tropopause or at the top of the atmosphere. In the traditional RF concept employed in previous IPCC reports, all surface and tropospheric conditions are kept fixed. In calculations of RF for well-mixed greenhouse gases and aerosols in this report, physical variables, except for the ocean and sea ice, are allowed to respond to perturbations with rapid adjustments. The resulting forcing is called effective radiative forcing (ERF) in the underlying report. This change reflects the scientific progress from previous assessments and results in a better indication of the eventual temperature response for these drivers. For all drivers other than well-mixed greenhouse gases and aerosols, rapid adjustments are less well characterized and assumed to be small, and thus the traditional RF is used [4].

## 4.3 Global Warming Potential and Its Limitations

In the earlier sections of this chapter, global warming potential (GWP) is a measurement of how well heat is absorbed by greenhouse gases. The IPCC defines global warming potential (GWP) as "the ratio of the time integrated radiative forcing from a pulse emission of 1 kg of a substance, relative to that of 1 kg of carbon dioxide, over a fixed horizon period. GWP is a relative index used to compare the climate impact of an emitted greenhouse gas, relative to an equal amount of Carbon Dioxide" [10]. Also, the IPCC examines the GWP for 1 g of carbon dioxide at a 20-, 100-, and 500-year time horizon in comparison to other greenhouse gases [9]. The six major greenhouse gases are determined by the Kyoto Protocol. The Kyoto Protocol is an international treaty that sets obligations on industrialized countries to lower the

**Table 4.1** IPCC global warming potential consensus [2, 8, 9]

| Six greenhouse gases listed designated by the Kyoto Protocol | Lifetime (years) | Global warming potential for the given time horizon | | |
|---|---|---|---|---|
| | | 20 years | 100 years | 500 years |
| Carbon dioxide, $CO_2$ | ~150 | 1 | 1 | 1 |
| Methane, $CH_4$ | 12 | 72 | 25 | 7.6 |
| Nitrous oxide, $N_2O$ | 114 | 289 | 298 | 153 |
| HFC-23 | 270 | 12,000 | 14,800 | 12,200 |
| PFC-116 | 10,000 | 8630 | 12,200 | 18,200 |
| Sulfur hexafluoride, $SF_6$ | 3200 | 5210 | 7390 | 11,200 |

emissions of greenhouse gases. The GWP values can be seen in Table 4.1; the table shows the six major greenhouse gases from the Kyoto Protocol: carbon dioxide ($CO_2$), methane ($CH_4$), nitrous oxide ($N_2O$), HFCs, PFCs, and sulfur hexafluoride ($SF_6$). The Intergovernmental Panel on Climate Change (IPCC) includes many greenhouse gases in their consensus and a list of HFCs and PFCs. The HFC and PFC chosen in Table 4.1 are for the greatest GWP values within the consensus list. The lifetime among the six gases ranges from 12 to 10,000 years. The value closest to the median is sulfur hexafluoride, with a lifetime of 3200 years. At a time horizon of 500 years, while carbon dioxide releases 1 g, sulfur hexafluoride releases 11,200 g of carbon dioxide (11,200 times more) for the same time horizon. A stronger greenhouse gas can easily leak and create a major impact.

The global warming potential has limitations where radiative properties are uncertain and nonlinear ($CO_2$, $CH_4$, $N_2O$); the actual resident life of greenhouse gases and how long it actually stays in the atmosphere vary and some are unknown ($CO_2$, ozone precursors, diesel PM, and PM); if the resident lifetimes are short-lived in the atmosphere, the GWP is not useful; there are not only direct radiative forcings but also indirect radiative forcings with uncertainties (i.e., ozone precursors are not only a gas, but they also form ozone) [2]. While the graphs and data interpretations are accepted, people have challenged methodologies and how data is used and interpreted; however, in this case, the US Environmental Protection Agency (USEPA), the United Nations (UN), and the Intergovernmental Panel Data Analysis reports and technical books [3–6, 8–11, 16–17, 19–25] are widely accepted. Comparing global warming potentials to carbon dioxide, greenhouse gases are better understood when examining why carbon dioxide absorbs heat on a molecular level.

## 4.4 Heat Absorption by Carbon Dioxide

Carbon dioxide's ability to absorb heat is characterized by the molecular structure, the wavelength, and radiative properties. Visible light from the Sun is able to pass the carbon dioxide molecules without its energy being absorbed since the frequency of visible light does induce a dipole moment on the atmospheric $CO_2$ molecules.

Carbon dioxide does however absorb "infrared" radiation (heat from the Earth's surface) and also re-emits that energy at the same wavelength as what was absorbed (also as heat) [6]. As for its molecular structure, "Carbon dioxide doesn't have a molecular dipole in its ground state. However, some $CO_2$ vibrations produce a structure with a molecular dipole. Because of this, $CO_2$ strongly absorbs infrared radiation" [7].

The energy of a molecule can change due to a change in the energy state of the electrons of which it is composed. Thus, the molecule also has electronic energy. The energy levels are quantized and take discrete values only. Absorption and emission of radiation take place when the atoms or molecules undergo transitions from one energy state to another. In general, these transitions are governed by selection rules. Atoms exhibit line spectra associated with electronic energy levels.

The dipole moment is determined by the magnitude of the charge difference and the distance between the two centers of charge. If there is a match in frequency of the radiation and the natural vibration of the molecule, absorption occurs and this alters the amplitude of the molecular vibration. This also occurs when the rotation of asymmetric molecules around their centers results in a dipole moment change, which permits interaction with the radiation field. Dipole moment is a vector quantity and depends on the orientation of the molecule and the photon electric vector [12].

In accordance with Kirchhoff's laws, the following are noted:

1. Materials that are strong absorbers at a given wavelength are also strong emitters at that wavelength; similarly weak absorbers are weak emitters.
2. Emission, reflection, and transmission account for all the incident radiation for media in thermodynamic equilibrium [2, 6].

## 4.5 Rising Temperature Trend in the Environment

### *4.5.1 Atmosphere Temperature Increase*

As a result of increased human activity, more greenhouse gases are warming the Earth, resulting in an increased temperature trend around the world. The following graphs suggest the increase in emissions has led to the increase in $CO_2$ in the atmosphere, thereby increasing the temperature of the Earth's surface on land. Figure 4.4 shows the global per capita carbon emission estimates versus years. It appears that the global per capita carbon emission increases significantly after the year 2000 [15].

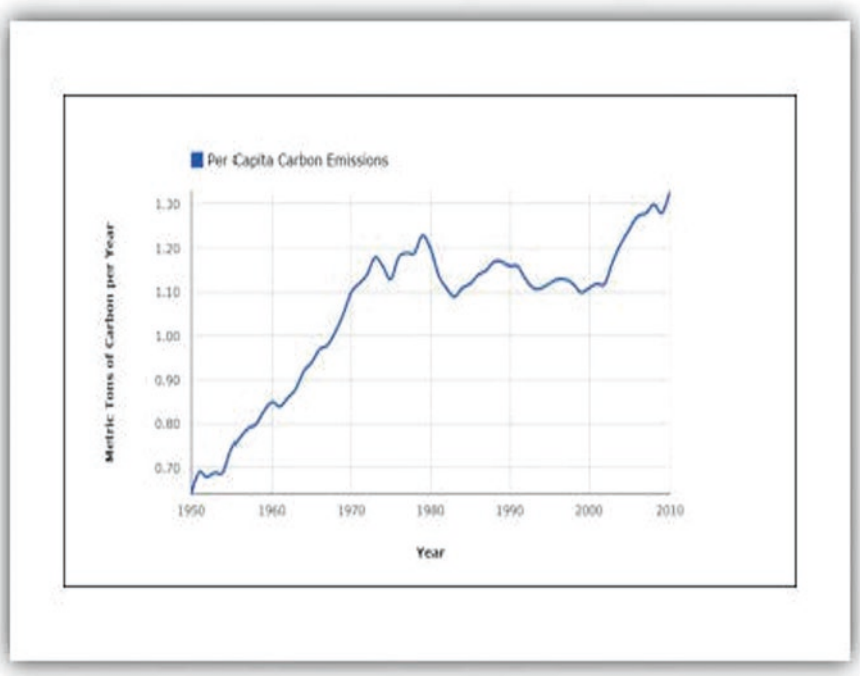



**Fig. 4.4** Global per capita carbon emission estimates versus years [15]

### *4.5.2 Land and Ocean Temperature Increase*

As land temperatures rise, we find ocean temperatures rise as well. "The globally averaged combined land and ocean surface temperature data as calculated by a linear trend, show a warming of 0.85 [0.65–1.06] °C, over the period 1880–2012, when multiple independently produced datasets exist. The total increase between the average of the 1850–1900 period and the 2003–2012 period is 0.78 [0.72–0.85] °C, based on the single longest dataset available. There are two methods: The first calculates the difference using a best fit linear trend of all points between 1880 and 2012. The second calculates the difference between averages for the two periods 1850–1900 and 2003–2012 [4].

Figure 4.5 shows an increase of carbon dioxide in the atmosphere from 1958 to 2012 [4], while Fig. 4.6 shows the annual temperature anomalies from land ocean in the period of 1880–2012 [16]. Based on the presented figures, an increase of global carbon emissions shown in Fig. 4.4 leads to an increase of carbon dioxide in the atmosphere shown in Fig. 4.5 and finally results in a temperature increase on land and ocean shown in Fig. 4.6.

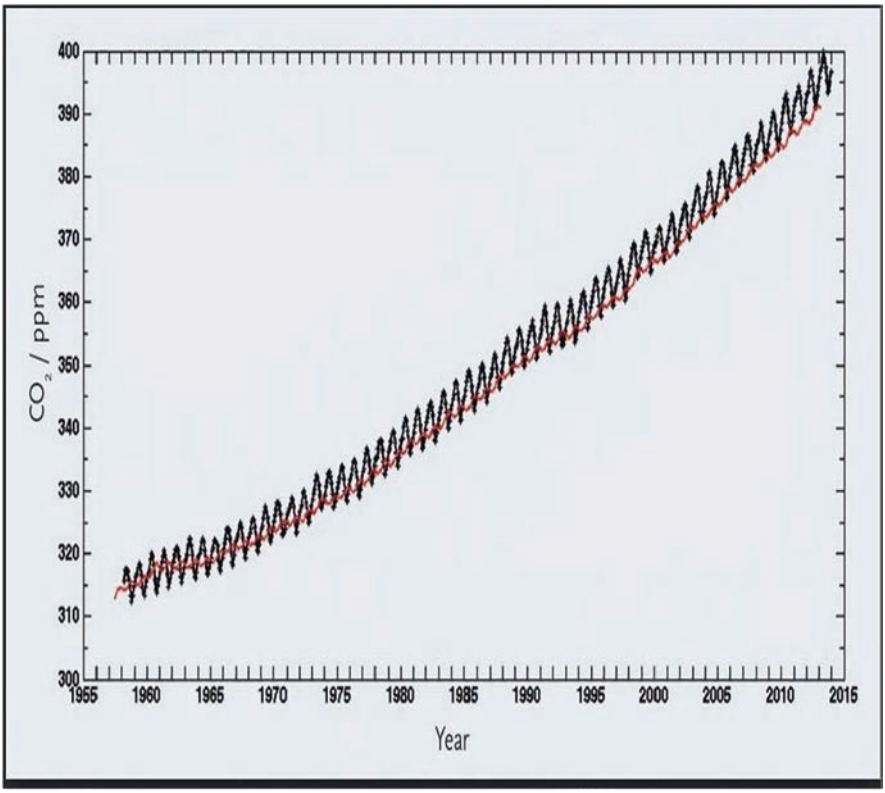



**Fig. 4.5** Increase of carbon dioxide in the atmosphere [4]

### *4.5.3 Rising Temperatures of Land, Air, Sea, and Ice*

Figure 4.7 summarizes the temperatures of land, air, and sea, with a dramatic increase beginning in 1980. Similarly, 1920–1940 also experienced an upward trend; however, from 1940 approaching 1980, the temperatures slightly decreased. The reason the temperatures dropped is due to the industrial revolution's emissions, where manufacturers and factories sent a layer of soot in the atmosphere. The layer of soot became a barrier and blocked solar radiation from hitting the Earth's surface, causing a cooling effect. However, when the Clean Air Act of 1970 was enforced, the layer of soot moved out of the atmosphere and so began the true and actual warming trend [2, 18, 19].

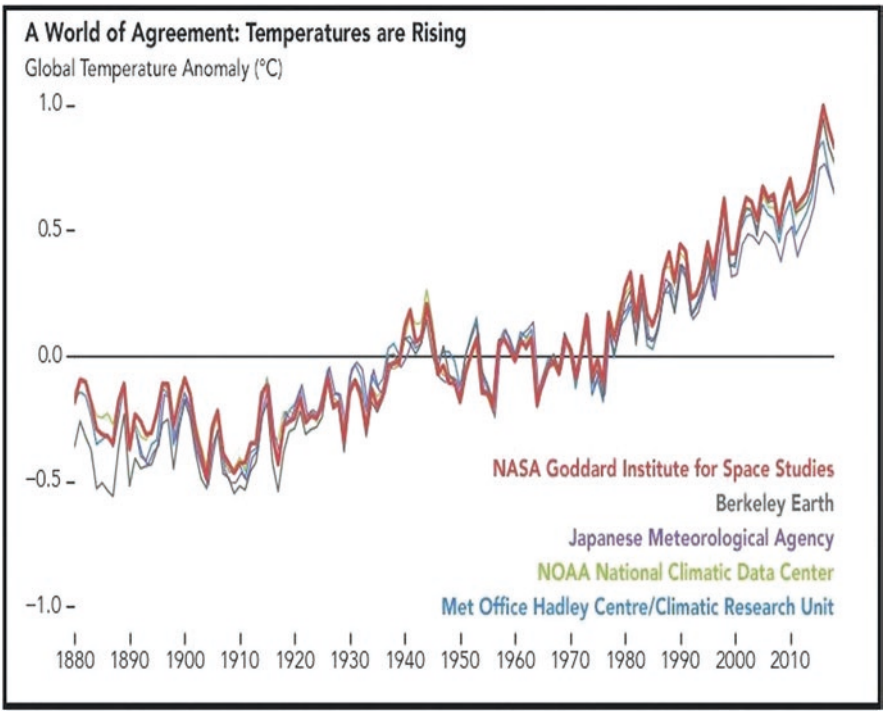



**Fig. 4.6** Land and ocean temperature increase: annual temperature anomalies from land and ocean 1880–2012 [16]

## 4.6 Increased Temperatures on Land and Its Impacts on Agriculture

As discussed previously, climate change results in increased temperatures in the atmosphere, land, and sea. Weather patterns will become more extreme and forceful where storms become cyclones such as Hurricanes Katrina, Irene, and Sandy in the United States. Winters will reach record cooling temperatures, and summers will reach record hotter temperatures. Dry land will become more dry, and droughts will become so severe, agriculture and food shortages may eventually lead to famine. Arid regions will be impacted where there once was water, will be no more water at all, or will experience significantly lower water levels. Agriculture will become a great challenge as soil becomes too dry or arid to harvest food [34].

Table 4.2 demonstrates the impacts of increased temperatures to our food supply. Table 4.2 illustrates the types of impacts that could be experienced as the world comes into equilibrium with more greenhouse gases. The top panel shows the range of temperatures projected at stabilization levels between 400 ppm and 750 ppm $CO_2$ at equilibrium. The solid horizontal lines indicate the 5–95% range based on climate

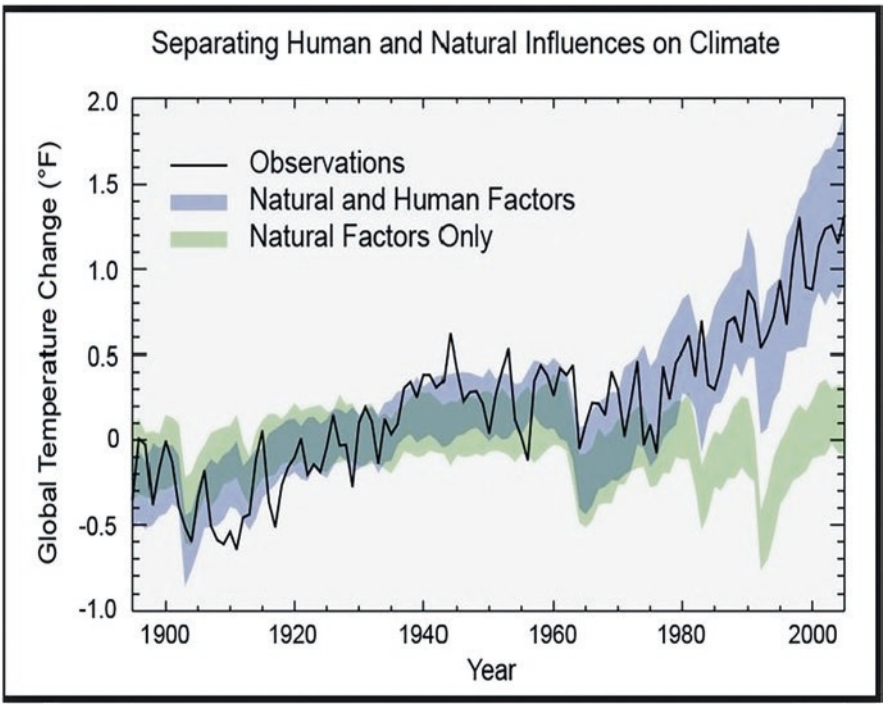


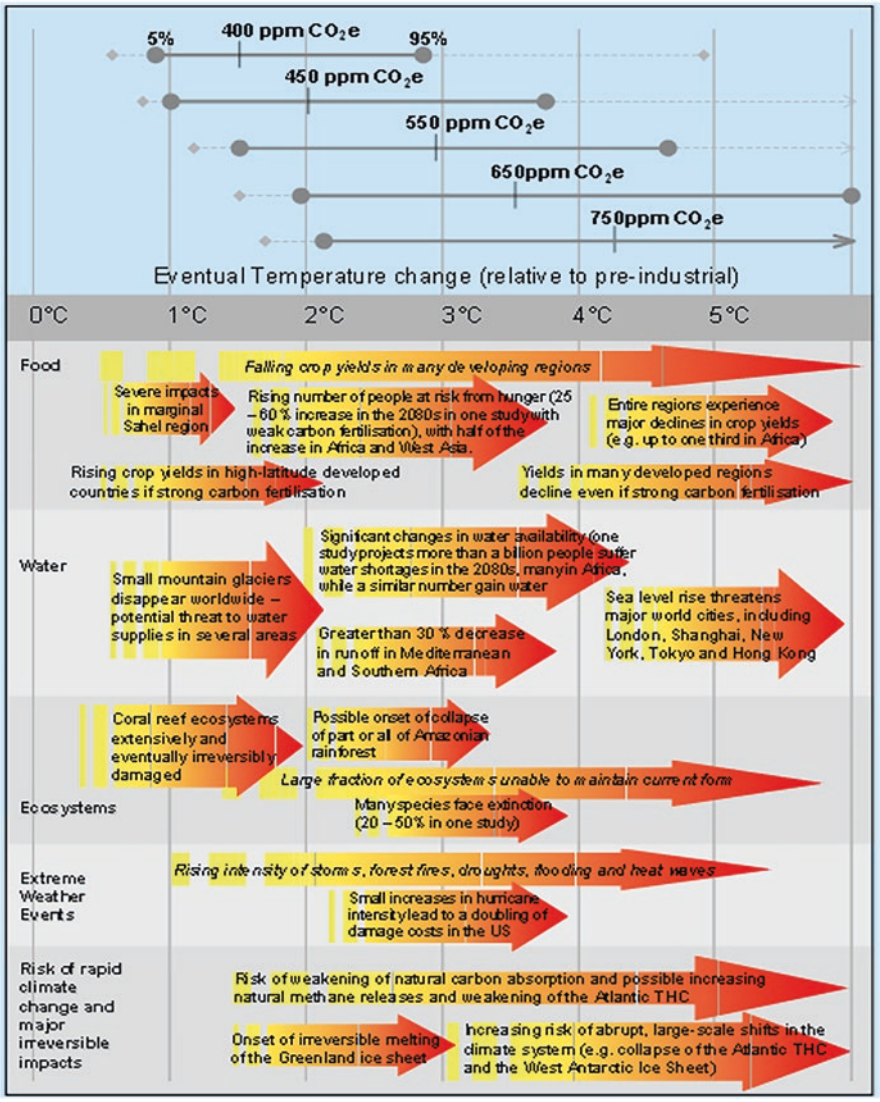

**Fig. 4.7** Observed globally averaged combined land and ocean surface temperature anomaly 1895–2012

sensitivity estimates from the IPCC 20012 and a recent Hadley Centre ensemble study. The vertical line indicates the mean of the 50th percentile point. The dashed lines show the 5–95% range based on 11 recent studies. The bottom panel illustrates the range of impacts expected at different levels of warming. The relationship between global average temperature changes and regional climate changes is very uncertain, especially with regard to changes in precipitation. This figure shows potential changes based on current scientific literature [34].

## 4.7 Effect of Global Warming and Climate Change on Sea Level Rise

As global average surface temperatures rise, and global average sea levels increase, the snow cover and ice will decrease and melt. Figure 4.8 reports the changes of temperature, sea level, and Northern Hemisphere snow cover. Specifically observed changes in Fig. 4.8(a) show global average surface temperature versus years. Figure 4.8(b) shows global average sea level from tide gauge (blue) and satellite (red) data versus years. Figure 4.8(c) shows Northern Hemisphere snow cover for

**Table 4.2** Stabilization levels and probability ranges for temperature increases




March–April versus years. All differences are relative to corresponding averages for the period 1961–2000. The smoothed curves represent decadal averaged values, while the circles show yearly values. The shaded areas are the uncertainty intervals estimated from a comprehensive analysis of known uncertainties shown in the figures.

The impacts of sea level rise include increased flood risk, infrastructure investment implications around the world as seen in other countries like Italy, Netherlands,

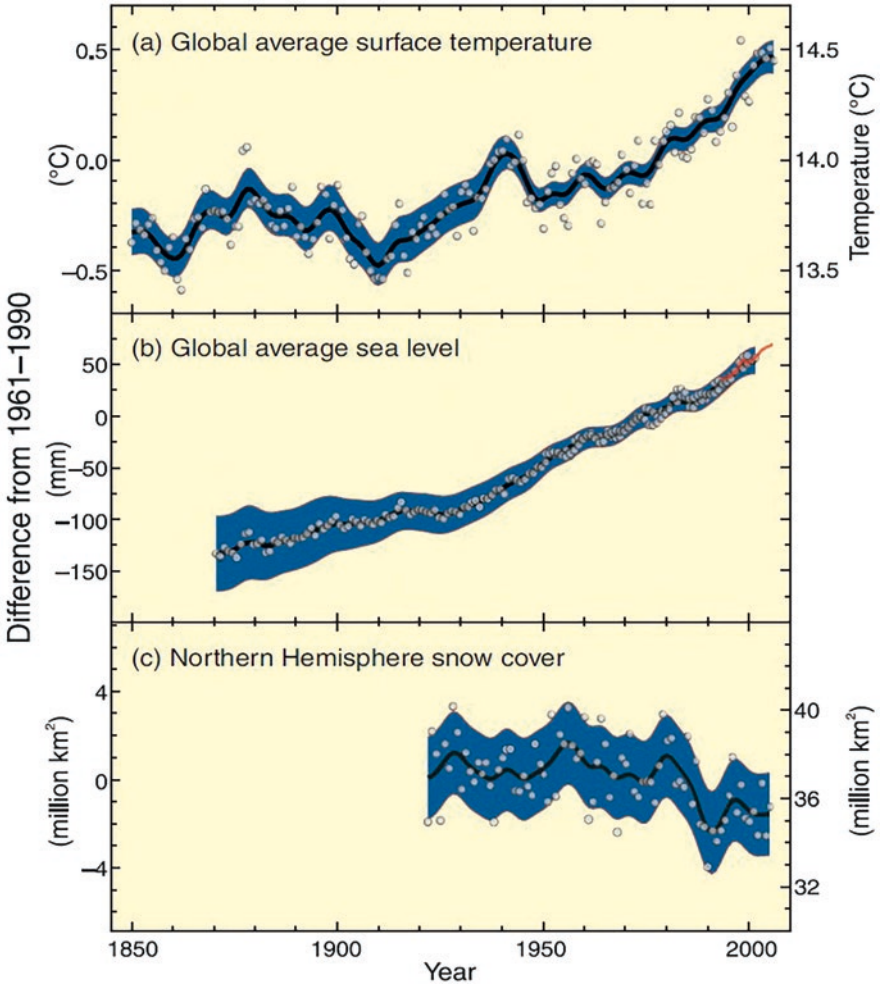



**Fig. 4.8** Changes in temperature, sea level, and Northern Hemisphere snow cover

and the South Pacific Island. Sea level rise will also contribute to increases in salinity of rivers and estuaries, saltwater intrusion, as seen in the United States and all around the world. Saltwater intrusion can affect the water supplies for drinking and irrigation water and depletes the available freshwater habitat, as seen in the United States.

The Asia-Pacific Region's climate change adaptation involves the risk insurance scheme as a social safety net through risk transfer and creates resilient societies. The advantages of risk transfer are that it promotes risk mitigation compared to current response-driven mechanisms, provides a cost-effective way to deal with expensive impacts from the effects of climate change, "supports climate change adaptation by covering the residual risks uncovered by other risk reduction mechanisms such as

building regulations, landuse planning and disaster risk management plans," stabilizes incomes in rural areas and minimizes the gap in income fluctuation and socioeconomic development, provides partnerships between public and private sectors, reduces government dependence after a disaster to reconstruct, helps people and communities to recover and get back to everyday life quickly, and addresses various risks from climatic and non-climatic origin, depending on how insurance is set up.

One of the greatest challenges to sea level rise impacts is increased salinity intrusion in rivers and estuaries, putting our water supply at risk.

## 4.8 Increased Salinity Intrusion in Rivers and Estuaries

According to the IPCC, studies have shown freshwaters to become more saline over time and seawater has become fresher. This has affected rivers and estuaries, drinking water supplies, irrigation, sea levels, and ecosystems.

### *4.8.1 Salinity Intrusion in Rivers and Estuaries*

Saltwater intrusion (or salinity intrusion) is the movement of saline water into freshwater aquifers, which can become contaminated and undrinkable. Freshwater aquifers can experience saline intrusion due to the hydraulic connection between groundwater and seawater. Because saltwater is more mineral rich than freshwater, it is denser and has higher water pressure. And so the heavier saltwater is able to push inland beneath the freshwater.

Sources of saltwater intrusion include, but are not limited to, (a) activities like groundwater pumping from coastal freshwater wells as seen in coastal areas; (b) water extraction which drops the level of fresh groundwater, reducing its water pressure and allowing saltwater to flow inland; and (c) water channels or agricultural and drainage channels, carrying saltwater inland, and causing sea level rise. Saltwater intrusion can also be worsened by extreme weather events like tropical cyclones and hurricane storm surges. All over the world, rivers and estuaries experience salinity intrusion as a result of rising sea levels.

A case study to consider is California's Sacramento-San Joaquin Delta. The Sacramento-San Joaquin Delta is at the heart of most discussions about water in California. The 1153 square mile of twist-and-turn islands and interconnected waterways is located where the Sacramento and San Joaquin Rivers converge and flow into San Francisco Bay through the Golden Gate Bridge. About 42% of the state's annual runoff flows through the Delta serving more than 23 million Californians and irrigating millions of acres in the Central Valley. Two-thirds of Californians get all or part of their drinking water from the Delta by government water projects that export water to the San Francisco Bay Area and Central and Southern California. The Delta is also the largest estuary on the West Coast with

hundreds of species of birds that travel along the Pacific Flyway and dozens of fish species including salmon and steelhead that migrate through the Delta on their journey to and from the ocean. The Delta is strongly influenced by freshwater inflow from tributary rivers, by tides in the SF Bay, and by salinity upstream. Since 1860, the Delta waters have seen an increase in salinity [1, 2].

Plant pollen revealed the Delta was mainly a freshwater marsh for the past 2500 years; however, in the past 100 years, because of human activity, the Delta has become more saline. Today, salinity intrusion is approximately 3–15 miles deeper into the Delta than the early twentieth century. Between 1860 and 1920, human activity modified the Delta when marshland was reclaimed, hydraulic mining caused increased deposition and erosion sediment, and the expansion of the Delta channel's width, depth, and connections took place.

Before freshwater diversions increased in the 1940s, the Delta and Suisun Bay would freshen every winter, even during extreme droughts as seen in the 1930s. However, the Delta did not freshen during recent droughts (1976–1977, 1987–1994, 2007–2009, and 2014–2015), resulting in contaminants and toxins accumulating in the system. The past 25 years have been relatively wet; the Delta's autumn salinity levels have shown to be in drought-like conditions due to human activity and water diversions.

The historical record and published studies demonstrate that the Delta is far saltier now due to human interference. Starting in 1917, local industries and residents observed unprecedented salinity levels, causing a local sugar refinery to find a new water supply, the Town of Antioch to file a lawsuit against upstream water users, and the State of California to start a salinity monitoring program and investigation (Fig. 4.9).

The colored portion on each chart represents the amount of freshwater available within Suisun Bay downstream of the Delta boundary (approximately 18 miles above Crockett). From 2001 to 2005, freshwater was seldom available below the Delta boundary, indicating that the Delta did not "flush" as it used to. Without the seasonal freshening of the Delta, contaminants and toxics can accumulate in the system; and in this case, toxics were found to be a factor in the decline of the Delta ecosystem. Note: While hydrological conditions were similar in the three time periods shown in the table, the sequence of wet and dry periods differs.

### *4.8.2 Water Quality and Water Supply Impacted by Climate Change and Salinity Intrusion*

Because of salinity intrusion, water quality impacts to the water supply are affected. Many coastal cities in the United States have experienced saltwater intrusion through water supply wells. Impacts of saltwater intrusion depend on how far the intrusion extends, the plans for the water use, and how concentrations exceed the standard of dissolved ions for its intended use.

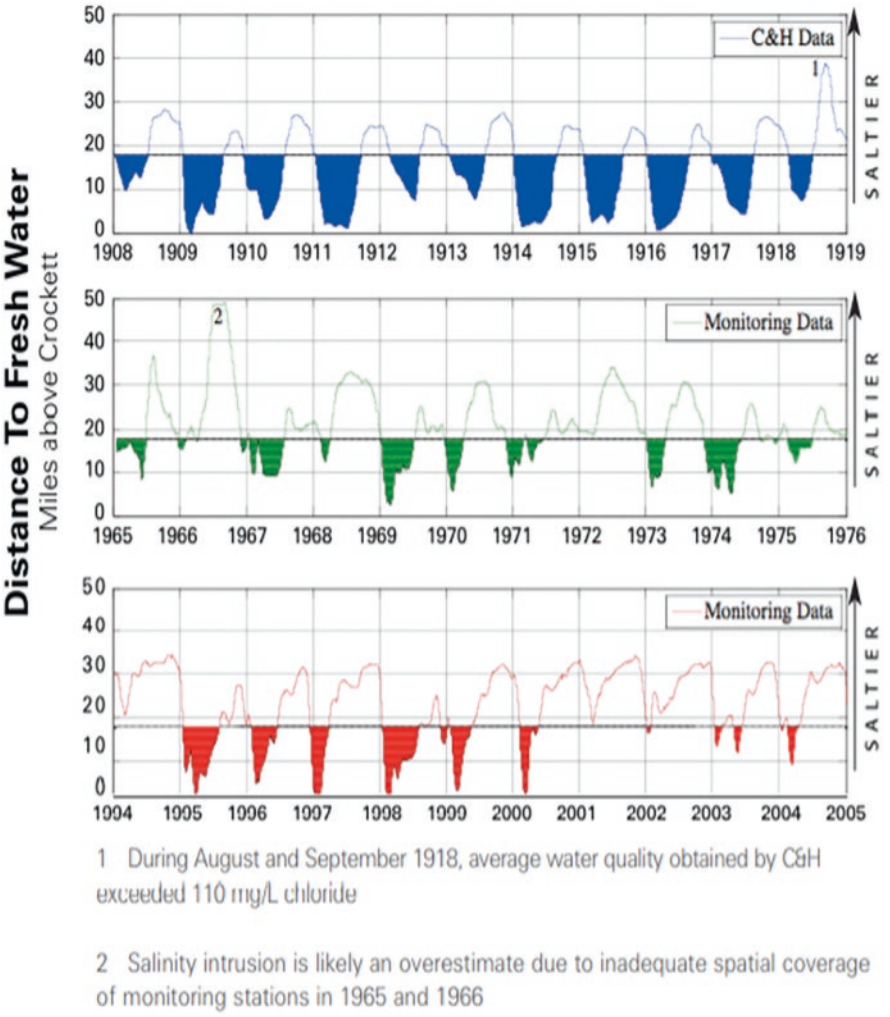



**Fig. 4.9** Freshwater was available within Suisun Bay for a longer time period each year during the early 1900s

For example, a coastal state such as Washington State and Southern California reaches portions of the aquifer, affecting only certain water supply wells, whereas Cape May, New Jersey, United States, experienced saltwater encroachment laterally within each aquifer; it led to closing 20 or more public and industrial supply wells.

Not only in the United States, but salinity intrusion is a threat to water quality and water supply all over the world. Since the autumn of 2003, a strong salinity intrusion has caused a serious threat to the water supply in the Pearl River Delta, in Macau and Zhuhai, cities in China. The salinity intrusion is caused by rapid industrialization and urbanization, leading to greater water demand in the middle and

upper stream of the river basin. When the flow runs low, either by water demand or during a dry season, salinity intrusion often occurs. Other sources of salinity intrusion include the rise of temperatures and sea levels, thereby affecting the water supply and quality.

Also, in Bangladesh, drinking water from natural sources by the coast has become contaminated by varying degrees of salinity due to saltwater intrusion from rising sea levels, cyclone and storm surges, and upstream withdrawal of freshwater. Not only in Asia, but also in Africa, the Ada peninsular in Ghana has suffered rapid coastal erosion and inundation for over 50 years. As a result, it has led to loss of property and livelihoods, economic stagnation, and saltwater intrusion. While property loss and economic opportunities are addressed, the solutions do not benefit increasing salinity intrusion in the Volta River. In fact, it aggravates the salinity intrusion, increasing salt in the Volta Estuary. The government intervened to implement a sea defense project to keep seawater from intruding and is concerned about the water supply and water quality due to saltwater intrusion.

Ada, situated at the mouth of the Volta Estuary, the Impact Assessment Report writes there is a likely significant impact on the physicochemical water characteristic of the estuary: "The most significant change will be an increased salinity in this zone, whereby the salinity gradient will shift to the north… The intrusion of salt water further into the estuary will probably lead to local changes in water quality. In places where the fresh water is high in particulate organic matter content comes in contact with salty coastal water, the organic matter starts to flocculate creating depositions of dark material in which toxins and nutrients tend to accumulate. This compromises the quality of water in the far southern part of the estuary, even more so because it creates nutrient rich conditions in which many bacterial and viral organisms, capable of causing diseases thrive. Hence, by opening up the estuary, this zone could be drawn into the estuary, negatively affecting water quality. However, the spatial extent of salinity shift is uncertain, and rated low to moderate compared to some natural phases in the estuary dynamics where openings have been created naturally that are far larger than the planned access channel."

In the case of Australia's dry weather challenges, droughts and extreme low flows in the water systems reduce ecosystem capacity to absorb and process contaminated water. As sea levels rise, the estuaries in the Murray-Darling Basin experience an increase in saltwater intrusion, affecting major urban water supplies, as well as freshwater ecosystem stability and productivity.

According to a case study of salinity of Israel's Lake Kinneret, the conclusion was made that increased salinity did not show obvious signs to effects on the lake ecology; however, the study showed a reduced water quality; this brought attention to implement changes in the Israeli water supply system.

All around the world, water quality impacts to water supply are affected by increased salinity in drinking water, such as well water, aquifers, and other sources, where the salt concentrations exceed the allowable. In the United States, a secondary maximum contaminant level is applied to 15 contaminants as a nonmandatory measure.

Water naturally accumulates a variety of dissolved solids, or salts, as it passes through soils and rocks on its way to the sea. These salts typically include such cations as sodium, calcium, magnesium, and potassium and anions such as chloride, sulfate, and bicarbonate. A careful analysis of salinity would result in a list of the concentrations of the primary cations and anions, but a simpler, more commonly used measure of salinity is the concentration of total dissolved solids (TDS). As a rough approximation, freshwater can be considered to be water with less than rough approximation, freshwater can be considered to be water with less than 1500 mg/L TDS, brackish waters may have TDS values up to 5000 mg/L, and saline waters are those with concentrations above 5000 mg/L, whereas seawater contains 30,000–34,000 mg/L TDS.

In some parts of the country, salty water may be encountered. Since the saltwater generally is overlain by freshwater, the lower part of the well in the saltwater zone can be sealed off. But when this is done, the yield of the well is decreased.

Sometimes, waste saltwater resulting from the backwashing of a home ion exchange water softener is discharged close to the well. Since saltwater is not filtered out in seeping through the soil, it may find its way into the well. The best thing to do is to discharge the wastewater as far as possible and downgrade from the well or utilize a commercial water softener service. Saltwater is corrosive; it will damage grass and plants and sterilize soil. Road salting or salt storage areas may also contribute to well pollution.

Special desalting units (using distillation, deionization, and reverse osmosis) are available for residential use, but they are of limited capacity and are relatively expensive, and pretreatment of the water may be needed. Complete information, including effectiveness with the water in question and annual cost, should be obtained before purchase.

The US Environmental Protection Agency (USEPA) has established National Primary Drinking Water Regulations (NPDWR) that set mandatory water quality standards for drinking water contaminants. One enforceable standard is the measurement of MCL (maximum contaminant levels), which were established to protect the public against consumption of drinking water contaminants that present a risk to human health. An MCL is the maximum allowable amount of a contaminant in drinking water, which is delivered to the consumer.

### *4.8.3 Agricultural Irrigation and Operations Impacted by Climate Change and Salinity Intrusion*

According to Hung and Forrider [37], the impacts of global warming and climate change include degradation of natural resources, reduced agricultural production, and human dislocation, and these impacts as a driver of future forced migration depend on several factors:

1. Quantity of future GHG emissions

2. Rate of future population growth and distribution
3. Meteorological evolution of climate change
4. Effectiveness of local and national adaption strategies

Climate change has affected the food security either directly or indirectly causing stress on the production of food [39]. Climate change has also created pressures on the hydrological cycle and impacts water availability, which strongly influences agriculture. Effects on crop production are hard to predict as it depends on the frequency or intensity of extreme weather events. Global aridity has increased since the 1970s due to desertification. The areas under aridity have increased from 17 to 27% from the 1950s until now. This has notable effect on crop production and decreasing crop yields. For example, maize yields diminish up to 1.7% under drought conditions [39].

Higher $CO_2$ levels can positively affect food crop growth. However, other factors such as temperature, ozone, water availability, and nutrient constraints may counteract potential increases in yield. Many weeds, pests, and fungi thrive under warmer temperatures and wetter climates. Currently, farms spend more than $11 billion per year to fight weeds which compete with crops.

Drought may threaten livestock yield. In 2011, exposure to temperature events caused over $1 billion in heat-related losses. Heat stress affects animals directly and indirectly, making them vulnerable to disease and infertility. The prevalence of parasites increases, and the productivity of pastures is affected. The quality of the forage found in pastureland decreases with higher GHG; as a result, the cattle would need to eat more to get the same nutritional value. This can lead to overgrazing and misuse of land management [40].

Fisheries are also impacted [30]. American anglers catch or harvest five million metric tons of seafood each year and contribute $1.5 billion to the US economy annually. Aquatic species migrate to colder waters, and shell-building animals decrease in number. Fishermen experience decreases in harvest, which increase the price and availability of seafood. American fisheries are analogous to the global fishing economy.

Salt affects plant growth in three ways: (a) osmotic effects, caused by the total dissolved salt concentration in the soil water; (b) specific ion toxicity, caused by the concentration of individual ions; and (c) soil particle dispersion, caused by high sodium and low salinity. With increasing soil salinity in the root zone, plants expend more of their available energy on adjusting the salt concentration within the tissue (osmotic adjustment) to obtain needed water from the soil. The consequence is less energy is available for plant growth.

In irrigated areas, salts originate from the local groundwater or from salts in the applied water. Salts tend to concentrate in the root zone due to evapotranspiration, and plant damage is tied closely to an increase in soil salinity. Establishing a net downward flux of water and salt through the root zone is the only practical way to manage a salinity problem. Under such conditions, good drainage is essential to allow a continuous movement of water and salt below the root zone. Long-term use

of reclaimed water for irrigation in which only the conventional constituents have been removed is not possible without adequate drainage.

Specific ion toxicity is another factor to be studied. If the decline of crop growth is due to excessive concentrations of specific ions, rather than osmotic effects alone, it is referred to as "specific ion toxicity." The ions of most concern in wastewater are sodium, chloride, and boron. The most prevalent toxicity from the use of reclaimed water is boron. The source of boron is usually household detergents or discharges from industrial plants. The quantities of chloride and sodium also increase as a result of domestic usage, especially where water softeners are used.

For sensitive crops, specific ion toxicity is difficult to correct without changing the crop or the water source. The problem is also accentuated by hot and dry climatic conditions due to high evapotranspiration rates. Regulations for maximum trace element concentrations for irrigation water are reported. In severe cases, these elements tend to accumulate in plants and soils, which could result in human and animal health hazards or cause phytotoxicity in plants.

The concentration of dissolved solids is an important indicator of the usefulness of water for various applications. Drinking water, for example, has a recommended maximum total dissolved solids (TDS) concentration of 500 mg/L. Many people will begin to notice water tastes salty at about 1000 mg/L of TDS, although this is very dependent on the particular dissolved constituents. Livestock can tolerate higher concentrations. Upper limits for stock water concentrations quoted by the US Geological Survey (1985) include poultry at 2860 mg/L, pigs at 4290 mg/L, and beef cattle at 10,100 mg/L. Of greater importance, however, is the salt tolerance of crops. As the concentration of salts in irrigation water increases above 500 mg/L, the need for careful water management to maintain crop yields becomes increasingly important. With sufficient drainage to keep salts from accumulating in the soil, up to 1500 mg/L TDS can be tolerated by most crops with little loss of yield, but at concentrations above 2100 mg/L, water is generally unsuitable for irrigation except for the most salt-tolerant crops. All naturally occurring water has some amount of salt in it. In addition, many industries discharge high concentrations of salts, and urban runoff may contain large amounts in areas where salt is used to keep ice from forming on roads in the winter. Although such human activities may increase salinity by adding salts to a given volume of water, it is more often the opposite process, the removal of freshwater by evaporation, that causes salinity problems. When water evaporates, the salts are left behind, and since there is less remaining freshwater to dilute them, their concentration increases.

Irrigated agriculture, especially in arid areas, is always vulnerable to an accumulation of salts due to this evapotranspiration on the cropland itself. The salinity is enhanced by the increased evaporation in reservoirs that typically accompany irrigation projects. In addition, irrigation drainage water may pick up additional salt as it passes over and through soils. As a result, irrigation drainage water is always higher in salinity than the supply water and, with every reuse, its salt concentration increases even more. In rivers that are heavily used for irrigation, the salt concentration progressively increases downstream as the volume of water available to dilute salts decreases due to evaporation and as the salt load increases due to salty drainage

water returning from irrigated lands. It has been estimated that roughly one-third of the irrigated lands in the western part of the United States have a salinity problem that is increasing with time, including regions in the Lower Colorado River Basin and the west side of the San Joaquin Valley in California, United States. An estimated 100,000 tons of salt are imported annually into Southern Arizona through its consumption of Colorado River water via the 300 mile long, Central Arizona Project canal. Salinity problems are also having major impacts on irrigated lands in Iraq, Pakistan, India, Mexico, Argentina, Mali, and North Africa, among others. The collapse of ancient civilizations, once known as the Fertile Crescent, and is now Iraq, is thought to have formed by accumulating salt from irrigated agriculture. Agriculture that depends on irrigation from affected rivers would be directly impacted by sea level rise. Crops do not grow as well with salty water, as seen in the resulting smaller leaves, shorter stature, and sometimes fewer leaves. The severity of salinity on crops is based on the environment's humidity, temperature, radiation, and air pollution. Some of the agricultural production could be shifted to salt-tolerant crops. Irrigation with salty water tends to accumulate salt in the soil, decreasing soil productivity. Note that this is not the only way that climate change can disrupt food production.

### *4.8.4 Food Production Impacted by Climate Change and Salinity Intrusion*

Drought and other climate extremes have a direct impact on food crop, food supply, and economics. During a dry spell, there will be excessive water loss from the plants; thus, the process of photosynthesis is greatly reduced and it is difficult for the plants to survive [36]. On the contrary, during a flooding event, plants will be inundated and damaged due to depleted oxygen (approaches zero after 24 h flooding event) and nitrogen levels in the flooded soils. In addition, the affected plant's stomata will be closed for a period of time which will subsequently reduce the respiration, transpiration. Loss in crop yield may lead to economic collapse (as the price of staple crops could rapidly escalate causing major inflation) and food shortage, where hunger will be the biggest battle and create conflicts in some countries.

Coming out of the last ice age, the climate change was maintained and steady and the human population was small and nomadic, whereas now, large communities of an increasing population live away from agriculture that naturally gets rain, but instead relies on irrigation. Demand for water is greater than the renewable supply of freshwater for a community's demand and supply needs. Irrigation demand is of poor water quality containing dissolved salts that collect in the soil. Irrigation and removing native perennial vegetation have led to rising water tables—some rise into the root zone and soak the land. Over 50% of groundwater is saline, especially in dry and semiarid regions, so as water tables rise, the saltwater gets brought into root zone areas. How sustainable are irrigated systems as we work through issues of water resource availability and allocation? The changes in climate and population

are projected to increase, and so irrigation and water supply would follow. Unless dramatic changes are made, continued increasing salinity will be found in agriculture.

### *4.8.5 Ecosystem Impacts Due to Loss of Freshwater Habitat (Recreation, Fishing)*

Salt accumulation in soils is often controlled by flushing the salts away with additional amounts of irrigation water. This increases costs; wastes water, which may not be abundantly available in the first place; and unless adequate drainage is available, increases the likelihood that a rising water table will drown plant roots in salt-laden water. Providing adequate drainage can be an expensive and challenging task involving extensive on-farm subsurface drainage systems coupled with a central drain and disposal system. Even when salt is removed from the agricultural land by good drainage, it can have later repercussions. In the mid-1980s, it was found that birdlife in the natural freshwater marshes of the Kesterson National Wildlife Refuge in Central California was being poisoned by selenium draining from the region's agricultural fields. Since irrigation return water contains not only salts but also fertilizers and pesticides, finding an acceptable method of disposal is difficult. These issues with salts highlight how important it is to not only deal with the immediate impacts of pollution but to develop the remedies so that further downstream impacts are not created.

## 4.9 Impacts of Solid Waste Landfill Gas on Sanitary Landfill Utility, Ecosystem, and Human

### *4.9.1 Impacts on Sanitary Landfill Operations and Surrounding Environment*

Shammas, Wang, Wang, and Chen [35] have discussed the ecological impact of sanitary landfill gas (LFG) on the landfill utility's operation as well as LFG collection, control, and utilization. The result of a 214-year study of the time phase evolution of various gases in a landfill has shown the following [38]:

1. Hydrogen, oxygen, nitrogen, carbon dioxide, and methane constitute the major gases.
2. Hydrogen in great quantities appears during the first 3 weeks (20% during the first and second weeks).
3. Hydrogen sulfide appears in a trace form during the first 2 years.
4. Carbon dioxide reaches 35% after 2 weeks (40% after 2 months).

5. Methane reaches 2.7% after 2 months, 6% in 6 months, 13% after 1 year, and 20% after 2 years.
6. Composition of gas is dependent on compaction densities. Higher compaction densities yield more gas per unit volume.
7. The most pronounced changes in the organic materials occur within the first 2 months.
8. A landfill is still far from being stabilized at the end of 2 years.
9. Dry refuse and saturated refuse produce 0.0022 and 0.0131 $m^3$ gas/kg refuse, respectively, on a dry basis.
10. Carbon dioxide increases the hardness and level of bicarbonates in groundwater. Depending on the pH, the water may become acidic and corrosive.

Aziz, Rosli, and Hung [36] have reported that methane is a shorter lifetime potent gas (9–15 years) with a high global warming potential due to a strong molar absorption coefficient. As the concentrations in the atmosphere increased due to uncontrolled anthropogenic methane production, it has become more long-lived and causes damages by creating an imbalance between methane emissions and removals. Their publication [36] discusses about methane generation in landfills (anaerobic decomposition process, source of methane in landfills, and methane reduction), methane emissions (mechanisms and factors influencing the mechanisms), methane in the atmosphere (methane sink and removal), and the impact of landfill methane emissions.

The migration and emission of LFG may potentially lead to negative effects in the surroundings, for example, fire and explosion hazards, health risks, damage to vegetation, groundwater contamination, and global climate effects. The main environmental hazards related to methane emissions are believed to be explosion hazards and global climate effects.

The potential for methane gas to explode is determined by its lower explosive limit (LEL) and upper explosive limit (UEL), which lies between 5 and 15% in air at ambient temperature and atmospheric pressure. Even though explosion will not occur if the concentration is above the UEL, methane concentrations equal to or greater than LEL will be considered hazardous as it exceeds the LEL. Thus, it is essential to monitor and keep the methane concentration below the LEL.

### *4.9.2 Impacts on Human Health*

Extreme climate affects human health with exposure to both extreme hot and cold weather being associated with cardiovascular disease (CVD) and mortality [36]. The Europe episode in summer 2013 is one example of mortality effect, when the temperature increased to 3.5 °C above normal and caused 22,000–45,000 heat-related deaths within 2 weeks in August 2003. In addition, changes in the rainfall pattern in many areas affect the distribution of infectious diseases/vector-borne diseases (malaria, dengue, plague, elephantiasis, and bluetongue disease) due to the

nature of the infectious agents (bacteria, virus, and protozoa) and their vector organisms (mosquitoes, snails, and other insects) that are temperature dependent, with a warm environment boosting their rate of reproduction. This was seen during an El Niño episode in Peru (1997–1998) when the ambient temperature increased more than 5 °C above normal and caused the number of daily admissions for diarrhea to increase by twofold from the previous rate.

At a low concentration in the air, methane and carbon dioxide do not affect the health. Nevertheless, high concentrations of methane and carbon dioxide in the atmosphere will contribute to adverse health effects, not by breathing the gases itself but through the displacement of oxygen, which can reduce the concentration of oxygen (below 16%) in the air [41]. As a result, there is a risk of asphyxiation, which can lead to dizziness, fatigue, vomiting, headache, visual disturbance, faster heartbeat, asthma, reduced lung function, unconsciousness, and even death if the condition is prolonged [42].

### *4.9.3 Impacts on Vegetation*

Methane does not have a direct toxicity effect on the plant or vegetation growth. Nevertheless, a high methane concentration in ambient air will result in a lack of oxygen in the root zone, and the displacement of oxygen by methane can cause anaerobic soil conditions which are detrimental to plants [35].

## 4.10 Natural Variability

Is it possible that the natural environment could make a small contributing factor toward climate change? When assessing climate change, one must also consider the impacts of the natural environment. Natural contributions to climate can happen through either internal impacts or external forces. Internal impacts are those factors that occur directly within a climate system. These can occur within the atmosphere, through entities within the climate system, or among phenomena that drive climate variations on Earth. The effects of internal impacts can happen almost immediately or incrementally over a long period. On the other hand, external forces are factors outside of the climate system that can result in changes in the climate. For example, ash and sulfuric aerosols from a volcanic eruption may cause temporary changes both locally and thousands of miles away from the eruption. The consequence of these natural particulate emissions is that these emissions create a layer of particulates that keep sunlight from penetrating the atmosphere. As a result, there is an expectation that temperatures will be cooler for a period before recovering back to levels experienced prior to the event [31–32].

The change to climate due to nature can happen over a short or long period of time. Changes that happen over thousands of years are known as millennial climate

cycles. These cycles can happen every 10,000–100,000 years and can cause significant periods of warming and cooling. The cause of these changes can be attributed to Milankovitch cycles, or changes in the Earth's orbit around the Sun. While these changes do not directly cause warming and cooling, they can provide a mechanism for these phenomena to take place. For example, a change in solar reflectivity can increase ice melt. Periods where warming and cooling can occur between 250 and 1000 years are known as century-scale climate changes. The shorter periods between events could be attributed to the Sun or ocean circulation patterns. Finally, there are periods where climate can change in as short of a time as year to every 10 years. Most of the time, this is caused by interactions between the ocean and the atmosphere. The most common example is the El Niño-Southern Oscillation (ENSO). This can bring warmer weather to some areas and an increase in precipitation in others.

It is important to quantify if climate change is directly related to the climate system or some external factors. The primary way to determine this is by having an understanding of the physical attributes of the climate. Data based on climatic observations is also beneficial. Models can also be considered because they provide a way to simulate the unpredictable effects of varying phenomena over time. Nevertheless, models must be compared with climatic observations and the known physical attributes of a climate to ensure that the models are put into proper context. This is done by placing proper boundaries around scenarios and outcomes that would not otherwise fit within what has been observed historically through data or by prior knowledge of the climate. This would in turn minimize potential biases that could occur in situations where data was unavailable [32].

So what have the models reported? The following is a summary of key points as described from the IPCC [31–32]:

1. With an exception of a few locals, the models show both model and observed data agree that warming occurs around the world. During the first half of the century, warming was due to a combination of anthropogenic and natural events (volcanic, solar, and internal). Anthropogenic sources caused warming during the second half of the century. The anthropogenic forcing appears to be primarily greenhouse gases (GHGs).
2. An increase in Northern Hemisphere (NH) temperatures has occurred within the last 50 years regardless of methods of reconstruction or external factors employed.
3. It is impossible to see a significant increase in NH temperatures without human influence, but natural variability would play some role since the warming is not consistent.
4. Greenhouse gas, volcanic eruptions, and solar irradiance have played some role in temperature change over the past 1000 years. Volcanic activity during 1675 and 1715 might have led to cooling during this time.
5. Models found that data that only considered natural variability did not match global mean surface temperature data. This data appeared to match better to what is seen in simulations comparing with what was observed.

6. There is uncertainty surrounding the effects from the Sun and volcanoes. This is primarily because of the changes to methods in modeling (e.g., number of sample sizes, scaling factors to account for unknown factors, and internal variability). Therefore, one must consider the assumptions and factors each author makes within a model.
7. Regional climate change may be hard to predict due to internal impacts that are unique to a particular area. These impacts will become more important at the regional level as opposed to considering the larger area. This is also true on shorter time scales of less than 50 years.
8. There has been documented evidence of a change in tropospheric height, ozone-induced stratospheric cooling, and tropospheric warming by GHGs. It appears that natural causes alone are simply unable to explain these changes.
9. Oceans have gained $14.2 \times 10^{22}$ J of energy from 1961 to 2003. The reason behind such a gain may be attributed to GHGs and sulfur aerosols. Volcanic eruptions can explain some cooling events within an ocean.
10. The sea level rise might be explained by anthropogenic reasons, specifically upper ocean and glacier loss. There have been small changes to sea level pressure changes due to ozone depletion. Anthropogenic impacts have affected Asian monsoon circulation (black carbon aerosols), an increase of tropical cyclones, atmospheric water vapor, and saturated vapor pressure. The combination of both anthropogenic and natural causes has also contributed to an increase in land mass mean precipitation.
11. Greenhouse gases may have also caused changes in precipitation values and glacial retreat. Warming may have altered the movement of water vapor from the tropics to high-altitude regions may have led to changes in the precipitation values.

## 4.11 Applications to Take Action

To avoid the consequences, solutions begin with us. We can start by [2]:

1. Carpooling.
2. Get a vehicle with better gas mileage
3. Use compact florescent lights
4. Make your home more energy efficient by replacing appliances
5. Turn off your power strip when you are done; it conserves 25%
6. Be a better consumer by buying recycled things, and recycle simple things like the disposable coffee sleeve from your coffee shop
7. Get off junk mail
8. Stop buying bottled water and use a water filter

## 4.12 Summary

What is certain? It is certain that:

1. The study of climate change begun as early as the 1820s with scientists such as Fourier, Tyndall, and Arrhenius. Therefore, this field of science is older than the first trans-Atlantic flight and was before the invention of the atomic bomb!
2. Contemporary recognition of climate change began in the 1950s and continued to become a staple within the scientific community during the environmental movement of the 1960s and 1970s.
3. The increasing amount of human activity is changing the composition of the atmosphere with overwhelming supporting data.
4. Carbon dioxide, methane, and nitrous oxide are increasing dramatically because of human activities.
5. Greenhouse gases absorb heat and emit heat; since they get trapped in the atmosphere, the heat gets trapped in the atmosphere and warms the Earth.
6. Human activity produces greenhouse gases that remain in the atmosphere for years.
7. It is estimated that the average global temperatures have risen between 1 and 4 °F.
8. During the first half of the twentieth century, increases in temperature have been due to natural causes. During the latter half, temperature increases have been due to anthropogenic activities.
9. Greenhouse gases, global warming, and climate change have negative impacts on agricultural irrigation, agricultural operations, food production, water utility, and sanitary landfill utility.

What is uncertain? It is uncertain that:

1. Forecasting exact impacts to health, agriculture, water resources, forests, wildlife, and coastal areas in regional basis is difficult.
2. There is also uncertainty in quantifying the exact magnitude and extent of adverse effects, projecting the magnitude of sea level rise, and quantifying the indirect effects of aerosol particles to the Earth's energy balance (i.e., cloud formation and its radiative properties, precipitation efficiencies).
3. The negative impacts of greenhouse gases, global warming, and climate change on agricultural irrigation, agricultural operations, food production, water utility, and sanitary landfill utility cannot be quantified at present.

Adaptation/mitigation for the effects of climate change is necessary because evidence shows it is too late for complete prevention. The responsible thing to do is to start preparing now.

## Glossary

**Agricultural irrigation** It is a large-scale agricultural process of applying controlled amounts of water to land to assist in the production of crops, as well as to grow landscape plants. Small-scale irrigation applied to lawn is called watering. There are different types of irrigation, such as sprinkler irrigation, surface irrigation, drip irrigation, subirrigation, and manual irrigation.

**Climate** (a) Climate in a narrow sense is usually defined as the "average weather" or, more rigorously, as the statistical description in terms of the mean and variability of relevant quantities over a period of time ranging from months to thousands of years. The classical period is three decades, as defined by the World Meteorological Organization (WMO). These quantities are most often surface variables such as temperature, precipitation, and wind. Climate in a wider sense is the state, including a statistical description, of the climate system. (b) The average weather (usually taken over a 30-year time period) for a particular region and time period. Climate is not the same as weather, but rather, it is the average pattern of weather for a particular region. Weather describes the short-term state of the atmosphere. Climatic elements include precipitation; temperature; humidity; sunshine; wind velocity; phenomena such as fog, frost, and hailstorms; and other measures of the weather.

**Climate change** (1) Changes in average weather conditions that persist over multiple decades or longer. Climate change encompasses both increases and decreases in temperature, as well as shifts in precipitation, changing risk of certain types of severe weather events, and changes to other features of the climate system. (2) Climate change refers to any significant change in the measures of climate lasting for an extended period of time. In other words, climate change includes major changes in temperature, precipitation, or wind patterns, among others, that occur over several decades or longer.

**Enhanced greenhouse effect** The concept that the natural greenhouse effect has been enhanced by increased atmospheric concentrations of greenhouse gases (such as $CO_2$ and methane) emitted as a result of human activities. These added greenhouse gases cause the Earth to warm.

**Environment** The complex of physical, chemical, and biotic factors (as climate, soil, and living things) that act upon an organism (a living thing) or an ecological community (a collection of living things) and ultimately determine its form and survival. The circumstances, objects, and conditions that surround each of us.

**Public utility** (a) A public utility is an organization that maintains the infrastructure for a public service and, therefore, is subject to forms of public control and regulation ranging from local community-based groups to statewide government monopolies. (b) It is an organization supplying the community with electricity, gas, water, solid waste disposal service, or sewerage management service.

**Rain-fed agriculture** It is an agriculture that does not use irrigation but instead relies only on direct rainfall.

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# Chapter 5
# Innovative PACT Activated Sludge, CAPTOR Activated Sludge, Activated Bio-Filter, Vertical Loop Reactor, and PhoStrip Processes


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## Acronyms

| | |
|---|---|
| ABF | Activated bio-filters |
| BOD | Biochemical oxygen demand |
| CAST | CAPTOR in activated sludge treatment |
| CBOD | Carbonaceous biochemical oxygen demand |
| COD | Chemical oxygen demand |
| DAF | Dissolved air flotation |
| F/M ratio | Food-to-microorganism ratio |
| HRT | Hydraulic retention time, d |
| MF | Membrane filters |
| MG | Million gallons |
| MGD | Million gallons per day |
| MLSS | Mixed liquor suspended solids |
| $NH_3$-N | Ammonia nitrogen |
| $NO_2$-N | Nitrite nitrogen |
| $NO_3$-N | Nitrate nitrogen |
| NSFC | National Small Flows Clearinghouse |
| PAC | Powdered activated carbon |
| PACE | Effluent PAC concentration, mg/L |
| PACI | Influent PAC concentration, mg/L |
| PACR | Mixed liquor PAC concentration in the reactor, mg/L |

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| | |
|---|---|
| PACT | Powdered Activated Carbon Treatment |
| SRT | Design solids retention time, d |
| TKN | Total Kjeldahl nitrogen |
| TSS | Total suspended solids |
| UMIST | University of Manchester Institute of Science and Technology |
| UNIDO | United Nations Industrial Development Organization |
| USACE | US Army Corps of Engineers |
| USEPA | US Environmental Protection Agency |
| VLR | Vertical Loop Reactor |
| WRC | British Water Research Centre |

## 5.1 Powdered Activated Carbon Treatment (PACT)

### *5.1.1 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. 5.1). 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.

There are six types of combined biological and physicochemical PAC process systems [1–7]:

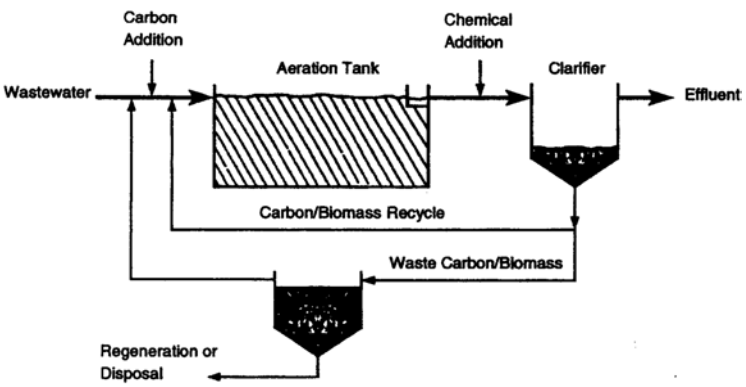


**Fig. 5.1** Powdered activated carbon activated sludge process (PACT) [10, 14]

(a) Continuous combined biological and physicochemical PAC process systems involving the use of sedimentation clarifiers
(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 the PACT process, in which PAC still stands for powdered activated carbon, while ACT stands for activated sludge.

### *5.1.2 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 [8]:

(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 900–1800 kg/day (2400–4000 lb/day), wet air oxidation/regeneration (WAR) is claimed to represent an economical approach to carbon recovery and waste biomass destruction [9]. However, an ash separation step is needed in this case, affecting the economics of carbon regeneration and recovery [10]. 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 [7, 10, 11].

In the United States, PACT systems for nitrification generally have 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 [12]. A summary of selected municipal PACT facilities is presented in Table 5.1.

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 [13]. According to the major supplier of the technology [12, 14], most 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 [12, 14]. 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 US Environmental Protection Agency [14], for practical engineering design considering the loss, the PAC concentration to be added can be calculated from Eq. (5.1):

**Table 5.1** Summary of PACT process systems using wet air oxidation for APC regeneration [10, 14]

| Facility | Current/design flow, $m^3/s$ | PAC/WAR[a] status | Reason for PAC[a] | 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 | 0.31/0.44 | MA | N | 10 | 12 | 1.5–8.0 |
| N. Olmsted,[b] 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 | SD[d] | SD | SD |

[a] C = Color Removal; S = Space; N = Nitrification; T = Toxics; O = Organics

[b] Plan to convert to NAC without regeneration

[c] 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

$$\mathrm{PACI} = \mathrm{PACE} + \left(\mathrm{PACR}\right)\mathrm{HRT}/\mathrm{SRT} \tag{5.1}$$

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, day
SRT is the design solids retention time, day

The value of PACE in Eq. (5.1) 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 [14]. Researchers have provided evidence that the addition of PAC to nitrifying activated sludge systems receiving industrial wastewaters improved nitrification rates [14–16]. 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 [17]. 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 [18].

### *5.1.3 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 3 major PAC producers, over 50 manufacturers of volumetric and gravimetric feeders, and over 50 manufacturers of slurry feeders [19–21]. There are also many manufacturers of sequencing batch reactors (SBR) [2], dissolved air flotation (DAF) clarifiers [7], and membrane filtration (MF) reactors [6].

### *5.1.4 Process Limitations*

The process limitations of PACT process systems are identical to that of the PAC physicochemical process. The 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 post-filtration 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 1 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 the 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. [22–31].

## 5.2 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 [32–34]. The increase in immobilized biomass reduces the process dependence on secondary settling basins for clarification. In such hybrid systems where attached growth coexists with suspended growth, one gets more stable systems which possess the combined advantages of both fixed and suspended growth reactors.

### *5.2.1 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:

(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 [35–39].

(b) Increased persistence in reactor. This leads to an increase in biomass of organisms, reduction of hydraulic retention time, and thus smaller reactor volumes [40–42].
(c) Higher growth rate [43–45].
(d) Increased metabolic activity. This leads to an increase in respiration and substrate utilization, hence higher removal rates [46–49].
(e) Better resistance to toxicity [50–53].

### *5.2.2 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 [54]. 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 US Environmental Protection Agency [55, 56].

### *5.2.3 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 [57–59].

### 5.2.4 Pilot-Plant Study

The conducted pilot-plant work indicated that it was possible to achieve the following [55, 56]:

(a) Biomass concentrations of 7000–10,000 mg/L
(b) Waste sludge concentrations of 4–6% dry solids using a special pad cleaner
(c) Improved oxygen transfer efficiencies
(d) High BOD volumetric removal rates

### 5.2.5 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. 5.2 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. 5.3), 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 $BOD_5$/day/kg MLSS. With the

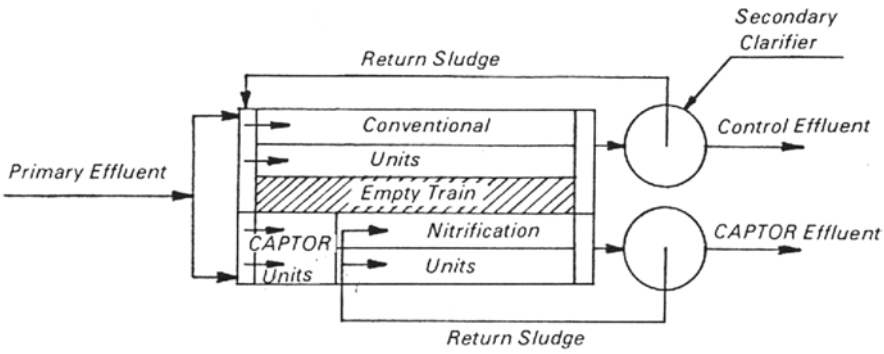


**Fig. 5.2** Schematic of treatment plant showing incorporation of CAPTOR [56]

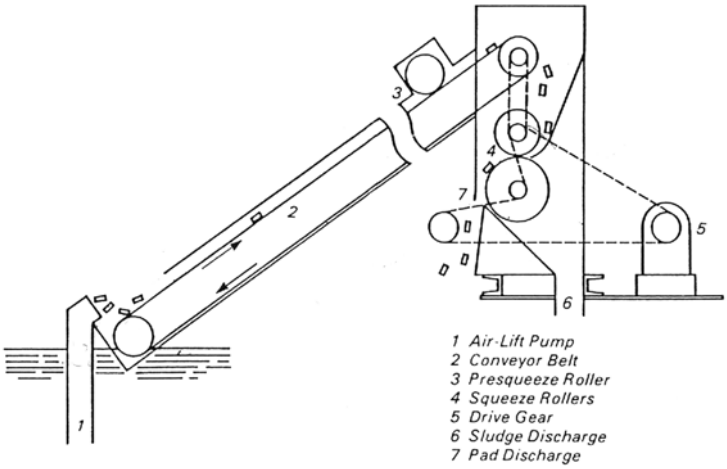


**Fig. 5.3** CAPTOR pad cleaner [56]

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 [56].

#### 5.2.5.1 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 maldistribution 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.

One 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.

#### 5.2.5.2 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 [60–62].

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. 5.3) of % $BOD_5$ removal (total and soluble) vs. volumetric organic loading rate over the range of 1–3.5 kg $BOD_5/day/m^3$ (62–218 $lb/day/1000\ ft^3$). High and low pad cleaning rates are differentiated in Fig. 5.4 as ≥16% and <16% of the total pad inventory/d, respectively [56].

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. 5.4, although low bulk liquid DOs may have adversely affected removals on some of the low cleaning runs. Low cleaning rates (<16%/day) were detrimental to soluble $BOD_5$ removal efficiency because of a gradual decline in activity of the biomass remaining in the pad.

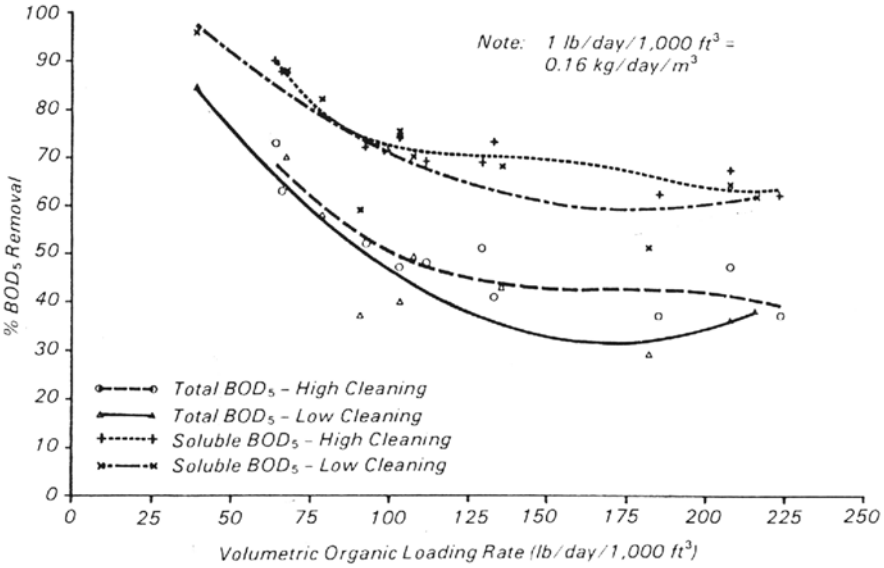


**Fig. 5.4** Pilot-scale CAPTOR $BOD_5$ removals as a function of organic loading rate [56]

Cleaning rates greater than 24%/day, however, resulted in reduced biomass levels in the pads and a reduction in performance.

The problem of maldistribution 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. 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 5.2 for two different volumetric loading rates [56].

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

**Table 5.2** Pilot-scale operating conditions and process performance [56]

| Parameter | Period | | | |
|---|---|---|---|---|
| | 1 | | 2 | |
| Volumetric loading (lb $BOD_5$/day/1000 $ft^3$)[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 $BOD_5$/day/kgMLSS) | 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/day/1000 $ft^3$ = 0016 kg/day/$m^3$

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 5.3 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 [63]. With a pad concentration of 40/L, equivalent MLSS levels varied from 3960 to 4960 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.

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/day/$m^3$ (15.6 lb/day/1000 $ft^3$) and 0.20 kg/day/$m^3$ (12.5 lb/day/1000 $ft^3$), respectively.

#### 5.2.5.3 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 [56]:

**Table 5.3** Pilot-scale CAST and activated sludge operating conditions and performance [56]

| Parameter | System | | | |
|---|---|---|---|---|
| | CAST | | Activated Sludge | |
| Volumetric loading (lb $BOD_5$/day/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) | 3930 | | – | |
| MLSS in suspension (mg/L) | 3720 | | 6030 | |
| Total MLSS (mg/L) | 7650 | | 6030 | |
| F/M loading (kg $BOD_5$/day/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/day/1000 $ft^3$ = 0.016 kg/day/$m^3$

(a) Splitting each of the CAPTOR trains, C1 and C2, into two compartments, C1A and C1B and C2A and C2B, as shown in Fig. 5.5
(b) Feeding influent flow along long weirs at the side of the trains instead of at the narrow inlet ends
(c) Modifying the aeration pipework 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 countercurrent 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
(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

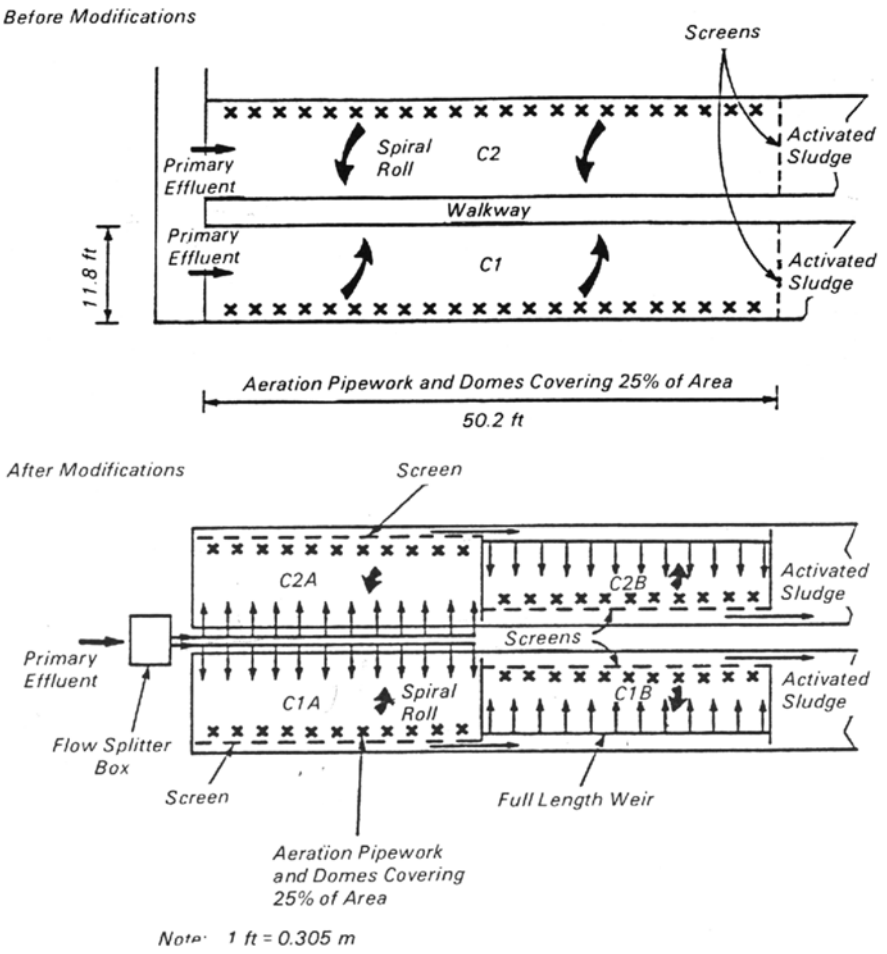


**Fig. 5.5** Modifications to full-scale CAPTOR system flow pattern [56]

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 average volumetric loading rate of 1.24 kg $BOD_5$/day/$m^3$ (77 lb/day/1000 $ft^3$), an average HRT (excluding sludge recycle) of 2.55 h, and an overall biomass concentration of 4830 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 $BOD_5$/day/$m^3$ (69 lb/day/1000 $ft^3$) and 3.40 h, respectively.

Performance data summarized in Tables 5.4 and 5.5 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%.

#### 5.2.5.4 Overall Conclusions

The US Environmental Protection Agency (USEPA) conclusions and recommendations for the CAPTOR/CAST treatment systems are as follows [55, 56, 64]:

(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 6000–8000 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

**Table 5.4** Full-scale modified CAPTOR performance results [56]

| 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 5.5** Full-scale modified CAST performance results [56]

| 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 |

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.

## 5.3 Activated Bio-filter (ABF)

### *5.3.1 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 (bio-cell) 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. No intermediate clarifier is utilized. Forward flow passes directly from the tower discharge to the aeration tank (Fig. 5.6). 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 [65]. 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 [66].

The bio-media in the bio-cell 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 sloughing of biota. Droplet formation and

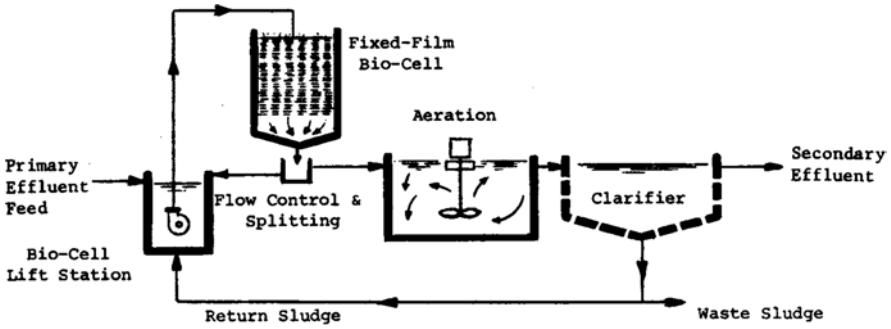


**Fig. 5.6** ABF process flow diagram [65]

breakup induced by wastewater dripping from lath to lath enhances oxygen transfer. Other types of material for the bio-media have also been reported by other researchers and equipment manufacturers [67–70]. 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 bio-cell 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 bio-cell acts as a first-stage roughing unit and the aeration basin as a second-stage nitrification unit [71, 72]. ABF bio-cells 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.

### *5.3.2 Applications*

Activated bio-filters can be used for treating municipal wastewater and biodegradable industrial wastewater. ABF systems are especially useful where [65, 66]:

(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 [73]. The plant was upgraded in the 1990s using ABF at a cost of $48 M. The facility currently serves a population of 180,000 with a flow of 43 ML

a day and has the capacity to treat 53 ML/day for a population of 220,000 in the year 2020. The bio-filter is 30 m in diameter and has a design organic loading of 3.2 kg $BOD_5/m^3$/day. 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 USEPA set limits.

### 5.3.3 Design Criteria

The design criteria for the ABF system are reported to be as follows [65, 74, 75]:

(a) Bio-cell organic load: 100–200 lb $BOD_5$/day/1000 $ft^3$
(b) Return sludge rate: 25–100%
(c) Bio-cell recycle rate: 0–100%
(d) Bio-cell hydraulic load: 1–5.5 gpm/$ft^2$
(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 $BOD_5$/day/lb MLVSS for BOD removal
0.18 lb $BOD_5$/day/lb MLVSS for two-stage nitrification.

### 5.3.4 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 [65]. The detailed results are shown in Table 5.6.

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.

**Table 5.6** Performance of BAF systems [65]

| 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

## 5.4 Vertical Loop Reactor (VLR)

### *5.4.1 Description*

A Vertical Loop Reactor (VLR) is an activated sludge biological treatment process similar to an oxidation ditch [76, 77]. 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. 5.7 [78]. 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 sidewall depths which range from approximately 10–22 ft [79]. 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 below the surface of the water. Because a VLR is typically deeper than an oxidation ditch, the VLR requires less land area.

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 [80]. 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 intra-channel 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.

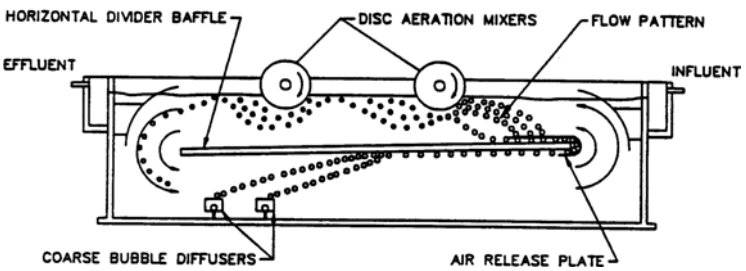


**Fig. 5.7** Diagram of the Vertical Loop Reactor [77, 78]

### 5.4.2 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, United States, wastewater treatment plant [81]. The facility is designed for an average daily flow of 4.5 MGD and is capable of handling a peak flow of 7.2 MGD.

The following advantages have been reported for VLR systems [82]:

(a) The 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.

### 5.4.3 Design Criteria

The design criteria for the VLR process are reported to be as follows [76]:

BOD loading: 14–22 lb $BOD_5$/1000 $ft^3$/day
SRT: 17–36 day
Detention Time: 12–24 h

### 5.4.4 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 5.7 indicates the percent of time the monthly average effluent concentration of the given pollutants was less than the

**Table 5.7** Reliability of the VLR treatment process [76]

| 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

concentration given in the first column. No significant difference in results was observed between winter and summer data.

### *5.4.5 USEPA Evaluation of VLR*

The following summarizes the major findings and conclusions of USEPA evaluation of VLRs [77]. 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 bypass design for treatment of high peak to average flows.
(b) There are currently over ten operating VLRs in the United States ranging in size from 0.22 to 5.0 MGD.
(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 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.
(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 lbs CBOD/1000 $ft^3$. 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 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 range. The reported VLR costs at plants ranging from 2 to 10 MGD 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.

### *5.4.6 Energy Requirements*

The VLR energy requirements are shown in Fig. 5.8. The requirements are based on the following assumptions [76]:

(a) Water quality
    $BOD_5$: influent = 200 mg/L, effluent = 20 mg/L
    TKN: influent = 35 mg/L, effluent = 1 mg/L
(b) Design basis

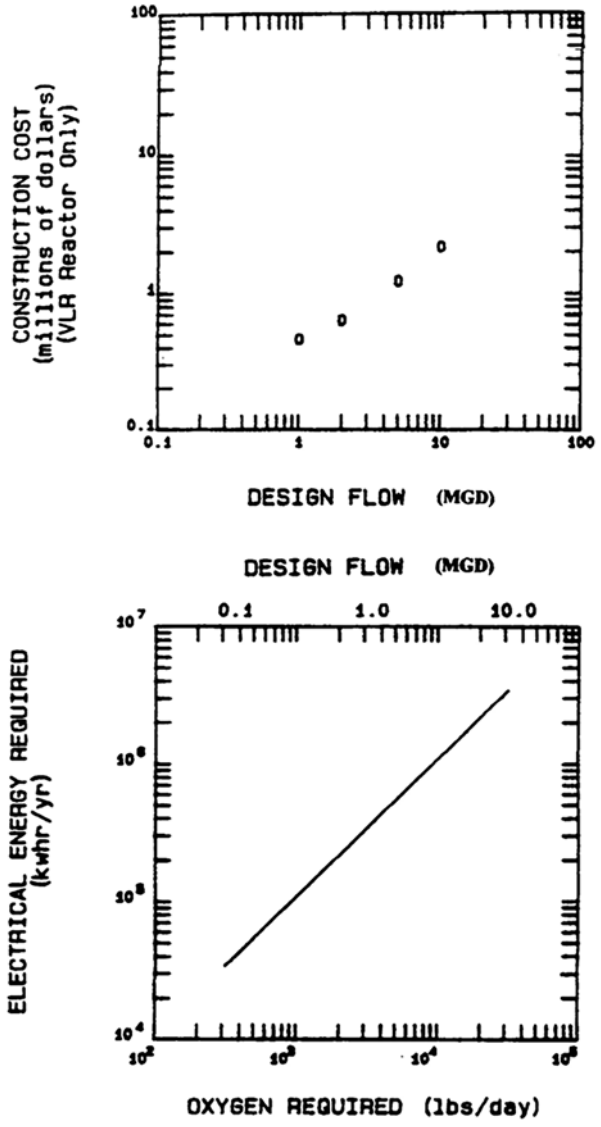


**Fig. 5.8** VLR energy requirements and construction cost [76, 77]

Oxygen transfer efficiency: 2.5 lb $O_2$/Hp hour
Nitrification occurs

(c) Operating parameters
Oxygen requirement: 1.5 lb $O_2$/lb $BOD_5$ removed
4.57 lb $O_2$/lb TKN removed

(d) Type of energy: electrical

### 5.4.7 Costs

The construction costs (1991 Dollars, Utilities Index = 392.35) for VLR are shown in Fig. 5.8. To obtain the values in terms of the present 2004 US Dollars, using the Cost Index for Utilities (Appendix 1), multiply the costs by a factor of 506.13/392.35 = 1.29 [83]. The operation costs are similar to oxidation ditch type treatment plant.

## 5.5 PhoStrip Process

### 5.5.1 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. 5.9, 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 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 [65]. The readers are referred to the

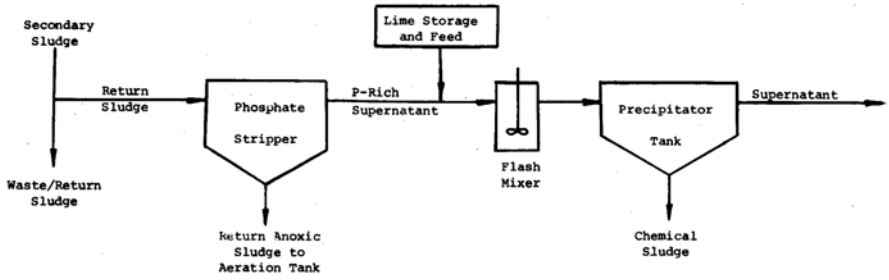


**Fig. 5.9** PhoStrip process flow diagram [65]

literature [84–97] for additional innovative wastewater and sludge treatment processes, such as biological sequencing batch reactor, physicochemical sequencing batch reactor, membrane bioreactor, flotation bioreactor, membrane flotation bioreactor, Symbio process, column bioreactor clarifier process, upflow sludge blanket filtration, deep well injection, land application, aerobic granulation technology, vertical shaft bioreactor, vertical shaft digestion, bioreactor landfill, post aeration, etc.

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, United States, and, more recently, Reno/Sparks, Nevada, United States. Nearly a dozen commercial installations are reported to be in the operational phase.

### *5.5.2 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 [65].

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.

**Table 5.8** Typical design criteria for the PhoStrip process [74]

| Design parameter | Unit | Value |
|---|---|---|
| Food-to-microorganism ratio (F/M) | lb BOD/lb MLSS/day | 0.1–0.5 |
| Solids retention time (SRT) | day | 10–30 |
| Mixed liquor suspended solids (MLSS) | mg/L | 600–5000 |
| 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 |

### *5.5.3 Design Criteria*

The fraction of the total sludge flow which 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. The 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 [65]. Typical design criteria for the PhoStrip process are shown in Table 5.8 [74].

### *5.5.4 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 [74] or even to 0.5 mg/L or less [75]. A plant-scale evaluation of the method treating 6 MGD 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 the entire system.

### *5.5.5 Cost*

#### 5.5.5.1 Construction Cost

The construction costs (1980 Dollars, Utilities Index = 277.60) for PhoStrip are shown in Fig. 5.10. To obtain the values in terms of the present 2004 US Dollars, using the Cost Index for Utilities (Appendix 1), multiply the costs by a factor of

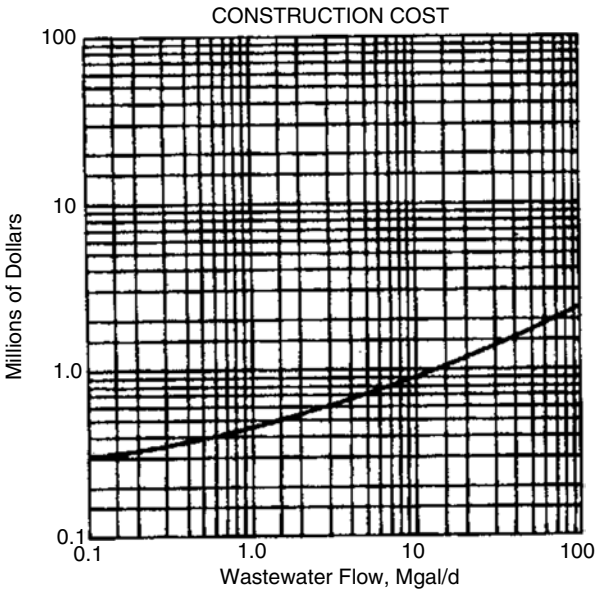


**Fig. 5.10** PhoStrip construction cost [65]

506.13/277.60 = 1.82 [83]. Construction costs include stripper (10 h detention time at 50% of return sludge), flash mixer, flocculator/clarifier, thickeners, and lime feed and storage facilities [65].

#### 5.5.5.2 Operation and Maintenance Cost

The electrical energy required for operation of pumps, lime mixing equipment, and clarifiers is shown in Fig. 5.11. The operation and maintenance costs (1980 Dollars, Utilities Index = 277.60) for PhoStrip are shown in Fig. 5.12. To obtain the values in terms of the present 2004 US Dollars, using the Cost Index for Utilities (Appendix 1), multiply the costs by a factor of 506.13/277.60 = 1.82 [83]. Operation and maintenance costs include labor for operation, preventive maintenance, and minor repairs; materials to include replacement parts and major repair work; and lime and power cost based on the electrical energy requirement shown in Fig. 5.11 [65].

## Glossary

**Activated bio-filter (ABF)** Activated bio-filters are a recent innovation in the biological treatment field. This process consists of the series combination of an aerobic tower (bio-cell) with wood or other packing material, followed by an

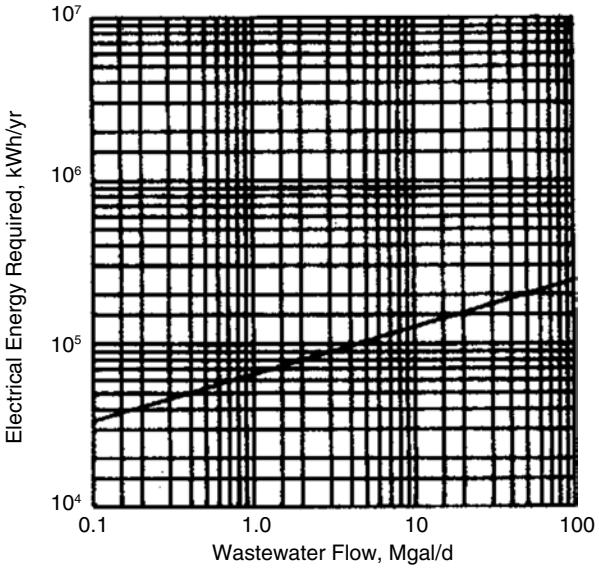


**Fig. 5.11** PhoStrip electrical energy requirement [65]

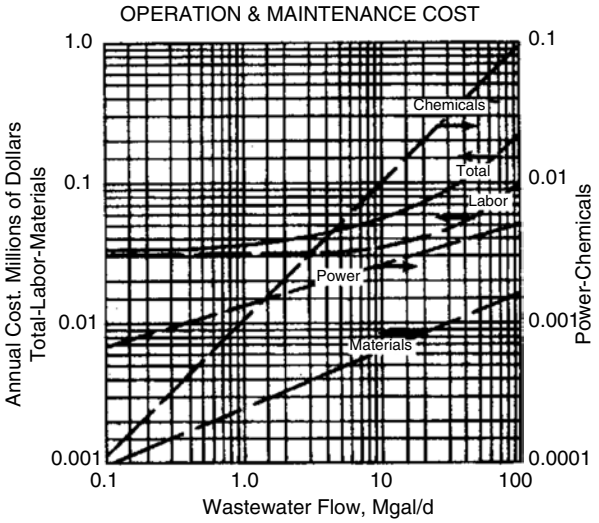


**Fig. 5.12** PhoStrip operation and maintenance cost [65]

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. No intermediate clarifier is

utilized. Forward flow passes directly from the tower discharge to the aeration tank. 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. 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.

**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. The increase in immobilized biomass reduces the process dependence on secondary settling basins for clarification. In such hybrid systems where attached growth coexists with suspended growth, one gets more stable systems which possess the combined advantages of both fixed and suspended growth reactors.

**PACT activated sludge process** 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. 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.

**PhoStrip process** "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. 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 phosphorus-rich superna-

tant, 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.

**Vertical Loop Reactor (VLR)** A Vertical Loop Reactor (VLR) is an activated sludge biological treatment process similar to an oxidation ditch. 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. 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 sidewall depths which range from approximately 10–22 ft. 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 below the surface of the water. Because a VLR is typically deeper than an oxidation ditch, the VLR requires less land area.

## Appendix 1: US Yearly Average Cost Index for Utilities [83]

| Year | Index | Year | Index |
|---|---|---|---|
| 1967 | 100 | 1995 | 439.72 |
| 1968 | 104.83 | 1996 | 445.58 |
| 1969 | 112.17 | 1997 | 454.99 |
| 1970 | 119.75 | 1998 | 459.40 |
| 1971 | 131.73 | 1999 | 460.16 |
| 1972 | 141.94 | 2000 | 468.05 |
| 1973 | 149.36 | 2001 | 472.18 |
| 1974 | 170.45 | 2002 | 486.16 |
| 1975 | 190.49 | 2003 | 497.40 |
| 1976 | 202.61 | 2004 | 563.78 |
| 1977 | 215.84 | 2005 | 605.47 |
| 1978 | 235.78 | 2006 | 645.52 |
| 1979 | 257.20 | 2007 | 681.88 |
| 1980 | 277.60 | 2008 | 741.36 |
| 1981 | 302.25 | 2009 | 699.70 |
| 1982 | 320.13 | 2010 | 720.80 |
| 1983 | 330.82 | 2011 | 758.79 |
| 1984 | 341.06 | 2012 | 769.30 |
| 1985 | 346.12 | 2013 | 776.44 |
| 1986 | 347.33 | 2014 | 791.59 |
| 1987 | 353.35 | 2015 | 786.32 |
| 1988 | 369.45 | 2016 | 782.46 |
| 1989 | 383.14 | 2017 | 803.93 |

(continued)

| Year | Index | Year | Index |
|---|---|---|---|
| 1990 | 386.75 | 2018 | 841.84 |
| 1991 | 392.35 | 2019 | 866.18 |
| 1992 | 399.07 | 2020 | 867.71 |
| 1993 | 410.63 | 2021 | 893.02[a] |
| 1994 | 424.91 | 2022 | 918.91[a] |

[a]Projected future cost index values

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# Chapter 6
# Agricultural Waste Treatment by Water Hyacinth Aquaculture, Wetland Aquaculture, Evapotranspiration, Rapid Rate Land Treatment, Slow Rate Land Treatment, and Subsurface Infiltration


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## Acronyms

| | |
|---|---|
| $Al_2(SO_4)_3$ | Aluminum sulfate |
| $BOD_5$ | 5-day biochemical oxygen demand |
| $CaCO_3$ | Calcium carbonate |
| COD | Chemical oxygen demand |
| ET | Evapotranspiration |
| $FeC1_3$ | Ferric chloride |
| FWS | Free water surface |
| MG | Million gallons |
| MPN | Most probable number |
| NEHA | National Environmental Health Association |
| SFS | Subsurface flow system |
| TDH | Total dynamic head |
| TSS | Total suspended solids |
| UNIDO | United Nations Industrial Development Organization |
| USACE | US Army Corps of Engineers |
| USEPA | US Environmental Protection Agency |
| VSB | Vegetated submerged bed |

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## 6.1 Aquaculture Treatment: Water Hyacinth System

### *6.1.1 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. 6.1), where the plants remove nutrients, $BOD_5$ (5-day biochemical oxygen demand), TSS (total suspended solids), heavy 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].

### *6.1.2 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 during

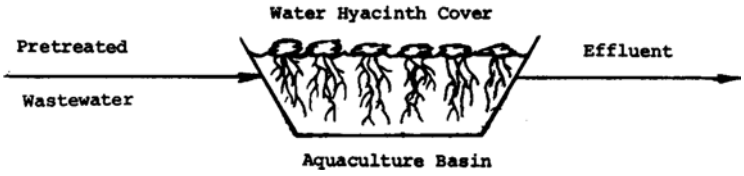


**Fig. 6.1** Aquaculture treatment: water hyacinth system. Source: U. S. EPA [2]

active growth periods and some phosphorus and retard algal 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) Stormwater treatment
(e) Demineralization of water
(f) Recycling fish culture water
(g) For biomonitoring purposes

### *6.1.3 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 seven times greater than evaporation alone.

### *6.1.4 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 6.1 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, 2–15 acres/MG/day.

### *6.1.5 Performance*

The 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 coworkers [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 1000–10,000 lb/day/acre. Basin cleaning at least once per year results in harvested hyacinths. For

**Table 6.1** Design criteria for water hyacinth systems. Source: U. S. EPA [4]

| Factor | Aerobic non-aerated | Aerobic non-aerated | Aerobic aerated |
|---|---|---|---|
| Influent wastewater | Screened or Settled | Secondary | Screened or settled |
| Influent $BOD_5$, mg/L | 130–180 | 30 | 130–180 |
| BOD5 loading, kg/ha day | 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 day | >200 | <800 | 550–1000 |
| Harvest schedule | Annually | Twice per month | Monthly |

further detailed information on water hyacinth systems, the reader is referred to Refs. [5–13].

## 6.2 Aquaculture Treatment: Wetland System

### *6.2.1 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 (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 (chemical oxygen demand), TSS, 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. 6.2). 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

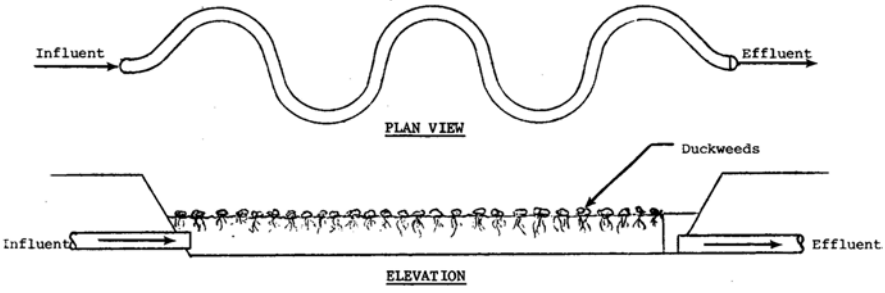


**Fig. 6.2** Aquaculture treatment: wetland system. Source: U. S. EPA [2]

(b) Dried or otherwise processed for use as animal feed supplements
(c) Digested to produce methane

### *6.2.2 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 plant 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 occur 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 mixing represent some of the enhancements that naturally follow [17].

### *6.2.3 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 waste discharge technology to lengthy outfalls extended into rivers, etc. and as a method of pretreatment of surface waters for domestic supply, stormwater treatment, recycling fish culture water, and biomonitoring purposes.

### *6.2.4 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.

### 6.2.5 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. The 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/day.
(c) Depth may vary with type of system, generally 1–5 ft.

### 6.2.6 Performance

The process appears reliable from mechanical and performance standpoints, subject to seasonality of vegetation growth. Low operator attention is required if properly designed.

Tables 6.2 and 6.3 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]:

(a) $BOD_5$, 80–95%
(b) TSS, 29–87%
(c) COD, 43–87%
(d) Nitrogen, 42–94% depending upon vegetative uptake and frequency of harvesting
(e) Total P, 0–94% (high levels possible with warm climates and harvesting)
(f) Coliforms, 86–99%
(g) 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.

**Table 6.2** Nutrient removal from natural wetlands. Source: U. S. EPA [4]

| Project | Flow, $m^3$/day | Wetland type | Percent reduction | | | |
|---|---|---|---|---|---|---|
| | | | 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 | 2309 | 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 |

[a] Total dissolved phosphorus
[b] Total nitrogen
[c] Nitrate and nitrite
[d] May–November only

**Table 6.3** Nutrient removal from constructed wetlands. Source: U. S. EPA [4]

| Project | Flow, $m^3$/day | Wetland Type | $BOD_5$, mg/L | | SS, mg/L | | Percent reduction | | Hydraulic surface loading rate, $m^3$/ha day |
|---|---|---|---|---|---|---|---|---|---|
| | | | Influent | Effluent | Influent | Effluent | $BOD_5$ | SS | |
| Listowel, Ontario [12] | 17 | FWS[a] | 56 | 10 | 111 | 8 | 82 | 93 | – |
| Santee, CA [10] | – | SFS[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 | 1543 |
| Gustine, CA | 3785 | FWS | 150 | 24 | 140 | 19 | 84 | 86 | 412 |

[a] Free water surface system
[b] Subsurface flow system

Residuals are dependent upon the type of system and whether or not harvesting is employed. Duckweed, for example, yields 50–60 lb/acre/day (dry weight) 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 Refs. [19–23].

## 6.3 Evapotranspiration System

### *6.3.1 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. 6.3) consists of a 1½ to 3 ft 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 flatland or a series of trenches on slopes. The surface area of the bed must be 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-fourths of all the ET beds in operation were designed to use both disposal methods. Mechanical evaporators have been developed, but are not used at full scale.

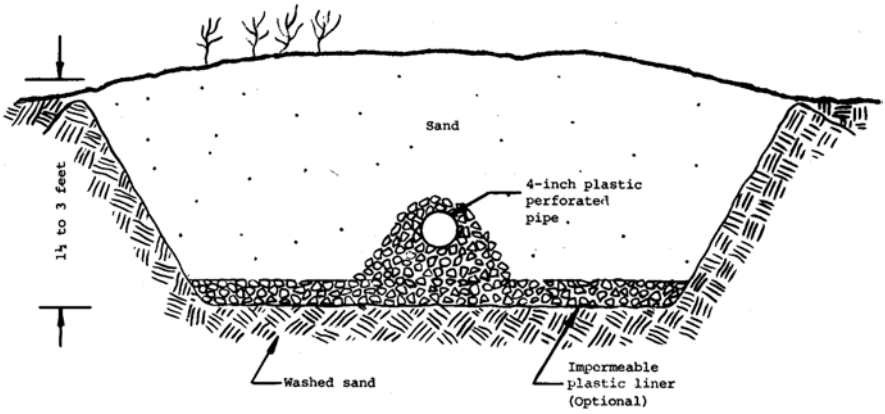


**Fig. 6.3** Section through an evapotranspiration bed. Source: U. S. EPA [2]

### *6.3.2 Applications*

There are estimated to be 4000–5000 year-round evapotranspiration beds 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.

### *6.3.3 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 and high latitudes greatly limits its use. Snow cover reflects solar radiation, which reduces EF. 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 watertight to prevent the possibility of groundwater contamination. Therefore, proper

construction methods should be employed to keep the liner from being punctured during installation.

### 6.3.4 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 non-growing season. Uniform sand in the size range of $D_{50}$ of approximately 0.10 mm is capable of raising water about 3 ft to the top of the bed. The polyethylene liner thickness is typically greater than or equal to 10 mil. Special attention should be paid to stormwater drainage to make sure that surface runoff is drained away from the bed proximity by proper lot grading.

### 6.3.5 Performance

Performance is a function of climate conditions, volume of wastewater, and physical design of the system. Evapotranspiration (ET) 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, does conserve the open space. Neither energy is required, nor is head loss of any value incurred.

### 6.3.6 Costs

The following site-specific costs serve to illustrate the major components of an evapotranspiration bed in Boulder, Colorado, United States, with an annual net ET rate in the range of 0.04 gpd/ft$^2$ [2]. A 200 gpd household discharge would require a 2 ft deep bed with an area of approximately 5000 ft$^2$. All costs have been adjusted to 2020 US Dollars using the Cost Index for Utilities shown in Appendix 1 [24].

| **Construction cost (2020)** | |
|---|---|
| Building sewer with 1000 gal septic tank, design, and permit | $2616 |
| Excavation and hauling (375 $yd^3$) | $3767 |
| Liner (5200 $ft^2$) | $2442 |
| Distribution piping (625 ft) | $1099 |
| Sand (340 $yd^3$) and gravel (38 $yd^3$) | $6557 |
| Supervision and labor | **$1831** |
| Total | $18,312 |
| **Annual operation and maintenance cost (2020)** | |
| Pumping septage from septic tank (every 3–5 years) | **$17–73** |
| Total | $17–73 |

The 2020 construction cost for this particular system would be approximately $3.66/$ft^2$, which is consistent with a reported national range of $2.72–5.86/$ft^2$. The cost of an evapotranspiration bed is highly dependent upon 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 $600–6000 to the 2020 construction cost and an amount of $218–750/year to the annual operation and maintenance cost.

## 6.4 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 groundwater regulations.

### 6.4.1 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 to deep and permeable deposits such as sand or sandy loam usually by distributing in basins (Fig. 6.4) 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 topsoil 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:

(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]:

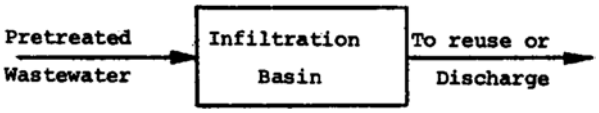


**Fig. 6.4** Flow diagram of land treatment using rapid rate system. Source: U. S. EPA [2]

(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
(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.

### *6.4.2 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 on-site.

### *6.4.3 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
(e) The slope of the land

Nitrate and nitrite removals are low unless special management practices are used.

### 6.4.4 Design Criteria

The design criteria for the rapid rate system can be summarized as follows [2]:

(a) Field area, 3–56 acres/MG/day
(b) Application rate, 20–400 ft/year, 4–92 in./week
(c) $BOD_5$ loading rate, 20–100 lb/acre/day
(d) Soil depth, 10–15 ft or more
(e) Soil permeability, 0.6 in./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, at least 2 basins/site
(i) Height of dikes, 4 ft; underdrains, 6 or more ft 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 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 6.4.

### 6.4.5 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

**Table 6.4** Loading cycles for high rate infiltration systems. Source: U. S. EPA [25]

| Loading cycle objective | Applied wastewater | Season | Application period, day[a] | Drying period, day |
|---|---|---|---|---|
| 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 |

[a] Regardless of season or cycle objective, application periods for primary effluent should be limited to 1–2 days to prevent excessive soil clogging

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].

### *6.4.6 Costs*

The construction and operation and maintenance costs are shown in Figs. 6.5 and 6.6, respectively [2]. The costs are based on 1973 (Utilities Index = 149.36, USEPA Index 194.2, ENR Index = 1850) figures. To obtain the values in terms of the present 2020 US Dollars, using the Cost Index for Utilities (Appendix 1), multiply the costs by a factor of 5.81 [24].

Assumptions applied in preparing the costs given in Figs. 6.5 and 6.6:

(a) Application rate, 182 ft/year.
(b) Construction costs include field preparations (removal of brush and trees) for multiple unit infiltration basins with 4 ft dike formed from native excavated material, and storage is not assumed necessary.
(c) Drain pipes buried 6–8 ft with 400 ft spacing, interception ditch along length of field, and weir for control of discharge; gravel service roads and 4 ft 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 pipes every 5 years, annual cleaning of interceptor ditch, and major repair of ditches, fences, and roads after 10 years.

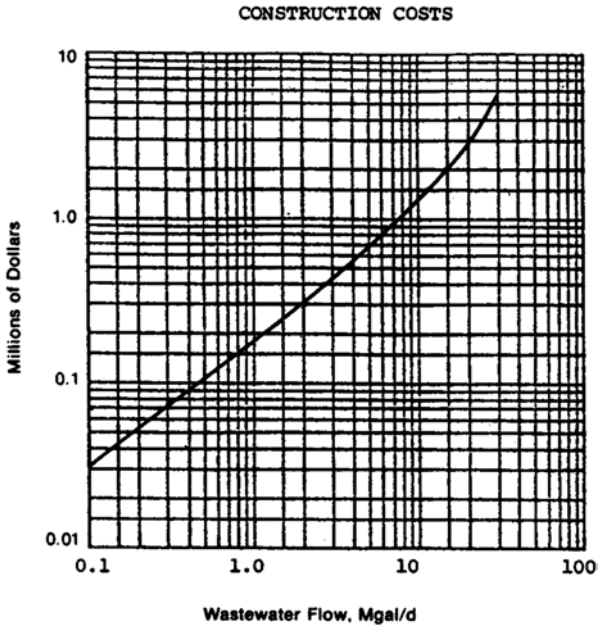


**Fig. 6.5** Construction costs for rapid rate system. Source: U. S. EPA [2]

(e) Costs of pretreatment monitoring wells, land, and transmission to and from pretreatment facility not included.

## 6.5 Land Treatment: Slow Rate System

### *6.5.1 Description*

Slow rate land treatment system represents the predominant municipal land treatment practice in the United States. In this process, wastewater is applied by sprinkling 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 plant uptake (Fig. 6.7). An underdrainage system consisting of a network of drainage pipe buried below the surface serves 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.

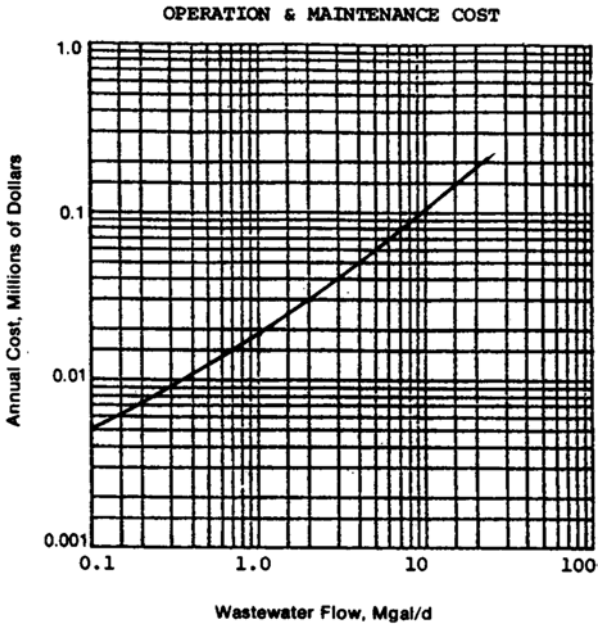


**Fig. 6.6** Operation and maintenance costs of rapid rate system. Source: U. S. EPA [2]

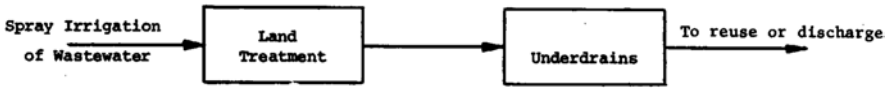


**Fig. 6.7** Flow diagram of land treatment using slow rate system. Source: U. S. EPA [2]

Sprinklers 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
(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
(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 1000 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 6.5 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.

### *6.5.2 Applications*

Slow rate systems produce the best results of all the land treatment systems. Advantages of sprinkler application over gravity methods include [34]:

**Table 6.5** Potential adverse effects of wastewater constituents on crops. Source: U. S. EPA [25]

| 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 (see Table 9.4) |
| 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 application |
| Chloride, mg/L | <106 | >106 | – | |
| Miscellaneous | | | | |
| NH4-N + $NO_3$-N, mg/L | <5 | 5–30 | 30 | Sugarbeets, potatoes, cotton, grains |
| HCO3, mg/L | <90 | 90–520 | >520 | Fruit |
| pH, units | 6.5–8.4 | 4.2–5.5 | <4.2 and > 8.5 | Most crops |

[a] Adjusted sodium adsorption ratio

(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
(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
(b) Water and nutrient conservation when utilized for irrigating landscaped areas

### *6.5.3 Limitations*

The slow rate process is limited by [2]:

(a) Soil type and depth
(b) Topography
(c) Underlying geology
(d) Climate
(e) Surface and groundwater hydrology and quality
(f) Crop selection
(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.

### 6.5.4 Design Criteria

The design criteria for the slow rate system can be summarized as follows [2]:

(a) Field area, 56–560 acres/MG/day
(b) Application rate, 2–20 ft/year, 0.5–4 in./week
(c) $BOD_5$ loading rate, 0.2–5 lb/acre/day
(d) Soil depth, 2–5 ft or more
(e) Soil permeability, 0.06–2.0 in./h
(f) Minimum preapplication treatment, primary
(g) Lower temperature limit, 25 °F
(h) Particle size of solids, less than one-third of the sprinkler nozzle diameter
(i) Underdrains, 4–8 in. diameter, 4–10 ft deep, 50–500 ft apart; pipe material, plastic, concrete (sulfate resistant, if necessary), or clay

### 6.5.5 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 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 groundwater and establishing nitrogen limits in either the percolate or groundwater as it leaves the property site. Nitrogen loading to the groundwater 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].

### 6.5.6 Costs

The construction and operation and maintenance costs are shown in Figs. 6.8 and 6.9, respectively [2]. The costs are based on 1973 (Utilities Index = 149.36, USEPA Index 194.2, ENR Index = 1850) figures. To obtain the values in terms of the present

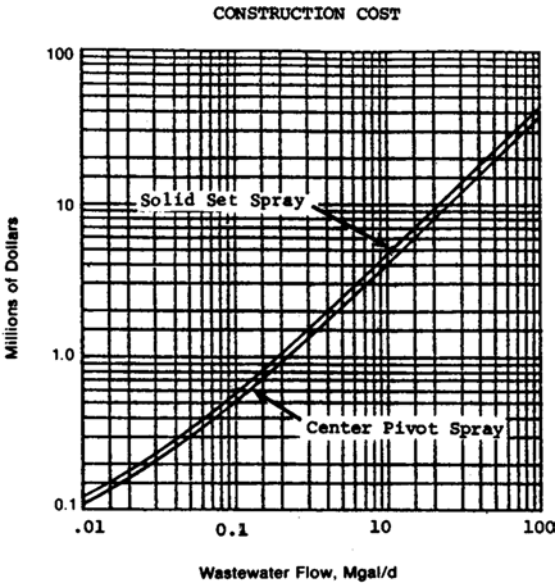


**Fig. 6.8** Construction cost of slow rate system. Source: U. S. EPA [2]

2020 US Dollars, using the Cost Index for Utilities (Appendix 1), multiply the costs by a factor of 5.81 [24].

Assumptions applied in preparing the costs given in Figs. 6.8 and 6.9:

(a) Yearly average application rate: 0.33 in./day.
(b) Energy requirements: Solid set spray distribution requires 2100 kwh/year/ft of TDH/MG/day capacity. Center pivot spraying requires an additional $0.84 \times 10^6$ kwh/year/acre (based on 3.5 days/week operation) for 1 MG/day or larger facilities (below 1 MG/day, additional power = $0.84–1.35 \times 10^6$ 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 between drain pipes. Drain pipes are buried 6–8 ft 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.

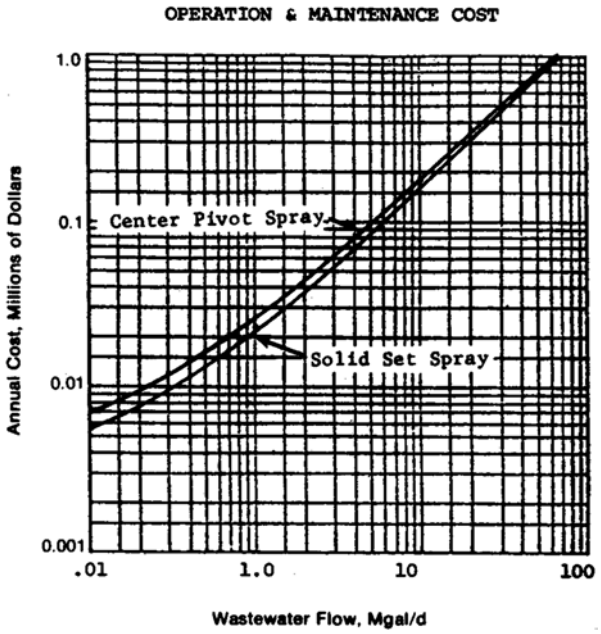


**Fig. 6.9** Operation and maintenance cost of slow rate system. Source: U. S. EPA [2]

(h) Labor costs include inspection and unclogging of drain pipes at outlets and dike maintenance.
(i) Material 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 dikes (12 ft wide at crest) are formed from native materials (inside slope 3:1, outside 2:1); rectangular shape on level ground; 12 ft water depth; multiple cells for more than 50 acre size; asphaltic lining; 9 in. riprap on inside slope of dikes.
(k) Cost of pretreatment, monitoring wells, land, and transmission to and from land treatment facility not included.

## 6.6 Land Treatment: Overland Flow System

### *6.6.1 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. A flow diagram of the system is shown in Fig. 6.10. Wastewater is applied over the upper reaches of sloped terraces and is treated as it flows across the vegetated surface to runoff collection ditches. 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, redtop, 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 are 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

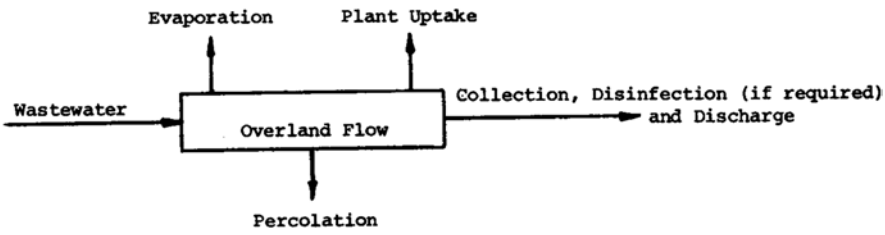


**Fig. 6.10** Flow diagram of land treatment using overland flow system. Source: U. S. EPA [2]

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 and 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 prior to overland flow permits reduced (as much as two-thirds reduction) land requirements. Effluent disinfection is required where stringent fecal coliform criteria exist.

### *6.6.2 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.

### *6.6.3 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. Flatland 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.

### *6.6.4 Design Criteria*

The design criteria for the overland flow system can be summarized as follows [2]:

(a) Field area required, 35–100 acres/MG/day
(b) Terraced slopes, 2–8%
(c) Application rate, 11–32 ft/year, 2.5–16 in./week
(d) $BOD_5$ loading rate, 5–50 lb/acre/day
(e) Soil depth, sufficient to form slopes that are uniform and to maintain a vegetative cover
(f) Soil permeability, 0.2 in./h or less
(g) Hydraulic loading cycle, 6–8 h application period, 16–181 weeks' resting period
(h) Operating period, 5–6 days/week
(i) Soil texture clay and clay loams

Below are representative application rates for 2–8% sloped terraces:

| in./week | Pretreatment | Terrace length, ft |
|---|---|---|
| 2.5–8 | Untreated or primary | 150 |
| 6–16 | Lagoon or secondary | 120 |

Generally, 40–80% of applied wastewater reaches collection structures, lower percent in summer and higher in winter (southwest data). Table 6.6 shows the required pretreatment and allowed application and hydraulic rates [48].

### 6.6.5 *Performance*

Percent removals for comminuted or screened municipal wastewater over about 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 ($CaCO_3$) prior to application will increase phosphorus removals.

**Table 6.6** Design loadings for overland flow systems. Source: U. S. EPA [48]

| Preapplication treatment | Application rate $m^3/h$ m | Hydraulic loading rate cm/day |
|---|---|---|
| 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 |

[a] $m^3/h$ m × 80.5 = gal/h ft
[b] cm/day × 0.394 = in./day
[c] Does not include removal of algae
[d] Recommended only for upgrading existing secondary treatment

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 Refs. [12, 50–52].

### *6.6.6 Costs*

The construction and operation and maintenance costs are shown in Figs. 6.11 and 6.12, respectively [2]. The costs are based on 1973 (Utilities Index = 149.36, EPA Index 194.2, ENR Index = 1850) figures. To obtain the values in terms of the present 2020 US Dollars, using the Cost Index for Utilities (Appendix 1), multiply the costs by a factor of 5.81 [24].

Assumptions applied in preparing the costs given in Figs. 6.11 and 6.12:

(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 (1400 yd/acre of cut). Costs include surveying, earthmoving, finish grading, ripping two ways, disking, land planning, and equipment mobilization.

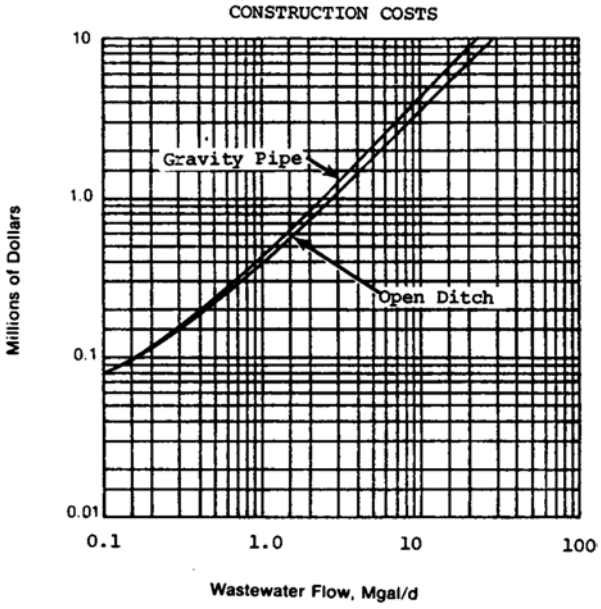


**Fig. 6.11** Construction cost of overland flow treatment system. Source: U. S. EPA [2]

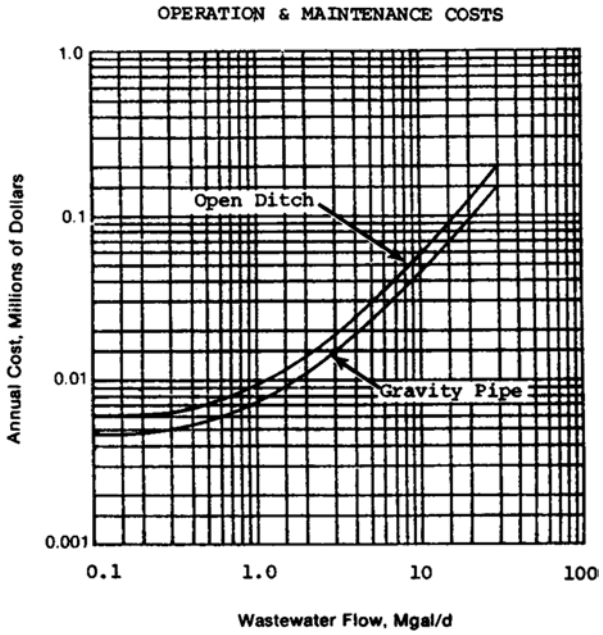


**Fig. 6.12** Operation and maintenance cost of overland flow treatment system. Source: U. S. EPA [2]

(c) Distribution system: application rate, 0.064 in./h; yearly average rate of 3 in./week (8 h/day; 6 days/week); 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 pipe 4 in. diameter and smaller is PVC; all larger pipe is 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 1000 ft intervals.
(e) Gravity pipe collection: network of gravity pipe interceptors with inlet/manholes every 250 ft along submains; storm runoff is allowed to pond at inlets; each inlet/manhole serves 1000 ft of collection ditch; manholes every 500 ft 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 and also includes dike maintenance and scraping and patching of storage basin liner every 10 years.
(g) Costs for pretreatment, land, transmission to site, disinfection, and service roads and fencing not included.

## 6.7 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 [53].

Subsurface infiltration wastewater management practices are embodied in the horizontal leach fields that routinely serve almost one-third of the US population that use more than 20 million septic tanks in their individual non-sewered establishments and homes [2]. In recent years, they have also been advanced for collective service in small isolated communities.

### *6.7.1 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 subsurface drainage system where clarified effluent percolates into the soil. Precast concrete tanks with a capacity of 1000 gallons are commonly used for house systems. Solids are collected and stored in the tank, forming sludge and scum layers. 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 [53], seepage bed [53, 54], or seepage pits [55]. 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. 6.13). Required absorption areas are dictated by state and

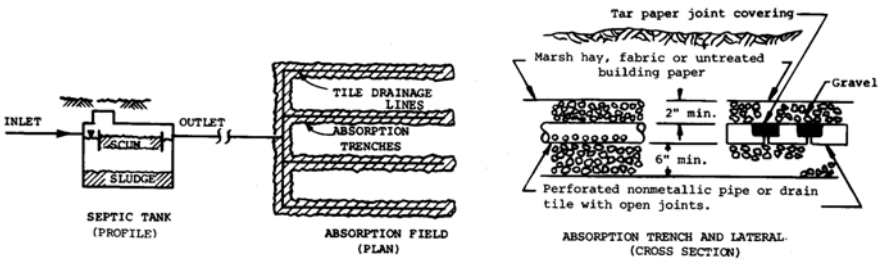


**Fig. 6.13** Septic tank absorption field. Source: U. S. EPA [2]

local codes. Trench depth is commonly about 24 in. to provide minimum gravel depth and earth cover. Clean, graded gravel or similar aggregate, varying in size from ½ to 2½ in., should surround the distribution pipe and extend at least 2 in. above and 6 in. below the pipe. The maintenance of at least a 2 ft 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.

In areas where problem soil conditions preclude the use of subsurface trenches or seepage beds, mounds can be installed (Fig. 6.14) 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, 56, 57]. A pressure distribution

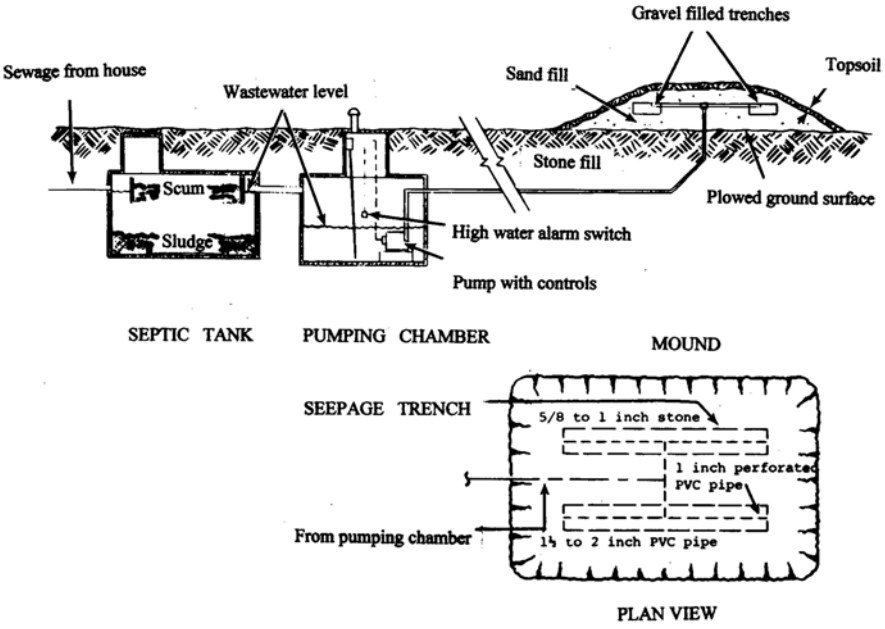


**Fig. 6.14** Septic tank mound absorption field. Source: U. S. EPA [2]

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 the conventional subsurface disposal system, along with higher construction costs. The system cannot be installed on steep slopes, nor over highly (120 mm/in.) impermeable subsurface. Seasonal high groundwater must be deeper than 2 ft 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 [58]. 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 effluent is then applied to the subsurface infiltration system.

### *6.7.2 Applications*

Subsurface infiltration systems for the disposal of septic tank 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.

### *6.7.3 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
(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.

**Table 6.7** Required areas of subsurface infiltration absorption fields. Source: U. S. EPA [2]

| 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 |

### *6.7.4 Design Criteria*

Absorption area requirements for individual residences are given in Table 6.7. The area required per bedroom is a function of the percolation rate; the higher the rate, the smaller is the required area [2].

The design criteria for the mound system are as follows [2, 56, 57]: design flow 75 gal/person/day, 150 gal/bedroom/day; basal area based on percolation rates up to 120 mm/in.; mound height at center approximately 3.5–5 ft; pump (centrifugal) that must accommodate approximately 30 gpm at required total dynamic head (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.

### *6.7.5 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
(d) Area geology and topography
(e) Physical and chemical composition of the soil mantle
(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, TSS, 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 groundwater. 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 and all other natural systems for treating agricultural wastes, the readers are referred to additional references [59–72].

## Glossary of Emerging Natural Waste Systems [69, 70]

**Evapotranspiration system** 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.

**Overland flow land treatment system** 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 treated as it flows across the vegetated surface to runoff collection ditches. 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, redtop, 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 are 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.

**Rapid rate land treatment system** 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 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. Most heavy 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. 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.

**Slow rate land treatment system** Slow rate land treatment system represents the predominant municipal land treatment practice in the United States. In this process, wastewater is applied by sprinkling 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 plant uptake. An underdrainage system consisting of a network of drainage pipe buried below the surface serves 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 can be categorized as hand moved, mechanically moved, and permanent set.

**Water hyacinth aquaculture treatment system** 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. Other organisms, such as duckweed, seaweed, midge larvae, alligator weeds, and a host of other organisms, are also used. 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, where the plants remove nutrients, $BOD_5$, TSS, heavy 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.

**Wetland aquaculture treatment system** 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 (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 (chemical oxygen demand), TSS, fecal coliforms, and pathogenic bacteria.

## Appendix 1: US Yearly Average Cost Index for Utilities [24]

| Year | Index | Year | Index |
|---|---|---|---|
| 1967 | 100 | 1995 | 439.72 |
| 1968 | 104.83 | 1996 | 445.58 |
| 1969 | 112.17 | 1997 | 454.99 |
| 1970 | 119.75 | 1998 | 459.40 |
| 1971 | 131.73 | 1999 | 460.16 |
| 1972 | 141.94 | 2000 | 468.05 |
| 1973 | 149.36 | 2001 | 472.18 |
| 1974 | 170.45 | 2002 | 486.16 |
| 1975 | 190.49 | 2003 | 497.40 |
| 1976 | 202.61 | 2004 | 563.78 |
| 1977 | 215.84 | 2005 | 605.47 |
| 1978 | 235.78 | 2006 | 645.52 |
| 1979 | 257.20 | 2007 | 681.88 |
| 1980 | 277.60 | 2008 | 741.36 |
| 1981 | 302.25 | 2009 | 699.70 |
| 1982 | 320.13 | 2010 | 720.80 |
| 1983 | 330.82 | 2011 | 758.79 |
| 1984 | 341.06 | 2012 | 769.30 |
| 1985 | 346.12 | 2013 | 776.44 |
| 1986 | 347.33 | 2014 | 791.59 |
| 1987 | 353.35 | 2015 | 786.32 |
| 1988 | 369.45 | 2016 | 782.46 |
| 1989 | 383.14 | 2017 | 803.93 |
| 1990 | 386.75 | 2018 | 841.84 |
| 1991 | 392.35 | 2019 | 866.18 |

| Year | Index | Year | Index |
|---|---|---|---|
| 1992 | 399.07 | 2020 | 867.71 |
| 1993 | 410.63 | 2021 | 893.02[a] |
| 1994 | 424.91 | 2022 | 918.91[a] |

[a] Projected future cost index values

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# Chapter 7
# Production and Applications of Crude Polyhydroxyalkanoate-Containing Bioplastic from the Agricultural and Food-Processing Wastes


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## 7.1 Biodegradable Plastics

The nonbiodegradable petrochemical plastics are permanently accumulated in the environment. Only in the USA, the quantity of plastics in municipal solid waste (MSW) in 2010 was 27 million tons [1]. A significant portion of these materials is incinerated or landfilled, which both are unsustainable and environmentally unfriendly solutions. Therefore, there is considerable interest in the development of biodegradable plastics. Their additional advantage is that they are producing from renewable sources so their production will increase environmental and economic sustainability.

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However, the cost of bioplastics produced by conventional biotechnologies is several times higher than the cost of petrochemical-based plastics. With the rise in oil prices, the cost of petroleum-based plastics has come more into line with that of the biodegradable alternatives. There are known predictions that the market for such biodegradable plastics as starch and cellulose derivates, polylactic acid (PLA), and polyhydroxyalkanoates (PHAs) will grow by about 20% a year but the reduction of the bioplastic production costs using cheap raw materials and technological innovations is still essential for the bioplastic industry and applications.

Polyhydroxyalkanoates (PHAs) are polyesters accumulated in bacterial biomass as a storage compound that can be used by a cell as intracellular carbon, energy, and reducing power reserve material. It is well known that PHAs are accumulated under excess of carbon and energy sources or shortage of oxygen and limitation of growth by low concentrations of such nutrients as sources of nitrogen, phosphorus, and others that are used for biomass synthesis. The most important polymers are poly-3-hydroxybutyrate (PHB) with monomer formula (-$OCH(CH_3)$-$CH_2$-C(O)-) and polyhydroxyvalerate (PHV) with monomer formula (-$OCH(CH_2CH_3)$-$CH_2$-C(O)-). PHAs are accumulated in the form of granules inside the cells of many bacterial species, for example, representatives of the bacterial genera *Acinetobacter*, *Alcaligenes*, *Alcanivorax*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Delftia*, *Klebsiella*, *Marinobacter*, *Pseudomonas*, *Ralstonia*, and *Rhizobium*.

The content of PHAs can be up to 80% of dry biomass. Accumulated PHAs can be extracted from bacterial biomass and used in practice as bioplastic with a melting temperature of 160–180°C, a tensile strength of 24–40 MPa, and an elongation at break of 3–142%. The last property, elasticity of the bioplastic, depends on the content of PHV in PHAs. These properties are comparable with the properties of petroleum-based thermoplastics. The most common commercial PHAs consist of a copolymer PHB and PHV together with a plasticizer/softener and inorganic additives such as titanium dioxide and calcium carbonate. The chemical and physical properties of PHAs can be found in numerous reviews [2–11].

The production of biodegradable plastic PHAs can provide many benefits to the industry and to the environment. However, known technologies of PHA production have three essential disadvantages:

- Use of aseptic culture of selected or genetically modified strains that requires high expenses for the sterilization of equipment and medium, as well as for the maintenance of aseptic conditions during biosynthesis of bioplastic
- Use of relatively expensive nutrients such as pure mineral salts and pure (defined) sources of carbon and energy
- Use of expensive, often flammable and toxic organic solvents or energy- and reagent-consuming methods for the extraction of PHAs from bacterial cells

The following options for raw materials, biotechnology of production, and applications of bioplastic can help to solve the problem of high cost of the bioplastic PHAs:

1. Use of organic fraction of food-processing or agricultural wastes for bioplastic production

2. Batch or continuous non-aseptic cultivation for the biosynthesis of bioplastic by mixed bacterial culture
3. Concentration and extraction of bioplastic using chemical treatment, filtration, centrifugation, or flotation for the production of crude bioplastic
4. Applications of crude bioplastic in the construction industry or in agriculture

The aim of this study is to analyze and examine the feasibility of these options for low-cost bioplastic production and its applications.

## 7.2 Nutrients for Non-aseptic Bioplastic Production

Cheap sources of carbon and energy were considered for the production of PHAs in several patents, reviews, and papers. These sources are mainly carbohydrates, organic acids, and proteins. Their origin is as follows:

– Food-processing wastes such as corn-steeped liquor, molasses, activated sludge, starch, and starch-containing wastes; even palm oil mill effluent can be used to produce PHAs [12, 13].
– Agricultural wastes such as unbaled straw; corn cobs, stalks, and leaves; silage effluent; horticulture residuals; farm yard manure; coconut fronds, husks, and shells; coffee hulls and husks; cotton (stalks), nut shells; rice hull, husk, straw, and stalks; sugarcane bagasse. Globally, 140 billion metric tons of biomass is generated every year from agriculture, which is equivalent to approximately 50 billion tons of oil. So, as raw materials, biomass wastes have attractive potentials for large-scale industries and community-level enterprises [14].
– Municipal wastes such as activated sludge of municipal wastewater treatment plant, sewage sludge, and reject water, which is liquid after separation and dewatering of sewage sludge from anaerobic digester of excessive activated sludge.

Wild strains of bacteria, including *Alcaligenes* spp., *Pseudomonas* spp., recombinant strains of *Alcaligenes eutrophus* [15, 16], and a number of filamentous genera can accumulate PHAs under limitation of growth with such essential nutrients for growth as $O_2$ (electron acceptor), N, P, Mg, K, or S, but at the same time there are a lot of mutant or recombinant strains that do not require nutrient limitations for the accumulation of PHAs [17, 18]. It is known that *Alcaligenes eutrophus* can accumulate PHAs up to 80% of dry biomass under excess of carbon source and limitation by P or N, and this stock can be oxidized after addition of P or N [17, 18].

There are some other storage compounds accumulating at nutrient limitation. These storage compounds are extracellular and intracellular polysaccharides in prokaryotes and intracellular polysaccharides and lipids in eukaryotes [19, 20]. Mono- and disaccharides, as well as easily hydrolyzing polysaccharides like starch and glycogen that are used as a source of carbon and energy, are transformed preferably to these storage compounds [19, 20]. Carbohydrates can be used for the production of PHAs mainly in aseptic culture because of the following reasons: (1) almost all

microorganisms can assimilate them; (2) during assimilation of carbohydrates, the major storage compounds will be extracellular and intracellular polysaccharides in prokaryotes or intracellular polysaccharides and lipids in eukaryotes. Excess of carbon and energy source could lead to the growth of glycogen-accumulating or slime-producing bacteria, but this problem can be easily overcome by acidification of the medium with a mixture of volatile fatty acids, which are readily converted to PHAs.

Meanwhile, organic acids, if they are sole carbon and energy source for the growth of bacteria, are accumulating preferably as PHA storage compounds. Therefore, organic acids must be used for non-aseptic cultivation of mixed culture to ensure selective conditions for the growth of PHA-accumulating bacteria. In case when carbohydrates of organic wastes are the major source of carbon and energy, these components should be converted to organic acids for the cultivation of PHA-accumulating bacteria under non-aseptic conditions.

Hydrolysis of polysaccharides and anaerobic acidogenic fermentation are the most acceptable bioprocesses for this transformation (Fig. 7.1).

There are many bacterial species able to hydrolyze polysaccharides, including cellulose and hemicellulose, and then ferment them to organic acids, hydrogen, and carbon dioxide as end products. Microorganisms participating in these processes depend on the sources of carbon and energy. For example, during acidogenic fermentation of cellulose, such representatives of the genera *Clostridium*, *Ruminococcus*, *Butyrivibrio*, *Acetivibrio*, *Fibrobacter*, *Eubacterium*, and *Bacteroides* could be dominating in population of acidogens [21]. The most typical material balance of this process can be shown by the following equation of acidogenic fermentation in rumen (molar ratios of volatile fatty acids (VFAs) were taken from [22]):

$$\begin{aligned} &C_6H_{12}O_6 + 0.82\,H_2O \rightarrow 1.13\,CH_3COOH + 0.35\,C_2H_5COOH + \\ &0.26\,C_3H_7COOH + 1.67\,CO_2 + 2.47\,H_2, \end{aligned} \tag{7.1}$$

where $C_6H_{12}O_6$ is a monomer of cellulose and $CH_3COOH$, $C_2H_5COOH$, and $C_3H_7COOH$ are acetic, propionic, and butyric acids, respectively.

Biotransformation of carbohydrates to VFA and hydrogen is most suitable in the utilization of organic wastes for PHA production because a huge diversity of

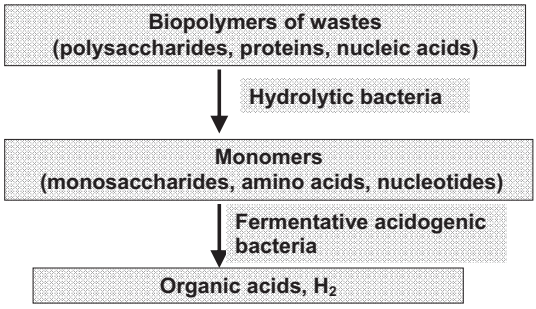


**Fig. 7.1** Microbial groups and substances of acidogenic fermentation

substances in organic waste can be transformed to VFA, which are selective and most favorable substances for the biosynthesis of PHAs under non-aseptic conditions. In this case, organic wastes can be converted to PAHs using a two-stage system including production of fermentative organic acids and biosynthesis of PHAs from these organic acids [23]. Different organic acids and polyols added to the medium are used by cells for copolymerization and enhancement of the PHA synthesis. For example, even a small concentration of caproic acid in the medium significantly increased the percentage of PHAs in bacterial biomass.

The system could contain two separate bioreactors, in order to satisfy the different physiologies and metabolic activities of the two types of microbes. One bioreactor is used for acidogenesis of organic wastes, and a second one for a mixed culture of PHA-producing bacteria. The fermentative acids should preferably be transferred from the first reactor to the second reactor without causing a solid mixing between the two reactors. VFA can be transferred through a membrane into a reactor where the acids can be utilized to produce PHAs.

However, in case when different organic wastes are used for fermentation, the remaining organic dissolved substances and particles can reduce the quality of produced PHAs. To solve this problem, it could be better to extract all volatile organic compounds and hydrogen from acidogenic bioreactor using recycling of biogas with absorption of VFA and using these VFA in the second bioreactor.

It is a well-known use of hydrogen for the production of PHAs [8, 24]. For example, it was proposed a process for converting organic materials, such as organic wastes, into a bioplastic through thermal gasification of the organic material into carbon monoxide and hydrogen, followed by photosynthetic bacterial assimilation of the gases into cell material [25, 26]. However, this gas is used by photosynthetic bacteria only and under anaerobic conditions. Some bacteria can transform gaseous hydrogen and carbon monoxide into PHAs [8, 24]. It is possible to perform acidogenic fermentation with sufficient yield of hydrogen [27] that will be used for the production of bioplastic. However, hydrogen-oxidizing bacteria cannot utilize simultaneously with hydrogen the wide range of organic compounds for the accumulation of PHAs. So, hydrogen produced during acidogenic fermentation of organic wastes could be a useful source for PHA production but has to be used altogether with VFA produced from organic wastes.

This point was used in the US Patent Application (provisional patent) 61/967616 (24 March 2014) “Method for Production of Biodegradable Plastic from Organic Wastes” [28]. The method for the production of biodegradable plastic including polyhydroxyalkanoates (PHAs) from organic wastes in the non-aseptic bioreactors differs from other methods because it includes four stages combined in a sequence and a cycle: (a) the stage of anaerobic transformation of organic matter into volatile fatty acids and hydrogen, which are supplied for stages (b) and (c); (b) the stage of aerobic production of microbial biomass which is supplied to stage (c); (c) the stage of microaerophilic accumulation of PHAs in produced microbial biomass and the recycle of the part of this biomass to stage (d); and (d) the stage of selection of PHA-producing microbial biomass through starvation of biomass supplied from stage (c) following with its recycling to stage (b).

Inorganic nutrients, such as N, P, S, Fe, and microelements, can be also supplied as the components of organic wastes. The ratio of the major inorganic nutrients and the sources of N and P to organic carbon should be in the range from 75:5:1 and 125:5:1 [27]. The typical C:N:P ratio for anaerobic acidogenic fermentation is 100:5:1. N could be from amines, nitrates, and ammonium. P is usually from nucleotides and orthophosphates. Suitable sources of inorganic nutrients could be reject water of municipal wastewater treatment plants (see below) and the mixtures of food-processing and agricultural wastes containing N, P, S, Fe, and microelements.

## 7.3 Food-Processing and Agricultural Wastes for Bioplastic Production

Almost all wastes of food-processing plants or from agricultural activities can be anaerobically transformed to VFA and hydrogen, which can be used further for the production of PHAs [29]. Fermented organic compounds containing in food processing wastes include carbonates, liquid and solid lipids, microbial biomass, glucose, saccharose, lactose, and starch. Food processing wastes include wastes from potato processing and starch manufacturing, molasses from sugar industry, dairy producing factories, fruits and vegetables processing plants, cheese whey, vinegar or acetate-containing waste, valeric acid or valerate-containing wastes, wastes of slaughterhouses or meat-processing plants.

Lipids are one of the major pollutants in food-processing wastewater. Wastewaters produced from edible oil refinery, slaughterhouse, wool scouring, and dairy products industry contain high concentration of lipids. Physicochemical treatment can remove 90% of lipids, but final biological treatment is necessary because of remaining emulsified and/or colloidal lipids. However, vegetable oils and fats will be transformed not too deeply in acidogenic anaerobic bioreactor. Fats are hydrolyzed to long-chain fatty acids (LCFA) and glycerol in the anaerobic digestion, but LCFA are inhibitors of both acidogenic fermentation and methanogenesis mainly because of their surface activity causing damage of cell membranes. Addition of calcium or dissolved ferrous/ferric salts reduced the inhibitory effect of LCFA because of precipitation of LCFA as calcium or iron salt. Iron(II) was used to reduce the inhibition caused by long-chain fatty acids to prokaryotes involved in anaerobic digestion. Degradation of stearic acid, one of model compounds of LCFA, was improved in the presence of divalent iron. The methane production rate was higher in the presence of iron (0.21 mL/L/h) as compared to control (0.17 mL/L/h) where iron was absent. The methane yield was 0.1 L/g COD in experiment and 0.08 L/g COD in control. Iron-containing clay was applied for degradation of vegetable oil. The methane production was increased 1.5 times as compared to control receiving no clay. The methane yield was 0.09 and 0.06 L/g COD in experiment and control, respectively. The COD removal efficiency was 98%, 80%, and 77%, when the iron

dosage was in the ratio of 20, 40, and 80 mg COD/mg Fe, respectively. Acetic and propionic acids were accumulated in reactors and inhibited the methanogenic process when iron was not present or when the COD/Fe ratio was higher than 20. However, no accumulations of acetic and propionic acids were observed, when the ratio of COD/Fe was 20. The presence of iron(II) significantly improved the anaerobic digestion of fat [30, 31]. Iron(II) can be produced in the treatment system from iron(III) hydroxide and iron-containing minerals. These results were confirmed by other researchers [32, 33].

Water-saving process could be shredding of organic wastes and performance of acidogenic fermentation in seawater. The complication of anaerobic acidogenic fermentation of organic fraction of MSW in seawater is sulfate reduction that is using organic acids formed by acidogenic bacteria for the production of toxic and bad-smelling dihydrogen sulfide:

$$CH_3COOH + SO_4^{2-} \rightarrow 2CO_2 + H_2S + 2OH^- \quad (7.2)$$

.

It could be possible to diminish sulfate reduction using Fe(III) compounds like iron ore to stimulate iron bioreduction that is competing with sulfate bioreduction [34, 35]:

$$CH_3COOH + 8Fe^{3+} + 2H_2O \rightarrow 2CO_2 + 8Fe^{2+} + 8H^+. \quad (7.3)$$

Fatty acids and hydrogen for PHA synthesis can be produced from food-processing wastes or agricultural wastes using fermenting bacteria from the genera *Acetobacter*, *Bacteroides*, *Clostridium*, *Citrobacter*, *Enterobacter*, *Moorella*, *Propionibacterium*, *Ruminococcus*, *Thermoanaerobium*, and many others. Initial inoculation of the bioreactor for acidogenic fermentation can be made using sewage sludge of municipal wastewater treatment plants (MWWTP), anaerobic sediments, wet soil, or manure. There must be anaerobic conditions in the acidogenic reactor, and oxidation/reduction potential should be from −50 mV to −400 mV.

The mass ratio of supplied carbohydrates, producing acids, and proteins, producing alkali, has to be in optimal region close to 3 to maintain near-neutral pH. However, if the content of wastes does not ensure self-maintenance of pH, for example, due to the high content of carbohydrates, an application of rotating drum bioreactor and addition of limestone or dolomite powder can ensure near-neutral pH stability in the acidogenic fermentation reactor. VFA produced in this reactor can be used as remaining culture liquid or can be separated from the culture liquid using membrane filtration or enhanced evaporation with recycling biogas as shown in Sect. 7.3.

Acidogenic fermentation of organic food-processing or agricultural wastes is a preferable process than methanogenesis because acidogenesis is significantly a faster process than methanogenesis. Methanogenesis requires long time of the waste treatment: hydraulic retention time (HRT) in anaerobic digester is about 10–20 days food-processing wastes and about 20–40 days for cellulose-containing wastes and manure [36]. Biotransformation of the same wastes to VFA and hydrogen, which

can then be used for the production of bioplastic, can be performed with HRT in anaerobic acidogenic reactor about 3–5 days.

Sugarcane or sugar beet molasses can be also used for PHA production by mixed microbial culture through acidogenic fermentation at first stage. At higher pH, acetic and propionic acids were the main products, while pH from 5 to 6 favored the production of butyric and valeric acids. The yield of PHA from carbon of VFA ranged from 37 to 62% [37].

Acidogenic fermentation of organic wastes for the synthesis of bioplastic should be done on centralized anaerobic digestion (CAD) plants with multiple digesters of about 2000–4000 $m^3$ [36]. Similar to anaerobic methanogenic digestion of organic wastes [38], CAD plants can accept animal manures, together with other waste arising from local food-processing plants, abattoirs, breweries, and municipal sewage sludge. Regarding the agricultural use of manures, sewage sludge or organic fraction of MSW, it is recommended pre-hygienization treatment at 50°C for several hours or at 70°C for 1 h [36]. The agricultural and food-processing wastes commonly used in AD plants include (1) cattle and swine manures and slurries, (2) poultry manure (with or without litter), (3) abattoir wastes, (4) potato and other vegetable processing residues, (5) maize and cereals, (6) silage effluent, (7) dairy processing residues, (8) brewery residues, (9) fish processing wastes, and (10) canning wastes and wastewaters [36].

The efficiencies of continuous acidogenic fermentation of food waste by rumen microorganisms were 71 and 82% at dilution rates 3 $d^{-1}$ and 1 $d^{-1}$, respectively. The final product contains mainly acetic acid (2.5 g/L) and propionic, butyric, and valeric acids with concentrations approximately 1.5 g/L each [39]. Liquid after acidogenic fermentation can be directed to aerobic bioreactor for biomass synthesis and then to microaerophilic reactor for PHA accumulation in biomass. Anaerobic acidogenic fermentation of organic wastes and biosynthesis of biomass and PHAs can be performed in one reactor with separated anaerobic and aerobic ones [23].

The remaining organic and inorganic particles of agricultural and food-processing wastes that are used for acidogenic fermentation and production of bioplastic from VFA can reduce the quality of produced PHAs. To solve this problem, a chain of membrane anaerobic and aerobic bioreactors can be used, similar to the described technology [40]. Another technological solution to avoid pollution of bioplastic or inhibition of microbial mixed culture with the toxic components of wastes is the recycling of biogas from anaerobic bioreactor with absorption of VFA and their supply to the stage of biomass and PHA production (Fig. 7.2).

This recycling of biogas can maintain pH in anaerobic acidogenic reactor due to permanent removal of VFA and $CO_2$ from culture liquid of the bioreactor. The recycling of the biogas through absorber of VFA and $CO_2$ will increase the concentration of $H_2$ in biogas. Therefore, at some concentration of hydrogen, determined by automatic device, the removal of the part of biogas will be used to supply hydrogen to the microaerophilic bioreactor for the accumulation of PHAs (Fig. 7.2). It is a well-known use of hydrogen for the production of PHAs [8, 24].

During acidogenic fermentation, the pH is dropped and the concentration of organic acids is increased. The microbial communities of acidogenic fermentation

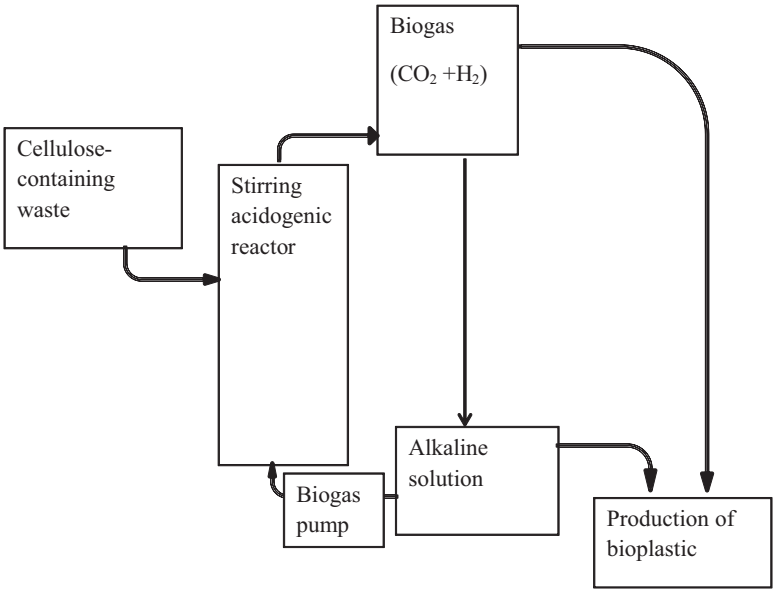


**Fig. 7.2** Maintenance of pH during acidogenic fermentation with recycle of biogas with adsorption of VFA

are active at high concentrations of organic acids, for example, at least of 20, 5, and 15 g/L of acetate, butyrate, and propionate, respectively [41]. Therefore, the pH of fermented organic waste can be dropped below 5.5 during acidogenic fermentation [42]; meanwhile, the optimal pH for acidogens is above 6.0 [43]. There are several ways to maintain optimal pH during acidogenic fermentation of organic waste:

1. For fast and effective acidogenic fermentation, pH must be controlled automatically by measuring and titration with alkali (NaOH). However, it will require reagent and the system of pH control, so the cost of products could be higher than the acceptable value.
2. The digestibility of carbohydrate-rich wastes can be improved by co-digestion with the wastes containing high amounts of protein [44, 45]. Thus, the additional source of protein containing waste, mixing of carbohydrate- and protein-containing waste could balance the concentration of protons results during anaerobic digestion of carbohydrates and hydroxide ions released during ammonification of protein.
3. VFA can be removed from the reactor of acidogenic fermentation using recycle of biogas, containing $CO_2$ + $H_2$, and absorption of VFA from biogas using alkaline solution (Fig. 7.2).
4. This recycle of biogas ensures pH maintenance and high mass transfer of VFA from acidogenic bioreactor to the tank for VFA collection and storage. This is similar to the use of biogas recycle for the removal of ammonia from the methanogenic fermentation reactor [46, 47].

The empirical formula of VFAs after acidogenic fermentation is $CH_2O_{0.57}$ (m.w. is 23.1) [48]. Considering that the growth yield ($Y_b$) from organic acids is 0.5 g of dry biomass/g of organic acid, the material balance of biomass synthesis from VFA of reject water could be written as:

$$CH_2O_{0.57} + x_1O_2 \rightarrow x_2CO_2 + x_3CH_{1.8}O_{0.48} + x_4\,H_2O, \tag{7.4}$$

where $CH_{1.8}O_{0.48}$ ("m.w." is 23.1) is the empirical CHO formula of microbial biomass without PHAs [49].

$$Y_b = 0.5 = x_3\,21.5/23.1, \text{so}\, x_3 = 0.54 \tag{7.5}$$

$$1 = x_2 + x_3\,(\text{balance of C}), \text{so}\, x_2 = 0.46 \tag{7.6}$$

$$2 = x_3 1.8 + 2x_4\,(\text{balance of H}), \text{so}\, x_4 = 0.54 \tag{7.7}$$

$$0.57 + 2x_1 = 2 \cdot 0.46 + 0.48 \cdot 0.54 + 2 \cdot 0.54, so\ x_1 = 0.84. \tag{7.8}$$

So,

$$CH_2O_{0.57} + 0.84\,O_2 \rightarrow 0.46CO_2 + 0.54\,CH_{1.8}O_{0.48} + 0.54\,H_2O. \tag{7.9}$$

The material balance of PHB (empirical formula is $CH_{1.5}O_{0.5}$, "m.w." is 21.5) synthesis from VFA after acidogenic fermentation of biomass (empirical formula is $CH_2O_{0.57}$) could be written as:

$$CH_2O_{0.57} + x_1\,H_2 \rightarrow CH_{1.5}O_{0.5} + x_2\,H_2O, \tag{7.10}$$

so from the balances of O and H $x_2 = 0.07$ and $x_1 = 0.18$

$$CH_2O_{0.57} + 0.18\,H_2 \rightarrow CH_{1.5}O_{0.5} + 0.07\,H_2O. \tag{7.11}$$

The equations of the production of bacterial biomass with PHB are as follows:

$$CH_2O_{0.57} + 0.84\,O_2 \rightarrow 0.46CO_2 + 0.54\,CH_{1.8}O_{0.48} + 0.54\,H_2O, \tag{7.12}$$

$$c\,CH_2O_{0.57} + c\,0.18\,H_2 \rightarrow c\,CH_{1.5}O_{0.5} + c\,0.07\,H_2O, \tag{7.13}$$

$$(1+c)\,CH_2O_{0.57} + 0.84\,O_2 + c0.18\,H_2 \rightarrow 0.54\,CH_{1.8}O_{0.48} + cCH_{1.5}O_{0.5} + 0.46CO_2 + (0.54 + 00.7c)\,H_2O. \tag{7.14}$$

The content of PHB in biomass, P, %, can be determined by the following equation:

$$\begin{aligned} &P = 100{\cdot}c\ CH_{1.5}O_{0.5} / \left(0.54\ CH_{1.8}O_{0.48} + c\ CH_{1.5}O_{0.5}\right) = \\ &2150c / \left(12.5 + 21.5c\right), \text{so } c = 0.58\ P / \left(100 - P\right). \end{aligned} \tag{7.15}$$

$$\text{The yield of biomass with PHB} = \left(0.54\ CH_{1.8}O_{0.48} + cCH_{1.5}O_{0.5}\right) / \left(1+c\right)CH_2O_{0.57} = \left(12.5 + 21.5c\right) / \left(1+c\right) 23.1 = \left(0.54 + 0.93c\right) / \left(1+c\right) \text{ g of PHB / g of consumed VFA.} \tag{7.16}$$

$$\begin{aligned} &\text{The yield of PHB} = cCH_{1.5}O_{0.5} / \left(1+c\right)CH_2O_{0.57} = \\ &0.93\,c / \left(1+c\right) \text{g of PHB / g of consumed VFA.} \end{aligned} \tag{7.17}$$

The yield of biomass with PHB and yield of PHB as the functions of PHB content in biomass are shown in Table 7.1.

The amount of PHAs, which can be accumulated by activated sludge of aerobic wastewater biotreatment systems, is from 15 to 62% of dry biomass weight. The addition of fatty acids to the medium enhances the synthesis of PHAs. These PHAs have useful functions and properties. The maximum accumulation of PHAs in activated sludge can be up to 37.4% in case when the C:N ratio will be increased to 144, and the maximum specific polymer production yield was 9.3% of consumed glucose at an optimum C:N ratio of 96 [50]. Accumulation of PHAs by activated sludge can be stimulated by the changes of the aerobic conditions to anoxic [51–55]. Sludge acclimatized with wastewater supplemented with acetate could accumulate PHAs up to 30% of sludge dry weight, and the production of PHAs was stimulated when the pH was kept at 8 or 9 [56, 57]. However, activated sludge, acclimatized in the microaerophilic aerobic process, accumulated PHAs up to 62% of dry activated sludge [58]. Essential conditions for the accumulation of PHAs, using mixed culture or activated sludge, are transient feeding of raw wastewater and the presence of electron donor and acceptor in the raw wastewater [59]. Activated sludge fed by VFA at alkaline pH accumulated up to 56% of PHAs in dry biomass probably due to permanent removal of phosphate and ammonium as struvite, $NH_4MgPO_4{\cdot}6H_2O$ [60]. Activated sludge with addition of valeric acid produced mainly poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) with the mole fraction 3-hydroxyvalerate (3 HV) up to 54% and a melting temperature of 99°C instead of 178°C for PHB [61].

**Table 7.1** Yields of biomass and PHB at different contents of PHB in biomass

| P (content of PHB in biomass, % w/w) | c | Growth yield of biomass with PHB, g/g of consumed VFA | Yield of PHB, g/g of consumed VFA |
|---|---|---|---|
| 0 | 0.00 | 0.54 | 0.00 |
| 20 | 0.14 | 0.59 | 0.29 |
| 40 | 0.37 | 0.64 | 0.25 |
| 60 | 0.87 | 0.72 | 0.43 |
| 80 | 2.32 | 0.81 | 0.65 |

Accumulation of PHB is possible also by the cultivation of fast-growing rhizobia *Sinorhizobium meliloti*, *Rhizobium leguminosarum* bv. *viciae*, *R. leguminosarum* bv. *phaseoli*, and *R. leguminosarum* bv. *trifolii* in sludge and in wastewater. Maxima HB yields were 7 or 11% w/w after 60 h of cultivation on sludge or 35 h of cultivation on slaughterhouse wastewater, respectively [17]. Growing rhizobia on sludge could be a way for bioplastic production from the wastes. However, feeding of activated sludge with VFA altogether with mineral nutrients could be the best way for the production of bioplastic from the organic wastes (Fig. 7.3).

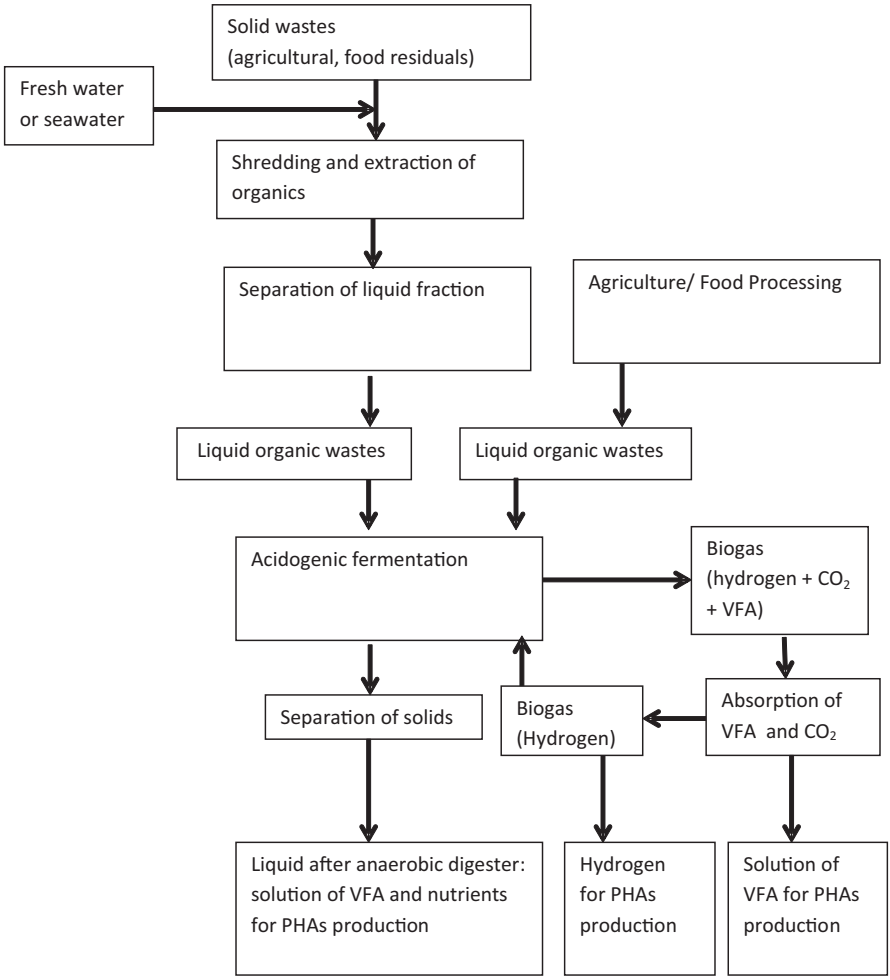


**Fig. 7.3** Preparation of nutrients for bioplastic production from wastes

## 7.4 Batch and Continuous Biosynthesis of PHA Bioplastic by Mixed Culture

Aseptic cultivation of selected or genetically recombinant strain of bacteria requires thermal sterilization of materials and equipment as well as specialized equipment. The cost of aseptic cultivation is several times higher than the cost of non-aseptic cultivation. Therefore, non-aseptic cultivation of mixed microbial culture, which is able to accumulate PHAs, could be a low-cost technology for the industrial production of PHAs.

The major points of using mixed bacterial culture for PHA production were considered in several patents, reviews, and papers and are shown below:

(a) Activated sludge, a well-known mixed culture, is able to store PHAs as carbon and energy storage material under unsteady conditions arising from an intermittent feeding regime and variation in the presence of an electron acceptor.
(b) Activated sludge accumulates PHAs to around 20% of dry weight under anaerobic conditions, but the content can be increased to 62% in a microaerophilic-aerobic sludge process. Oxygen management is crucial to conserving reducing power, as excessive aeration rates decrease the PHA yield and allow higher biomass growth.
(c) When compared with a pure culture accumulating up to 80% of cell dry weight, the merits of PHA production in mixed culture would be an enhanced economy, a simpler process control, no requirement of aseptic processing, and use of wastes. Nitrogen deficiency in wastewater is essential for the synthesis of PHAs using mixed culture [62].

It is expected that PHA-producing mixed culture will comprise representatives of the genera *Acinetobacter*, *Alcaligenes*, *Alcanivorax*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Delftia*, *Klebsiella*, *Marinobacter*, *Pseudomonas*, *Ralstonia*, *Rhizobium*, and others. Initial inoculation can be made using aerobic forest or garden soil suspension or activated sludge of MWWTP. Accumulation of PHAs by mixed microbial cultures occurs usually under transient conditions of carbon and energy sources, known respectively as aerobic dynamic feeding and anaerobic/aerobic process. In these processes, PHA-accumulating organisms, which are quite diverse in terms of phenotype, are selected by the dynamic operating conditions imposed to the reactor. The stability of these processes during longtime operation and the similarity of the polymer physical/chemical properties to the one produced by pure cultures were demonstrated. The accumulation of PHAs in mixed culture can be implemented at an industrial scale [63, 64].

A multistep cultivation process can be used for the production of bioplastic PHAs from organic raw materials and wastes as it was claimed in the US Patent Application 61/967616 (24 March 2014) “Method for Production of Biodegradable Plastic from Organic Wastes” [28]. The method for the production of biodegradable plastic includes production of polyhydroxyalkanoates (PHAs) from organic wastes

in the non-aseptic bioreactors. This can effectively reduce raw material and cultivation cost. This system includes following stages:

1. A process for producing VFA from anaerobically digested wastes is combination of wastes or addition of limestone/dolomite powder to maintain the neutral pH in the anaerobic reactor.
2. A separation of the liquid fraction after the anaerobic digester.
3. Continuous separation of VFA and hydrogen from the anaerobic digester, extraction, and use of VFA.
4. PHAs comprising the combination of the selection and culturing steps, which includes culturing PHA-producing microbes in a culturing zone and feeding said PHA-producing microbes into a selection zone. It includes a process for producing PHAs comprising the step of culturing PHA-producing microbes in a culture media containing hydrogen gas. It includes a process for extracting PHAs comprising the step of degradation of microbial cells into PHA granules and cell debris and the step of separation of the PHA granules from the cell debris.

The batch biosynthesis of bioplastic is simpler than the continuous one but could be less productive than the continuous process in large-scale applications. At the first stage of the batch process, mixed culture is growing in as rich as possible medium under intensive aeration and optimal temperature and pH but to ensure the fast growth of microbial biomass with the highest growth yield. After some period of cultivation, some essential nutrients, such as the sources of N or P, are consumed, and accumulation of PHAs is started [17]. Continuous systems of cultivation, for example, a two-stage chemostat with two reactors for the growth of bacteria and for the production of PHAs, are giving highest PHA accumulation [17]. A batch process was used by Imperial Chemical Industries for the large-scale production of PHB. A continuous process was used by the Austrian Company Chemie Linz GmbH for the pilot production of PHB. The productivity in the last case was about 1 kg of PHB/day/$m^3$ of the bioreactor [17].

The growth of biomass must be performed at concentrations of oxygen higher than 1 mg/L. Using mixed culture, the growth parameters of conventional activated sludge can be used: 0.5 g BOD/g of dry biomass/day and 0.8 g of biomass/g of BOD consumed [65], where BOD is "biological oxygen demand," i.e., oxygen used for biooxidation of organic matter. So, to produce 1 kg of biomass in a 10 L bioreactor with a maximum oxygen transfer rate of 1 g/L/h (which is corresponding to a biomass growth rate of 0.8 g of dry bacterial biomass/L/h), it is needed about 5 days. Considering that the content of PHAs in dry bacterial biomass will be accumulated to the level of 30%, the maximum rate of PHA production in mixed culture could be at the level about 0.24 g of dry PHAs/L/h. So, 1 kg of PHAs in a 10 L bioreactor could be produced for about 17 days. The process of PHA synthesis in the second reactor of the continuous system or in the second stage of the batch process can be initiated by decreasing dissolved oxygen concentration to 0.5 mg/L and by increasing the concentration of VFA as well as supply of hydrogen gas, which is produced during acidogenic fermentation.

Another technological approach for the production of PHAs is semicontinuous cultivation of a mixed culture using a feast-famine cycle comprising a feast phase and a famine phase in one bioreactor. This cycling process promotes not only accumulation of PHAs in biomass but also selection of PHA-producing microorganisms [66–68]. The continuous process can be used not only because of higher productivity but also because it can be used for permanent selection and maintenance culture with highest productivity of PHA synthesis. In every mixed culture, for example, in biological wastewater treatment, desired selection is ensured by the retention, recycling, or transfer of some microbial groups between different reactors [20, 65]. That is why the process for producing PHAs in continuous culture should comprise the combination of the selection, cultivation, and PHA synthesis stages with the recycling loop of PHA-accumulating cells as shown in Fig. 7.4.

The selector could be a bioreactor with intensive aeration but without supply of nutrients so that cells with intracellular storage of carbon and energy will have selective advantages for growth in such a bioreactor. This advantage will depend on the retention time in the bioreactor selector. There must be sufficient long time of starvation, approximately from 2 to 20 h, so that cells with accumulated PHAs will grow and dominate in the microbial population but cells without intracellular storage of PHAs will be suppressed in their growth or even die because of starvation. The hydraulic retention time (HRT) in the bioreactor for the growth of biomass should be in the range from 1 to 20 h, while the HRT in the bioreactor for PHA accumulation should be several times longer.

Additional selection factor, high salinity of the medium, could be used in case when seawater is used for the cultivation of PHA-producing mixed bacterial culture and osmotic shock is used to disrupt cells of halophilic bacteria and to release PHA granules in low-salinity fresh water. There are known halophilic bacterial species that accumulate PHAs, for example, representatives of the genera *Alcanivorax* [69, 70] and *Delftia* [71]. We isolated similar strains from the water of the Dead Sea in Jordan. These bacteria grew and accumulated intracellular PHAs in the medium

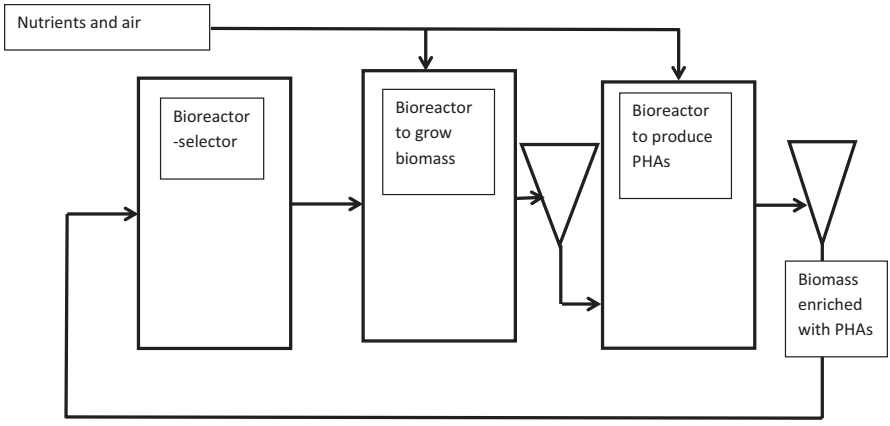


**Fig. 7.4** Continuous production of PHAs with selector

with 10% of NaCl. Organic acids for the cultivation of PHA-accumulating mixed halophilic culture also can be produced by acidogenic fermentation of organic substances dissolved or suspended in seawater.

## 7.5 Downstream Processes

A typical rod-shaped bacterial cell has a diameter of about 1–2 μm and a length of 2–5 μm and is covered with one or two membranes and thin or thick cell wall in Gram-negative or Gram-positive bacteria, respectively [20]. PHA granules in bacterial cell have diameters from 0.2 to 0.7 μm and are surrounded by a membrane [3]. So, to release the PHA granules from bacterial cell, the cell wall must be mechanically broken or (bio)chemically lysed.

According to the present state of the art, PHA-containing biomass is processed: (1) by extraction of PHAs from dried biomass with organic solvents following the separation of solution by centrifugation or decantation; (2) cell walls and membranes are degraded chemically by oxidants and surfactants and then granules of PHAs are concentrated by centrifugation or flotation; (3) cell walls and membranes are degraded enzymatically and then granules of PHAs are concentrated by centrifugation or flotation; and (4) cell walls and membranes of halophilic bacteria can be broken by osmotic shock. Different methods for isolation and purification of bacterial PHAs were described in detail in several reviews [3, 9, 72].

PHA solubilization by organic solvents occurs at low temperature [73] or cell wall breaking, adjustment of pH to alkali value, adding of surfactant, separating of coagulant from liquid, washing and drying of product [74]. Recovery and purification of PHAs can be performed also by solubilization of the non-PHA cell mass in an acidic solution, leaving a suspension of partially crystallized PHA granules, adjusting the pH of the suspension to 7–11 and separating the PHA solids from the dissolved non-PHA cellular mass, resuspending the PHA solids in a bleaching solution for decolorization, and drying the resulting PHA solids [75].

Different chemical pre-treatments can be used before and/or after enzymatic cell disruption to enhance extraction of PHAs [76]. It is well known that oxidation of the cell wall with hydrogen peroxide or other oxidants such as chlorine or chlorine-containing oxidation agents can be used for the recovery of PHAs. For example, one of the simplest ways could be mixing of biomass with bleach (solution of 5% of sodium hypochlorite with pH 13) so that the final concentration of hypochlorite will be about 1.2% and to pH about 11, and after 1 h of stirring, pH should be adjusted with HCl to 7. The suspension could be treated by continuous or batch centrifugation or flotation for separation of cell walls and PHA granules.

All known methods of PHA extraction suffer from high cost or environmental pollution and are difficult to be industrialized. To avoid disadvantages of chemical treatment, the proteolytic enzymes can be used [77]. The generated solid and liquid phases are separated by filtration or centrifugation. Instead of pure enzymes, it

could be possible to use crude enzymes or even intact microorganisms hydrolyzing bacterial cell walls. For example, there are known fungi, representatives of the genera *Absidia*, *Agaricus*, *Aspergillus*, *Chaetomium*, *Fusarium*, *Neurospora*, *Penicillium*, *Phanerochaete*, *Phialophora*, *Pleurotus*, *Rhizoctonia*, *Trichoderma*, and many others that are able to lyse bacterial cell walls. These microorganisms should be grown in a separate bioreactor using biomass remainder after separation or extraction of PHAs from biomass. When extracellular cellulolytic activity will be at the sufficient level, bacterial biomass containing PHAs can be treated with intact fungal suspension or its filtrate containing extracellular cellulolytic enzymes.

Disruption of biomass of halophilic bacteria, containing PHAs, can be performed just mixing with fresh or distilled water to break cells and to release PHA granules due to osmotic shock. Then suspension can be treated with fine dispersed air or by dissolved air flotation (DAF) at pH 3 to concentrate PHA granules in foam.

Separation of the PHA granules of PHAs from lysed or disrupted cells can be done using flotation, centrifugation, or membrane filtration. Probably, a low-cost technology could be flotative separation of PHA granules from destroyed cells. The bubbles of air have the hydrophobic surface, so at the pH about 3.0–3.5, the difference in hydrophobicity of the surface of PHA granules and cell walls ensures the preferable adhesion of PHA granules to the air bubbles [78]. This flotation process is affected by air bubbles, cells, and PHA granule interactions as well as dynamics of liquid and foam, sizes of the particles, hydrophobicity, and surface charge (zeta potential), which depends on pH of aqueous solution. Finally, a PHA purity of 86% (w/w) can be obtained using flotation separation and concentration [78].

However, flotative separation using just supply of air in the flotation tank with bacterial suspension after cell disruption has to be performed with small-diameter air bubbles and intensive aeration to have the sufficient specific surface for PHA granule adhesion and a fast foam formation rate for its continuous removal. Additionally, for the effective batch or continuous flotation, there must be automatic control of the level of liquid and liquid supply rate, as well as the level of foam and foam removal rate in the flotation tank to ensure proper separation of biomass and PHA granules. The simpler and more reliable way for PHA granule separation and concentration is dissolved air flotation (DAF) in batch mode. Usually, it involves supply of compressed air into the tank with bacterial biomass for dissolved oxygen saturation at excessive pressure, for example, at 5 atm, and then supply of this suspension to the flotation tank with atmospheric pressure. The smallest air bubbles are releasing in the flotation tank due to the difference of gas solubility at the excessive or atmospheric pressure. These small-size air bubbles adsorb most hydrophobic substances at their surfaces and float forming the foam. The foam, containing concentrated hydrophobic substances, is removed and collected. PHA granules are dried and then used as bioplastic. There are known many designs of DAF facilities for experimental and industrial applications.

## 7.6 Crude Bioplastic for Construction and Agricultural Applications

Potential market of bioplastics includes packaging materials, catering products, consumer electronics, medical materials, agriculture and horticulture (biodegradable mulch foil), toys, and textiles (en.european-bioplastics.org/market). PHA bioplastic has been used in the medical applications in skin substitutes, and drug delivery microspheres because of PHA biocompatibility and biodegradability [79]. However, PHAs producing by non-aseptic cultivation from waste materials using mixture cultures of bacteria cannot be used for biomedical, food packaging, or catering applications because chemical and physical properties of bioplastic are not controlled in non-aseptic cultivation and there may be present pollutants from wastes and microorganisms.

The type of application depends also on the mechanical properties of bioplastic. PHA mechanical properties depend very significantly on the chain length of the monomer. For example, PHB is stiffer and more brittle than for polypropylene, but copolymerization with hydroxyvalerate (PHB-co-PHV) makes bioplastic much more flexible [4]. This copolymer can be used for packaging material like films and bottles [17]. However, PHB applications are limited by its thermal degradation during molding and stiffness of bioplastic. For aseptic cultivation of genetically modified strains of PHA producers, it is possible to select specific medium and conditions for the production of PHAs with the desired mechanical properties, but for the non-aseptic cultivation of mixed culture, the chemical content of accumulated PHAs will be determined mainly by the spectrum of fatty acids in the medium.

The major advantage of PHAs for applications is biodegradability of bioplastic by soil and aquatic microorganisms. These microorganisms produce PHA depolymerases and other enzymes finally transforming PHA-made items to carbon dioxide and water for about 1.5 months in anaerobic sewage, 1.5 years in soil, and 6.5 years in seawater [4, 80, 81]. Dead bacterial biomass with PHAs contains also polysaccharides of the cell wall, proteins, polynucleotides, and phospholipids, where the contents are about 15%, 50%, 25%, and 10% of dry biomass without PHAs, respectively, and the biodegradation rates are higher than those of PHAs. Therefore, from the point of view of biodegradability in soil, there is no sense to extract PHAs from biomass but to use dry biomass with PHAs as crude nanocomposite from the granules of PHAs and interlayers of cellular biopolymers.

Speculatively, such nanocomposites should be more flexible and better biodegradable than extracted PHAs. There are known many natural biocomposites, where brittle nanocomponents like hydroxyapatite crystals in the bone or aragonite crystals in the pearls are composed of flexible biopolymer nanocomponents, usually nanolayers or nanoaggregates of protein molecules. Production of similar nanocomposite material from PHA-containing dry bacterial biomass will permit to exclude an expensive technological stage of PHA extraction and diminish additionally the cost of crude PHA-containing biodegradable material.

However, there are two potential problems in the applications of such crude PHA nanocomposite. The first problem is the temperature of malting for PHAs, which is in the range of 160–180°C [4–8]. The melting temperature of PHB is close to its thermal decomposition temperature $T_d$-10% [82]. Thermal decomposition temperatures of proteins, polysaccharides, and polynucleotides are also close to this value, i.e., all biopolymers have poor thermal stability at temperature of PHA melting. Natural antioxidants, which present in biomass, can reduce the rate of thermal destruction of biopolymers [83]. Protein itself can be considered as thermoplastic material but with additions of plasticizers, which inhibit the formation of cross-linking that can result in the formation of thermoset material from extruded protein [84]. Therefore, the molding of composite crude bioplastic material should be for as short process as possible to diminish the thermal decomposition of PHAs and other biopolymers of bacterial biomass.

There is a clear trend in the construction industry for using biodegradable materials and biopolymers [85–88]. One area of applications of nanocomposite bioplastic from bacterial biomass containing PHAs is the production and use of biodegradable construction materials. Actually, almost all construction activities started up from using the most abundant biodegradable polymeric material, wood, and other cellulose-containing natural materials. Currently, biodegradable construction material can diminish the area of land used for landfilling because they are degraded very quickly in soil or in the landfill.

For example, this sustainable, biodegradable bioplastic can be used for insulation walls and partitions, construction of nonstructural (internal) elements such as separating walls, and for manufacturing of geotextiles, drainage pipes, silt and dust fences, and different kinds of the temporarily constructions. Plastic foam and foam insulators that are used in the construction industry produce hazardous nonbiodegradable wastes after demolition of the buildings or temporal constructions. The non-biodegradability of these plastics limits their construction applications. The bioplastic could be used for new, green, sustainable construction materials because this easily biodegradable material can be being landfilled, composted, or even left on the site of use without excavation.

Other examples of potential application of crude nanocomposite from bacterial biomass containing PHAs are construction silt and dust fences that can be landfilled for fast biodegradation or composted as biomass. Sustainability of biodegradable construction materials is due to (1) production of bioplastic from renewable sources or even from organic wastes and (2) fast biodegradability of this material under the conditions of landfill or composting, so negative effect of construction waste on the environment will be minimized. There could be a big market for biodegradable bioplastic foam construction material, which does not require incineration after demolition.

There are also a lot of potential agricultural applications of nanocomposite bioplastic from bacterial biomass containing PHAs. One potential application is dark plastic mulch, which suppresses weeds, reduces water evaporation from soil, and warms soil for earlier planting. Millions of hectares of arable land are cultivated under plastic mulch. However, nonbiodegradable plastic mulch requires

labor-consuming annual removal from the field and disposal or energy-consuming and environmentally unfriendly recycling of the used film. The advantage of the film from biodegradable nanocomposite bioplastic from bacterial biomass containing PHAs is that this used mulch material can be left for natural biodegradation on the field.

Another agricultural application of nanocomposite bioplastic from bacterial biomass with PHAs is manufacturing of slow-release fertilizers using bioplastic coating or embedding of fertilizers in bioplastic granules, bars, or films. For example, fertilizer-embedding bioplastic bars can be used for oil palm plantations for slow release of fertilizers during 0.5–2 years of bioplastic biodegradation in soil.

## 7.7 Conclusions

It is possible to produce low-cost crude bioplastic using mixed microbial cultures under non-aseptic conditions of cultivation. Organic acids from liquid wastes or produced by acidogenic fermentation of solid or liquid organic wastes must be the dominating source of carbon for the biosynthesis of crude bioplastic.

There will be a lot of environmental benefits from production and use of crude bioplastic from organic fraction of solid municipal wastes: (1) reduction of the amount of municipal solid wastes to be incinerated, (2) reduction of the amount of ash to be landfilled, and (3) use of seawater for municipal solid waste separation that will save fresh water consumption.

Organic fraction of municipal solid and liquid wastes, as well as agricultural and food-processing wastes, can be used for the production of low-cost crude bioplastic with or without extraction of PHAs from microbial biomass. However, this crude bioplastic can be used only for specific applications in the construction industry or in agriculture. The applications in the construction industry could be the bioplastic foam, the foam insulators, the silt and dust fences, and the temporary constructions. The applications in agriculture could be the dark plastic mulch and different carriers for slow-release fertilizers.

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# Chapter 8
# Optimization Processes of Biodiesel Production from Pig and Neem (*Azadirachta indica* A. Juss) Seeds Blend Oil Using Alternative Catalysts from Waste Biomass


**T. F. Adepoju and Yung-Tse Hung**

## 8.1 Introduction

The important factors that affect the economic growth of a country are human resource, natural resources, capital formation, technological development, and social and political factors. Among the listed factors, natural resources come from nature, and this has become an important factor contending with human resource. As human growth increases, the needs for energy increase. Therefore, no country can achieve its economic growth and social stability without accessibility and optimality of energy.

Meanwhile, over 80% of energy utilization of a country comes from fossil fuel and its derivatives (natural gas, coal, and oil), and almost three-quarters of it originate from combustion of fossil fuel derived from carbon emission.

Recent reports by International Energy Statistics (IES) revealed that there has been an alertness to improve the biofuel production in the European Union Membership Countries and United States starting from 2019 to 2030. Countries such as China (the most populous country in East Asia), India (the second most populous country, and seventh largest country by area located in south Asia), Brazil (largest country in both South American and Latin America, the world fifth largest

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country by area and fifth most populous), and ASEAN (comprises ten countries in southern Asia, which promotes intergovernmental cooperation and facilitates economic, political, security, military, educational, and sociocultural) countries have increased the permitted feedstock base for biofuel and introduced subsidies to expand production capacity. Europe however has injected millions of euros in order to meet up with safety data sheet (SDS) level and is currently leading producer of biofuel follow by Brazil. Therefore, globally, working towards a secure, sustainable future for all through biofuel production is the technology roadmap.

For biofuel production, there exist more than 350 potential oil-bearing crops, excluding animals' fat and algae [1]. In China, popular biofuel feedstocks include sugar beet, sugarcane, tuber crops, sweet sorghum, *Jatropha curcas*, etc., while in India, the major feedstocks are palm oil, edible oil from oilseed crop, *Jatropha* oil, sugarcane, maize, sugar beet, and cassava [2]. In Brazil, the feedstocks include beef tallow, cottonseed oil, waste frying oil, pork lard, chicken fat, palm oil, etc. [3] while in the ASEAN countries, the first-generation biofuel feedstocks include sugarcane, wheat, corn, cassava, oil palm, soybean, rapeseed, and *Jatropha* [4]. Europe also used the feedstocks for the production of oil-based residue, lignocellulose, crops, algae, waste gases, etc. To continue to lead the world production in biofuel for 2020–2030, Europe identifies four primary sources that could provide additional biomass and supportive growth of bio-based industries, namely, agricultural residues, forest biomass, waste, and non-food crops [5].

Pig fat (lard), obtained from pig meat called pork, is the most commonly available waste, owing to the fact that pork meat is the most commonly consumed red meat worldwide (due to high in protein and rich in minerals and vitamin), especially in Eastern Asia, but its consumption is forbidden in certain religions (Islam and Judaism). High-quality protein is the main nutritional component of pork meat, making it useful for muscle growth and maintenance. However, pig contains varying amount of fat (lard) ranging from 10 to 32% depending on the level of trimming, but the fat is mainly composed of saturated and unsaturated fats, present in approximately equal amounts. Surprisingly, this fat causes harm to man, cattle, and other domestic animals when consumed. The risks in consumption include trichinosis, bovine spongiform, diarrhea, roundworm infestation, heart disease risk, bladder cancer risk, mad cow diseases, gastroenteritis, and many more [6]. Hence, it is needed to channel the fat as a feedstock for biofuel (biodiesel) generation.

The use of pig fat and other animal fats as a feedstock for biodiesel production have been reported by various researchers. Chinyere et al. [7] reported 96% of biodiesel yield in optimization of the methanolysis of lard oil in the production of biodiesel with response surface methodology using KOH. Chung et al. [8] produced 81.3% fatty acid methyl ester (FAME) content in a transesterification reaction of duck tallow with methanol using NaOH catalyst, while Anildo Jr. et al. [9] reported a total 83% conversion in a synthesis and characterization of ethylic biodiesel from animal fat wastes using KOH. Animal fat wastes for biodiesel production was reviewed by Vivian et al. [10], while Jishy and Sankar [11] gave a report on production of biodiesel from chicken fat, pork fat, and combination of the two feedstocks using KOH. The use of mixtures of waste frying oil and pork lard to produce

biodiesel using NaOH was also reported by Joana et al. [12]. Encinar et al. [13] reported a total of 89.0 wt.% ester content in a study of biodiesel production from animal fats with high free fatty acid content using $H_2SO_4$/NaOH.

*Azadirachta indica* belongs to the mahogany family Meliaceae, usually known as Neem, Nimtree, or India Lilac [14]. Its origin is from India and the Indian subcontinent which includes Nepal, Pakistan, Bangladesh, and Sri Lanka. It can also be found growing in tropical and semi-tropical regions, Islands in the southern part of Iran as well as Africa in Nigeria. Neem is considered a weed in many countries, including some parts of the Middle East, and most of Sub-Saharan Africa including West Africa and Indian Ocean states. Naturally, it lives well in similar environments to its own, but its weed potential has not been fully assessed [14]. Neem leaves are dried in India and placed in cupboards to prevent insects eating the clothes and also while storing rice in tins [15]. The tender shoots and flowers of the neem tree are eaten as a vegetable in India, a soup-like dish called *Veppampoo charu.* Products made from neem trees have been used in India for over two millennia for their medicinal properties. Neem seed contains 25–45% oil which is non-edible and has greater disadvantages when used. In small children it is highly toxic and can lead to death, in pregnant woman, it can cause miscarriages, in man, it can lead to infertility, and low blood sugar [16]. It is useful as a raw material in industries such as cosmetics, resin, soap, honey, neem blossom, fertilizer, and gum making.

Meanwhile, the use of mixture of oils in different proportions (70:30, 50:50, 25:75, 40:60) have been exploited for biodiesel synthesis, and the reports showed high yield of biodiesel when compared with the results obtained with single oil [12, 17–20], but all of these reports utilized homogeneous catalyst (KOH/NaOH) for biodiesel production apart from the work reported by Falowo et al. [17], where nanoparticles from elephant ear tree pod (*Enterolobium cyclocarpum*) husk was used as heterogeneous bio-base catalyst in biodiesel production intensification via microwave irradiation-assisted transesterification of oil blend.

However, the use of homogeneous catalysts for biodiesel synthesis produces good results, but not with its own shortcomings. The disadvantages include high cost price, toxic effects, inability to recycle, hygroscopic nature, time consuming in washing process making it difficult to separate glycerol from biodiesel and utility wastage.

Heterogeneous catalyst can be obtained from biomass waste, animal bones, activated carbon supported catalyst, and waste coral [21–24]. Direct burning of wastes does not decompose easily and substantial furnace modifications are usually required if they are to be used as firewood or fuel oil [25]. Heterogeneous catalyst however comprises many advantages including non-toxic easily recycle and reuse, non-hygroscopic, and availability with low or no cost.

Considering the large amounts of the solid waste that kola nut husk (KNH) and palm kernel shell husk (PKSH) constituted to the fruits, there is need for the process of recycling/recovery. It has been reported that using fermented kola nut husk (FKNH) before furnace modifications increases the calcium, potassium, phosphorous, and magnesium contents [26] enough to produce energy.

Therefore, this study synthesizes biodiesel from pig fat oil—neem oil blend using derived catalyst of the mixture of palm kernel shell husk (PKSH) and fermented kola nut husk (FKNH) as heterogeneous catalyst. Detailed characterization of the catalyst developed was carried out using SEM, FTIR, BET, and XRD. Process parameter optimization was investigated with a view to determine the optimum yield of biodiesel from miscible oil blend.

## 8.2 Materials and Methods

### *8.2.1 Materials*

#### 8.2.1.1 Pig Fat, Neem Oil, Palm Kernel Shell Husk, and Kola Nut Husk

Freshly harvested pig fat was obtained from slaughter house at Ete Market, Mkpat Enin L.G.A., Akwa Ibom State, Nigeria. Neem oil (5 L) was purchased from National Cereals Research Institute (NCRI), Abuja, while FKNH was freely obtained from a local market in Ikot Abasi Local Government Area, Akwa Ibom State, Nigeria, and PKSH was obtained from gasifier plant.

#### 8.2.1.2 Chemicals

99.5% pure methanol, 36 wt.% HCl, HPLC grade n-hexane, chloroform, H2SO4 acid, ethanol acetic acid, etc., were of analytical grade and need no further purification obtained from Finelib Nig. Ltd.

### *8.2.2 Methods*

#### 8.2.2.1 Oils Preparation

The method used by Anildo Jr. et al. [9] was adopted but with little modifications. To a flask of 5000 mL capacity, 2 kg of pig fat was washed with 1000 mL of disodium carbonate (1 mol/L) and was mechanically stirred for 25 min. The mixture was centrifuged for 10 min at a temperature of 15 °C using propylene tubes. The supernatant was separated by filtration, and 50 g of anhydrous disodium sulfate was added, stirred for another 10 min, and then centrifuged again for 5 min at a temperature of 15 °C. The pure pig fat oil (PFO) obtained was kept in a clean jar. Neem oil (NO) was heated at a temperature of 30 °C for 20 min, filtered to remove dirt, and then kept in a clean jar for further processing.

#### 8.2.2.2 Oil Blends and Their Characterization

Since neem oil is a non-edible oil with high free fatty acid [27] and pig fat oil is with low acid value [7, 8]; to achieved a mixture of the oil with low acid value, low viscosity, and low density, oils were blend in a ratio of neem oil:pig fat oil (v/v) as 10:90 ($NO_{10}$), 20:80 ($NO_{20}$), 30:70 ($NO_{30}$), 40:60 ($NO_{40}$), 50:50 ($NO_{50}$), 60:40 ($NO_{60}$), 70:30 ($NO_{70}$), 80:20 ($NO_{80}$), and 90:10 ($NO_{90}$), respectively, to enhance biodiesel synthesis using derived heterogeneous catalyst.

The blended oils were properly mixed at 35 °C in magnetic for easy miscibility considering the instability in fat nature. Each resulting mixture was examined for its acid value, density, and viscosity. The mixture with low acid value, density, and viscosity was used for biodiesel synthesis. Other properties of the oil mixture were further determined using association of official analytical chemists [28, 29]. The constituent of the volatile matter, long and branched chain hydrocarbons, esters and alcoholic acids, and others was determined using complete Agilent 5973 N gas chromatography–mass spectrometry instrument control and data storage. 10:90 ($NO_{10}$) is used as an abbreviation for 10 mL of neem oil:90 mL of pig fat oil and $NO_0$ is an abbreviation used for 100 mL of neem oil.

#### 8.2.2.3 Catalyst Preparation

A 500 g KNH was washed with distilled water twice in a cleaned bucket to remove unwanted materials. Solid state fermentation was carried out for 10 days on the sample. The fermented sample (FKNH) was oven dried to constant weight and then milled to powder of 0.5 mm particles size for easy calcination. 500 g of PKSH powder obtained from gasifier plant was made through the mesh size 0.5 mm and was mixed in the same ratio (1:1) with the FKNH powder; the mixed powder was calcined at 800 °C for 3 h in a muffler furnace at standard atmospheric pressure. The calcined powder obtained after calcination was characterized using scanning electron microscopy (SEM), energy dispersive spectroscope (EDS), X-ray diffraction (XRD) analysis equipped with Kά and Cu radiation source, accelerated at 20 mA and 30 kV, Fourier transform infrared spectroscopy (FTIR), and BET isothermal adsorption. The calcined mixed based catalyst was used for transesterification of mixed oil to biodiesel.

#### 8.2.2.4 Characterization of Calcined Powder

After calcination of the powder, the calcined sample was cooled at room temperature, and then characterized using scanning electron microscopy (SEM), to examine the surface morphology of the catalysts, energy dispersive spectroscope (EDS) to determine the elemental analysis of the samples and the quantitative composition of the catalysts, X-ray diffraction analysis (XRD) equipped with Kά and Cu radiation source, accelerated at 20 mA and 30 kV, to establish the angular scanning electron

performed in the range of $10^{o} < 2\phi < 70^{o}$ at a speed of 2 °C $min^{-1}$ [25], and Fourier transforms infrared spectroscopy (FTIR), to check the presence of functional group and verify the presence of characteristic absorption bands of major elements present. The surface area and the basicity of the catalysts were examined using BET isothermal adsorption and Hammett indicator method.

## *8.2.3 Biodiesel Production*

### 8.2.3.1 Esterifying the Oil Mixture

Considering the oil mixture, it was observed that the low viscous (21.50 $mm^2/s$) blended oil was obtained at 60:40 (NO: PFO), with acid value of 4.10 mg KOH/g oil (free fatty acid (FFA) = 2.05) and low density of 852 $kg/m^3$. This oil blend used for acid treatment stage is referred to as esterification. The method used can be found elsewhere [27]. The free fatty acid (FFA < 1.5) required for successful transesterification was obtained following the procedures already adopted by Adepoju et al. [30].

### 8.2.3.2 Transesterification of Esterified Oil Mixed to Biodiesel

The method used by Kostic et al. [31] was adopted with little modifications. The transesterification of the esterified mixed oils to biodiesel was carried out in a three necked reactor at the catalyst amount 2–5 g, reaction time of 50–80 min, methanol/oil ratio of 3–9, and reaction temperature of 50–65 °C. Measured derived catalyst amount was added to a known methanol in 250 mL flask, thermostated at 65 °C for 20 min, and then the predetermined mass of preheated mixed oil (thermostated at 60 °C for 1 h) was poured into the reactor and the reaction was monitored for a certain period. At the end of the reaction, the catalyst was separated by decantation and the biodiesel phase was separated from alcohol phase by separating funnel. The leach catalyst in the biodiesel was removed by washing with a mixture of 1.5 g sodium carbonate and 50 mL methanol, thermally heated at 65 °C for 3 h in a magnetic shaker. The mixture was filtered and then washed with ionized water twice for 20 min as earlier reported by Alba-Rubio et al. [32]. The biodiesel was separated through gravity settling and then dried over anhydrous sodium sulfate, which was separated by filtration to obtain mixed oil biodiesel (MOB). The yield of biodiesel was obtained using volume ratio of biodiesel produced to volume of oil used. The catalyst was collected for reuse at the end of the reaction, but there was reduction in the fourth and fifth cycle. Hence, the catalyst reusability was stopped after third usage. These processes were repeated based on experimental runs generated by deign expert.

#### 8.2.3.3 Experimental Design and Statistical Analysis

A three levels four factors was adopted for experimental design of transesterification of mixed oil to biodiesel, namely, catalyst amount (g), reaction time (min), reaction temperature (°C), and MeOH/OMR (mL/mL) (Table 8.1). Central composite design was used to produce a total of thirty (30) experimental runs so as to give room for a well-designed experiment, more interactive effects among the variables, higher number of runs to increase the axial and center points, and greater room for duplication.

For statistical analysis, the regression parameter and test of significance were used to confirm the level of contribution of each variable on the response (MOB). Also, the experimental results were compared with predicted value generated by design expert via a straight line plot of predicted against actual value (supplementary file). Process optimization was carried out by determining the significance of probability test value ($p$-value < 0.05), F-distribution (f-test), variance inflation factor (VIF), and the degree of freedom (df). The adequacy of the model was confirmed by determining the adjusted R-square $\left(R^2_{\text{adj.}}\right)$ and predicted R-square $\left(R^2_{\text{pred.}}\right)$ valuee. A graph of three-dimensional plots with contour lines was used for interactive effects among the variables.

The model fitting equation that correlates the relationship between the response variable and the independent variables is as given in Eq. (8.1).

$$\text{MOB} = \gamma_0 + \sum_{i=1}^{k} \gamma_i X_i + \sum_{i=1}^{k} \gamma_{ii} X_i^2 + \sum_{i<j}^{k} \gamma_{ij} X_i X_j + \text{T} \tag{8.1}$$

where MOB is the response, $\gamma_0$ is the intercept, $\gamma_i$ is the linear coefficient, $\gamma_{ii}$ is the interaction coefficient, $\gamma_{ij}$ is the quadratic coefficient terms, $X_i$, $X_j$ are the four factors, and $\epsilon$ is the residual error.

#### 8.2.3.4 Physicochemical Properties of MOB

Physicochemical properties of the pure MOB were determined by standard methods: viscosity (ASTM D6079), density (ISO 17828:2015), moisture content (EN ISO 12937:2000), iodine value (EN 14111), acid value (ASTM D664), cetane

**Table 8.1** Three-level four-factor central composite design for transesterification of mixed oils

| Variable | Units | Symbol | Levels | | | | |
|---|---|---|---|---|---|---|---|
| | | | -1(low) | 0 | +1(high) | -alpha | +alpha |
| Catalyst amount | (g) | $X_1$ | 2 | 3 | 4 | 1 | 5 |
| Reaction time | (min) | $X_2$ | 50 | 60 | 70 | 40 | 80 |
| Reaction temp | (°C) | $X_3$ | 50 | 55 | 60 | 45 | 65 |
| MeOH/OMR | (mL/mL) | $X_4$ | 3 | 5 | 7 | 1 | 9 |

Where MeOH/OMR = methanol/oil molar ratio

number (EN ISO 5165), higher heating value (ASTM D2015 [33]), oxidative stability (EN 14112), cold filter plugging (ASTM D6371), carbon residue (ASTM D4530), flash point (ASTM D93), cloud point (ASTM 2500), pour point (ASTM 97), and fatty acid methyl esters (FAME) (EN 14103:2003).

## 8.3 Results and Discussion

### *8.3.1 Physicochemical Properties and Fatty Acid Composition of Oils and Blend*

Table 8.2 shows physicochemical and fatty acid compositions of mixed oils. For comparative purposes, the mixture of the oil blend results are also presented. At 40 °C, the blended oil has low density and viscosity; this can be attributed to proper mixing, blend ratio, and the method adopted for extraction of oil from PFO. Low density and viscosity play very important roles in oil production, transportation

**Table 8.2** Properties and fatty acid compositions of oil and its blend

| Parameters | Pig fat oil | Neem oil | Blend ($NO_{60}$:$PFO_{40}$) |
|---|---|---|---|
| **Properties** | | | |
| Density (kg/m$^3$) at 40 °C | 860 | 890 | 852 |
| Viscosity at 40 °C/(mm$^2$/s) | 23.95 | 24.80 | 21.50 |
| Moisture content (%) | 0.024 | 0.031 | 0.011 |
| %FFA (as oleic acid) | 0.425 | 7.33 | 2.05 |
| Acid value (mg KOH/g oil) | 0.85 | 14.66 | 4.10 |
| Saponification value (mg KOH/g oil) | 201.6 | 199.16 | 198.30 |
| Iodine value (g $I_2$/100g oil) | 59.36 | 107.38 | 88.90 |
| Peroxide value (meq $O_2$/kg oil) | 77.00 | 4.40 | 34.30 |
| HHV (MJ/kg) | 40.27 | 42.88 | 56.94 |
| Cetane number | 59.81 | 46.33 | 45.83 |
| Mean molecular mass | 277.78 | 281.18 | 282.40 |
| Flash point (°C) | 205.00 | 207.00 | 208.00 |
| Cloud point (°C) | −5 | −5 | −5 |
| Pour point (°C) | −15 | −15 | −15 |
| **Fatty acid compositions (%)** | | | |
| Myristic acid (C14:0) | 1.40 | 1.30 | 1.80 |
| Palmitic acid (C16:0) | 22.60 | 23.10 | 18.20 |
| Palmitoleic acid (C16:1) | 1.80 | 2.70 | 3.10 |
| Stearic acid (C18:0) | 13.60 | 11.30 | 21.00 |
| Oleic acid (C18:1) | 43.10 | 50.60 | 40.42 |
| Linoleic acid (C18:2) | 15.70 | 8.70 | 14.40 |
| Linolenic (C18:3) | 1.50 | 2.10 | 1.40 |
| Others | 0.30 | 0.20 | 0.10 |

through the pipeline, and oil recovery processes [34]. The acid value obtained for neem oil (NO) is higher than that obtained for the blended oil (BO), but the value obtained for the PFO is less than that obtained for BO. The high acid value could be attributed to the percentage of NO present in the blend, storage duration of NO before purchase, and different agro-climatic conditions [35]. The iodine values obtained for NO (107.38 mg KOH/g oil) and BO (88.90 mg KOH/g oil) were high than that obtained for PFO (59.36 mg KOH/g oil). This observation supports the report earlier given by Anyaogu et al. [36], according to which the PFO is low in conjugated linoleic acid (CLA) and slightly richer in unsaturated acids. The oil is mainly composed of unsaturated and saturated fats, presently in approximately equal amounts. The peroxide values of NO and BO are less than that obtained for PFO, which showed that the NO and BO have high of rancidity and usually stable [37].

The high heating value and the high cetane number value proved that the BO serves as good feedstock for energy production with great combustibility. The flash point of BO takes into account the ignition ability of the BO. Oil is considered to be flammable and there is a risk of danger if its flash point is less than 60 °C. The value of 208 °C obtained for BO in this study showed that the BO is a safe feedstock and easy to handle with no risks. Cloud point is the temperature at which the wax begins to separate when oil is chilled to a low temperature; the value of −5 °C obtained in this study with low pour point showed that the BO is stable and of low wax formation at low temperature. The fatty acid compositions of mixed oil as presented in the table indicated that the oil is mainly made of saturated and monounsaturated acids.

### *8.3.2 Catalyst Characterization and Elemental Analysis*

Figure 8.1a shows the angular SEM images through the developed catalyst from PKSH and FKNH at different wavelengths of 50, 100, and 200 μm performed in the range of $10^{o} < 2\phi < 70^{o}$ at a rate of 2 $min^{-1}$. Observation from the images showed the powder was of various shapes, non-uniform in size, and less porous as the

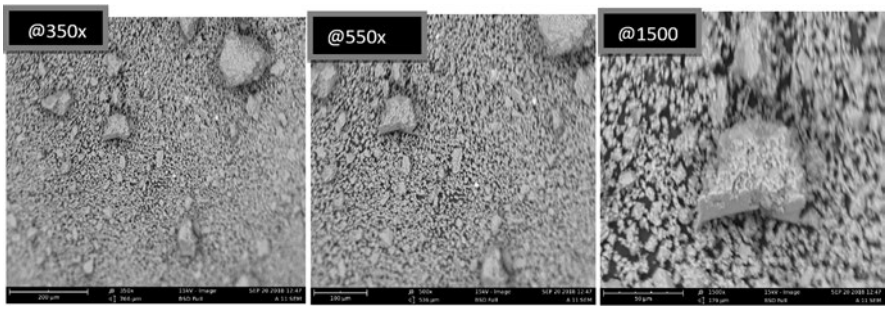


**Fig. 8.1** (**a**) SEM magnification of developed catalyst taken at resolution of 350×, 500×, and 1500×. (**b**) FTIR results of calcined kola nut husk powder (CKNHP) at 800 °C for 3 h

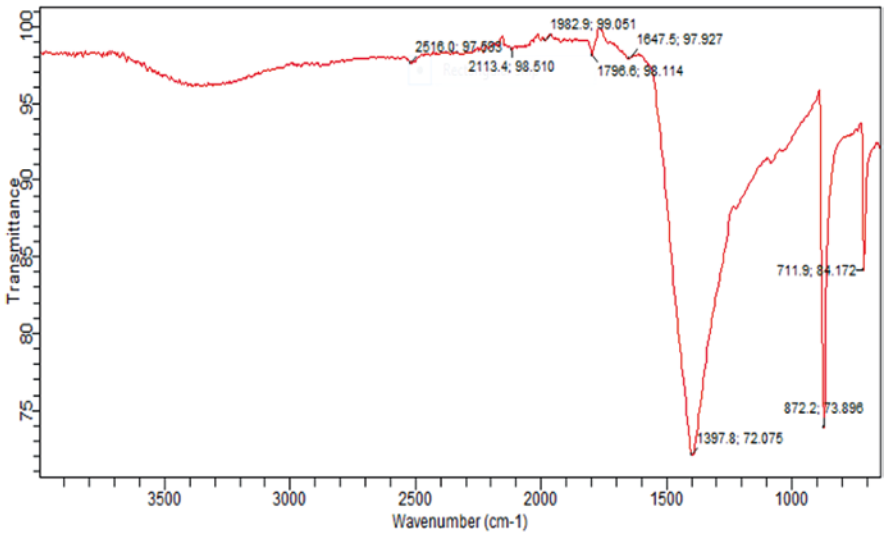


**Fig. 8.1** (continued)

magnification increases. Furthermore, the structure based on resolution showed a glossy nature, permeability, spongy nature of the particle present, and sintering of small mineral aggregates and agglomerated particles responsible for the spongy nature. This observation can be likened to the abundance of calcium and oxygen after calcination which proved that no carbon remains after the heat treatment that resulted in gaseous formation of $CO_2$ via decomposition of calcium carbonate.

Infrared spectroscopy (FTIR) is an amazingly useful materials analysis technique, assisting in detecting both organic and inorganic materials. It measures a sample's absorbance of infrared light at different wavelengths to decide the material's structural and molecular composition. The FTIR works to convert the raw data from the broad-band light source to obtain the absorbance level at each wavelength. The FTIR results of developed catalyst calcined at 800 °C for 3 h is displayed in Fig. 8.1(b) with the infrared spectrum, which plots the intensity of infrared spectra. The peaks (absorbance bands) correspond with the various vibrations of the sample's atoms when it is exposed to the infrared region of the electromagnetic spectrum. The spectrum stretching above 1500 $cm^{-1}$ in the infrared spectrum indicated the presence of two functional groups $CH_2$ and C=O, while the spectrum stretching below 1500 $cm^{-1}$ in the infrared spectrum indicated the presence of two functional groups aldehyde/ketone and ester. The wavelength band observed in the figure from 711.9–1397.8 $cm^{-1}$ stretches indicated the presence of aldehyde/ketone and ester while the spectrum stretching from 1796.6–2516 $cm^{-1}$ indicates the presence of two functional groups $CH_2$ and C=O, and when the wave length above 2526 $cm^{-1}$ suggest the absence of amide, catalytic acid and the presence of O-H.

Table 8.3 shows the elemental analysis results of the developed catalyst obtained using energy dispersive X-ray (EDX-ray). At the temperature of 550 °C, the main

**Table 8.3** Energy dispersive X-ray (EDX-ray) result of elemental analysis of the developed catalyst

| Constituents | | |
|---|---|---|
| Elements | Symbol | % Compositions |
| Calcium | CaO | 71.2 |
| Potassium | $K_2O$ | 14.1 |
| Sodium | $Na_2O$ | 3.0 |
| Phosphorus | $P_2O_5$ | 2.2 |
| Magnesium | MgO | 6.3 |
| Iron | FeO | 1.3 |
| Zinc | ZnO | 1.2 |
| | Other | <1.0 |

**Table 8.4** Properties of sample of catalysts activity calcined at 800 °C for 3 h

| Catalysts | $N_2$-AA ($m^2\ g^{-1}$) | TPV ($cm^3\ g^{-1}$) | %CaO | BS (μmole.$g^{-1}$) 400 < BS < 500 > 500 | | TBS | BSD (μmol/$m^2$) | Bio (wt.%) |
|---|---|---|---|---|---|---|---|---|
| *CPKSH* | 1.00 | 0.003 | 57.50 | 20 | 92 | 112 | 112.00 | 80.34 |
| *CFKNP* | 1.10 | 0.003 | 62.58 | 28 | 102 | 130 | 118.18 | 85.78 |
| *CMCP* | 1.20 | 0.003 | 71.20 | 42 | 116 | 158 | 131.67 | 94.52 |

*$N_2$-AA* nitrogen adsorption analysis, *TPV* total pore volume, *BS* basic site, *TBS* total basic site, *BSD* basic site density

composite is calcium carbonate; when there is an increase in thermal temperature to 650 °C, decomposition of calcium carbonate to form calcium (II) oxide started occurring using a grain model [38]. It was observed that the thermal decomposition of calcium carbonate to calcium II oxide occurred at a higher temperature than that observed by [27, 30]. This observation may be related to the presence of some functional groups present in the kola nut plant during growth period which occurred as a result of carbon growth inhibition. Owing to the abundance of calcium and potassium in the developed catalyst, other metals (Na, Mg, Zn, P, Fe) are less discoursed, but could also help in the transesterification reaction process [39].

Table 8.4 represents sample catalysts indicating the BET surface, total pore volume, basicity, and percentage of CaO converted through $N_2$ adsorption–desorption isotherm Brunauer-Emmett-Teller (BET) analysis. Observation from the results shows the calcined mixed catalyst powder (CMCP) has high total basic site (TBS) and high basic site density (BSD) than the individual catalysts (calcined palm kernel shell husk (CPKSH) and calcined fermented kola nut husk (CFKNH)). However, the BSD and the TBS of CFKNH are higher than that of CPKSH. However, the table also reflects the result of biodiesel yields; at the same process conditions (run 27) tested for each catalyst, the yields of biodiesel based on the nature of catalyst showed that the CPKSH, CFKNH, and CMCP catalyst have high basicity for conversion of mixed oil to biodiesel, but the CMCP produced highest yield (95.52% wt.), due to the percentage of CaO in the catalyst and high basic site density. The

value of biodiesel yield (85.78% wt.) obtained when CFKNP was used (80.34% wt.) was higher than the value obtained when CPKSH was used. This could be due to fermentation process adopted which increases the surface area after thermal treatment, thereby increasing the rate of reaction during transesterification.

## *8.3.3 Conversion of Oils to Biodiesel*

### 8.3.3.1 Oil Blend

The results of mixture of oils (PFO and NO) in proper ratio are displayed in Table 8.5. The initial acid value of NO was 14.66 mg KOH/g oil, having a density of 902 kg/m$^3$ at 40 °C and viscosity of 25.50 mm$^2$/s at 40 °C, while PFO has the initial acid value of 0.85 mg KOH/g oil, having a density of 870 kg/m$^3$ at 40 °C, and viscosity of 23.95 mm$^2$/s, respectively. The results of the blend of the oil are displayed in the table. It was observed that the low viscous oil (21.50 mm$^2$/s) with moderate low density (852 kg/m$^3$) was obtained at $NO_{60}$; the oil is free-flow oil, and with light hydrocarbon and low wax content.

### 8.3.3.2 Esterification of BO

The acid value of the low viscous BO/MO (4.10 mg KOH/g oil) was reduced to minimum value (0.586 mg KOH/g oil) within the reaction time of 1 h, MeOH/OMR of 4:1, and $H_2SO_4$ of 1.00 (v/v). Other methanol-to-oil ratio could further reduce the acid value, but the ratio used in this study is sufficient for successful conversion of esterified BO to a resulting mixed oil biodiesel (MOB) [30].

**Table 8.5** Acid values, densities, and viscosities of different oil blend

| Blends | Acid value (mg KOH/g oil) | Density at 40 °C (kg/m$^3$) | Viscosity at 40 °C (mm$^2$/s) |
|---|---|---|---|
| 100: 0 ($NO_{100}$) | 14.66 | 902 | 25.50 |
| 10:90 ($NO_{10}$) | 13.24 | 890 | 23.00 |
| 20:80 ($NO_{20}$) | 12.82 | 882 | 23.10 |
| 30:70 ($NO_{30}$) | 11.54 | 878 | 23.40 |
| 40:60 ($NO_{40}$) | 9.65 | 875 | 22.50 |
| 50:50 ($NO_{50}$) | 6.60 | 870 | 22.10 |
| 60:40 ($NO_{60}$) | 4.10 | 852 | 21.50 |
| 70:30 ($NO_{70}$) | 3.26 | 854 | 23.40 |
| 80:20 ($NO_{80}$) | 3.01 | 850 | 23.40 |
| 90:10 ($N0_{90}$) | 2.98 | 850 | 22.10 |
| 0:100 ($PFO_{100}$) | 0.85 | 870 | 23.95 |

#### 8.3.3.3 Statistical Optimization of Base-Catalyzed Transesterification

For statistical optimization of base-catalyzed transesterification of BO to biodiesel, build information was developed, design expert version 12.0.3.0 was used, the study type was response surface, the design type was central composite, the design model chosen was quadratic while the build time was 15 min, the subtype was randomized, and runs generated were 30, and these runs were carried out based on four-factor three-level variable and the results are presented in Tables 8.6 and 8.7. From the table, the highest MOB yield of 98.05 (wt. %) was obtained at catalyst amount of 2.0 (g), reaction time of 70 min, reaction temperature of 60 °C, and MeOH/OMR of 7:1 (mL/mL). The results in the table were optimized, based on the constraints

**Table 8.6** Transesterification of mixed oil (MO) to mixed oil biodiesel (MOB)

| Std. runs | $X_1$ | $X_2$ | $X_3$ | $X_4$ | MOB (wt. %) | PMOB (wt. %) | Residual |
|---|---|---|---|---|---|---|---|
| 1 | −1.000 | −1.000 | −1.000 | −1.000 | 83.83 | 83.81 | 0.0158 |
| 2 | 1.000 | −1.000 | −1.000 | −1.000 | 78.58 | 78.65 | −0.0717 |
| 3 | −1.000 | 1.000 | −1.000 | −1.000 | 89.50 | 89.50 | −0.0017 |
| 4 | 1.000 | 1.000 | −1.000 | −1.000 | 82.00 | 89.50 | −0.0017 |
| 5 | −1.000 | −1.000 | 1.000 | −1.000 | 88.15 | 88.23 | −0.0817 |
| 6 | 1.000 | −1.000 | 1.000 | −1.000 | 84.63 | 84.57 | 0.0558 |
| 7 | −1.000 | 1.000 | 1.000 | −1.000 | 90.16 | 90.14 | 0.0158 |
| 8 | 1.000 | 1.000 | 1.000 | −1.000 | 84.08 | 84.15 | −0.0717 |
| 9 | −1.000 | −1.000 | −1.000 | 1.000 | 88.09 | 88.17 | −0.0783 |
| 10 | 1.000 | −1.000 | −1.000 | 1.000 | 86.71 | 86.66 | 0.0492 |
| 11 | −1.000 | 1.000 | −1.000 | 1.000 | 93.93 | 93.92 | 0.0092 |
| 12 | 1.000 | 1.000 | −1.000 | 1.000 | 90.01 | 90.08 | −0.0683 |
| 13 | −1.000 | −1.000 | 1.000 | 1.000 | 96.22 | 96.15 | 0.0692 |
| 14 | 1.000 | −1.000 | 1.000 | 1.000 | 96.00 | 96.15 | −0.1483 |
| **15** | **−1.000** | **1.000** | **1.000** | **1.000** | 98.05 | 98.13 | −0.0783 |
| 16 | 1.000 | 1.000 | 1.000 | 1.000 | 95.84 | 95.79 | 0.0492 |
| 17 | −2.000 | 0.000 | 0.000 | 0.000 | 92.71 | 92.69 | 0.0225 |
| 18 | 2.000 | 0.000 | 0.000 | 0.000 | 85.25 | 85.19 | 0.0625 |
| 19 | 0.000 | −2.000 | 0.000 | 0.000 | 84.08 | 84.03 | 0.0525 |
| 20 | 0.000 | 2.000 | 0.000 | 0.000 | 89.39 | 89.36 | 0.0325 |
| 21 | 0.000 | 0.000 | −2.000 | 0.000 | 87.65 | 87.62 | 0.0325 |
| 22 | 0.000 | 0.000 | 2.000 | 0.000 | 97.80 | 97.75 | 0.0525 |
| 23 | 0.000 | 0.000 | 0.000 | −2.000 | 80.25 | 80.22 | 0.0292 |
| 24 | 0.000 | 0.000 | 0.000 | 2.000 | 96.27 | 96.21 | 0.0558 |
| 25 | 0.000 | 0.000 | 0.000 | 0.000 | 94.32 | 94.49 | −0.1667 |
| 26 | 0.000 | 0.000 | 0.000 | 0.000 | 94.52 | 94.49 | 0.0333 |
| 27 | 0.000 | 0.000 | 0.000 | 0.000 | 94.52 | 94.49 | 0.0333 |
| 28 | 0.000 | 0.000 | 0.000 | 0.000 | 94.52 | 94.49 | 0.0333 |
| 29 | 0.000 | 0.000 | 0.000 | 0.000 | 94.52 | 94.49 | 0.0333 |
| 30 | 0.000 | 0.000 | 0.000 | 0.000 | 94.52 | 94.49 | 0.0333 |

*PMOB* predicted mixed oil biodiesel

**Table 8.7** Predicted and validated MOB and its variable conditions

| Predicted MOB (wt.%) yield | catalyst amount | reaction time | reaction temperature | MeOH/OMR |
|---|---|---|---|---|
| 98.05 (wt.%) | 2.179 g | 57.45 min | 59.91 °C | 5.9:1 (mL/mL) |

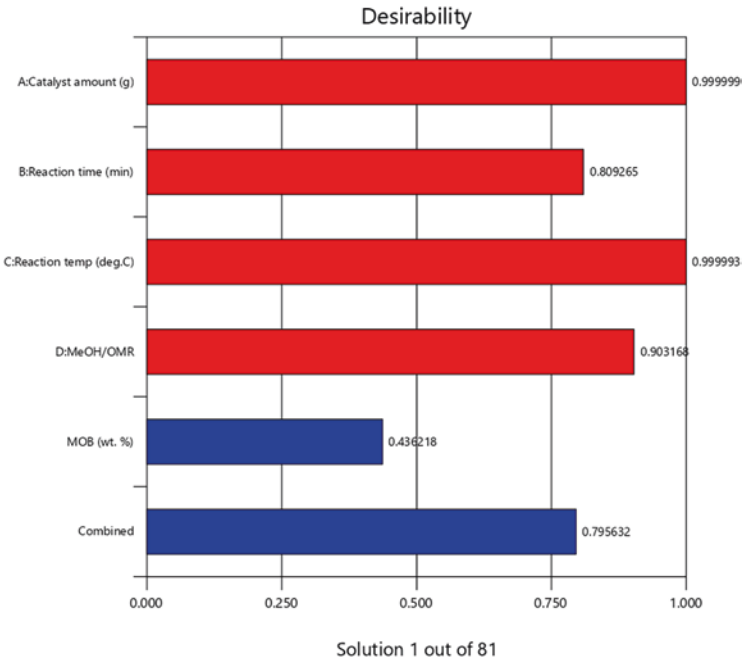


**Fig. 8.2** Variable level of contribution to the response in solution of 81 outcomes

(variable factors), 81 solutions (Fig. 8.2) were randomly provided, and the optimum predicted yield of MOB was 98.05 (wt. %) at catalyst amount of 2.179 (g), reaction time of 57.45 min, reaction temperature of 59.91 °C, and MeOH/OMR of 5.9:1 (mL/mL). The predicted value was validated in triplicate and an average MOB yield of 98.03 (wt. %) was obtained. This showed that the base-catalyzed transesterification of mixed oil to MOB is successive and the CaO derived from PKSH and FKNH proved to be a suitable heterogeneous catalyst for the reaction process.

Furthermore, the results of experimental MOB yield and the selected factors were fitted into second-order polynomial through regression coefficients and significance of response surface quadratic equation. The results of second-order polynomial equations obtained are presented in coded factor in Eq. (8.2) and actual factor in Eq. (8.3).

Final equation in terms of coded

$$\begin{aligned}\mathrm{MOB}(\mathrm{wt.\%}) &= +94.52 - 1.88X_1 + 1.33X_2 + 2.53X_3 + 4.00X_4 \\ &-0.5837X_1X_2 + 0.3763X_1X_3 + 0.9138X_1X_4 - 0.9438X_2X_3 \\ &+0.0163X_2X_4 + 0.8912X_2X_3 - 1.39X_1^2 - 1.95X_2^2 - 0.4510X_3^2 - 1.57X_4^2 \end{aligned} \quad (8.2)$$

Final equation in terms of actual

$$\begin{aligned}\mathrm{MOB}(\mathrm{wt.\%}) &= -121.51391 + 3.52813X_1 + 3.68069X_2 + 2.95221X_3 - 0.403854X_4 \\ &-0.058375X_1X_2 + 0.075250X_1X_3 + 0.456875X_1X_4 - 0.018875X_2X_3 + 0.000812X_2X_4 \\ &- + 0.089125X_3X_4 - 1.38729X_1^2 - 0.019485X_2^2 - 0.018042X_3^2 - 0.391823X_4^2 \end{aligned} \quad (8.3)$$

The coefficient is the direct measure of influence of each variable on the response (MOB). Following the second-order polynomial equation, negative coefficient decreases the response while a positive coefficient increases the response based on Eq. (8.2). All the linear variables have positive influence on the response; however, $X_4$ (4.000) with $F$-value = 48539.79 and $p$-value < 0.0001 is the most positively significant variable among the selected variables (Table 8.8). Furthermore, the **model *F*-value** of 8034.35 implies the model is significant. There is only a 0.01% chance that an $F$-value of this large could occur due to noise. Further analysis proved that all the selected variables considered for MOB production via methanolysis of developed catalyst were remarkably significant except the interaction of $X_2X_4$ (with $p$-value = 0.4760) that was non-significant term. The lack of fit $F$-value of 0.4151 with $p$-value = 1.28 implies the lack of fit is not significant relative to the pure error. There is a 77.68% chance that a lack of fit $F$-value could occur due to noise. Non-significant lack of fit is good [40]. Based on fit statistics and model comparison statistics, the coefficient of determination ($R^2$) of 0.9999 obtained showed the proportion of the variances in the dependent variable that is predictable from independent variable.

Predicted $R^2$ of 0.9994 was in reasonable agreement with the adjusted $R^2$ of 0.9997. Meanwhile the difference is less than 0.200 with high adequate precision greater than 4, which is required for a fit model.

Further process optimization was evaluated through the plot of predicted vs. actual MOB yield; a straight line passing through the points indicated that the predicted values agreed with the experimental results, and the differences is negligible (supplementary file: SF2). Also, the interaction among the variables was tested through the three-dimensional plots. Fig. 8.3(a–f) shows the interactions among the selected variables on the MOB yield. A plot of interaction between the reaction time and catalyst amount on MOB yield is shown in Fig. 8.3(a); Fig. 8.3(b) depicts a plot of interaction between reaction temperature and catalyst amount on MOB yield, while a plot of interaction between reaction time and MeOH/OMR on MOB yield is showed in Fig. 8.3(c). Fig. 8.3(d) shows a plot of interaction between MeOH/OMR and reaction temperature on MOB yield, and Fig. 8.3(e) is the plot of interaction

**Table 8.8** Test of Significance for Every Regression Coefficient for FAME production

| Source | Sum of squares | df | Mean square | *F*-value | *p*-value |
|---|---|---|---|---|---|
| *Model* | 889.10 | 14 | 63.51 | 8034.35 | <0.0001 |
| $X_1$ | 84.38 | 1 | 84.38 | 10674.37 | <0.0001 |
| $X$ | 42.61 | 1 | 42.61 | 5391.06 | <0.0001 |
| $X_3$ | 153.93 | 1 | 153.93 | 19473.27 | <0.0001 |
| $X_4$ | 383.68 | 1 | 383.68 | 48539.79 | <0.0001 |
| $X_1X_2$ | 5.45 | 1 | 5.45 | 689.77 | <0.0001 |
| $X_1X_3$ | 2.27 | 1 | 2.27 | 286.55 | <0.0001 |
| $X_1X_4$ | 13.36 | 1 | 13.36 | 1690.07 | <0.0001 |
| $X_2X_3$ | 14.25 | 1 | 14.25 | 1802.86 | <0.0001 |
| $X_2X_4$ | 0.0042 | 1 | 0.0042 | 0.5345 | 0.4760 |
| $X_1^2$ | 12.71 | 1 | 12.71 | 1607.86 | <0.0001 |
| $X_2^2$ | 52.79 | 1 | 52.79 | 6678.32 | <0.0001 |
| $X_3^2$ | 104.14 | 1 | 104.14 | 13175.02 | <0.0001 |
| $X_4^2$ | 5.58 | 1 | 5.58 | 705.94 | <0.0001 |
| *Residual* | 67.38 | 1 | 67.38 | 8523.77 | <0.0001 |
| *Lack of Fit* | 0.1186 | 15 | 0.0079 | | |
| *Pure Error* | 0.0852 | 10 | 0.0085 | 1.28 | 0.4151 |
| *Cor Total* | 0.0333 | 5 | 0.0067 | | |
| **Fit statistics** | | | | | |
| $R^2$ | | | 0.9999 | | |
| *Adjusted* $R^2$ | | | 0.9997 | | |
| *Predicted* $R^2$ | | | 0.9994 | | |
| *Adeq. precision* | | | 309.8089 | | |

between MeOH/OMR and catalyst on MOB yield, while a plot of interaction between the reaction temperature and reaction time on MOB yield are depicted in Fig. 8.3(f).

From the plots, it was noticed that there were mutual interactive effects of variables on the MOB yield; however, Fig. 8.3b, c and e plots showed perfect interactive effects than Fig. 8.3a, d, f. The interactive effects observed in Fig. 8.3(f) is less than that observed in Fig. 8.3(a and d). These showed that with respect to quadratic equation of second order, single variables played significant roles, making positive contribution to MOB yield than the quadratic and the interactive variables.

#### 8.3.3.4 Properties of MOB

The characteristics of mixed oil biodiesel (MOB), obtained from the transesterifications, of mixture of the oils via derived CaO base catalyzed derived from PKSH and FKNH, along with the data reported for the biodiesel standard ISO 17828, EN 14112; 14,214, and ASTM D97; D664; D2500; D4530; D6079; D6751

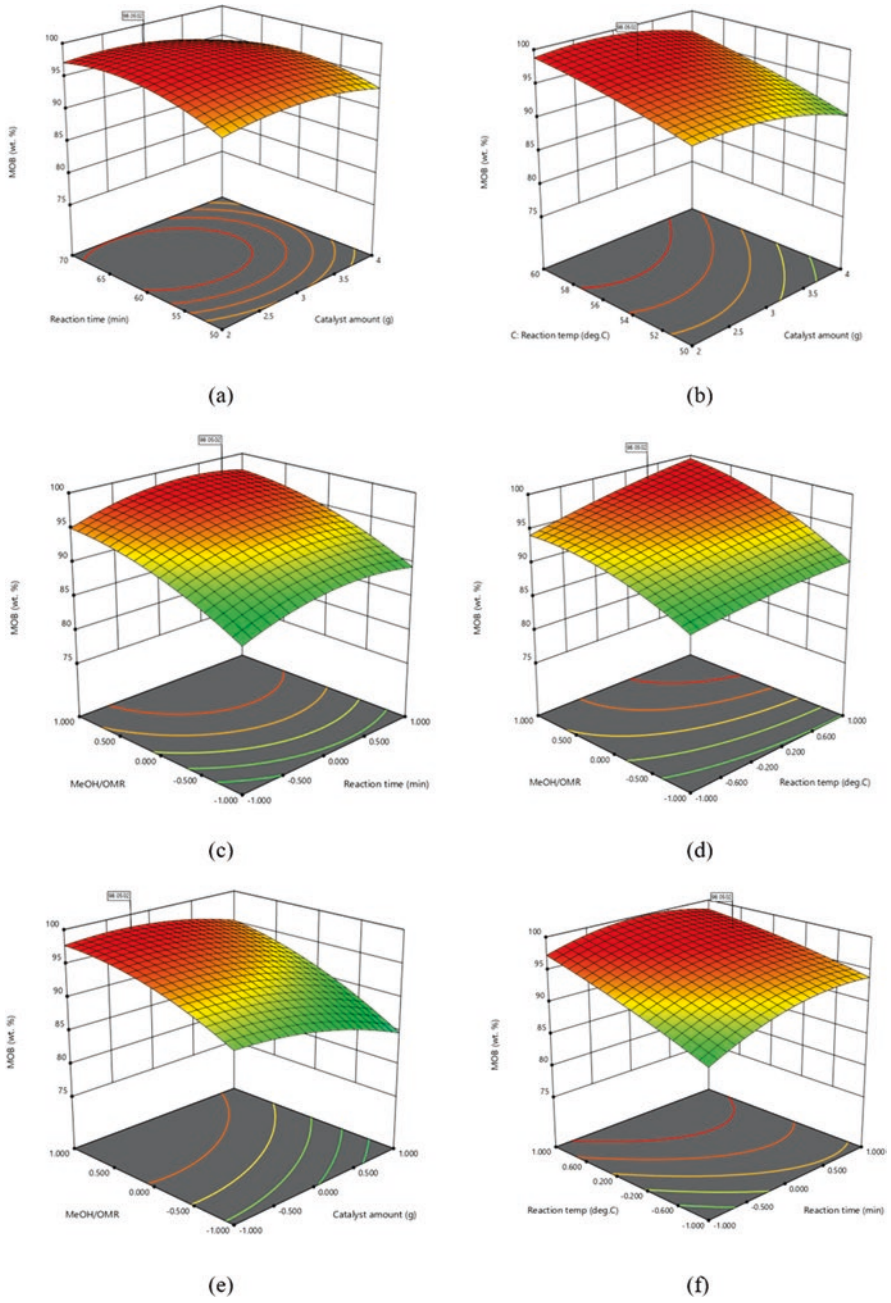


**Fig. 8.3** (**a**–**f**) Three-dimensional plots of interaction among the selected variables

**Table 8.9** Physicochemical and fatty acid composition of MOB

| Parameters | This study | Biodiesel standard | |
|---|---|---|---|
| **Physicochemical properties** | | | |
| Density (kg/m$^3$) at 40 °C | 825.20 | 880 | ISO 17828 |
| Viscosity at 40 °C/(mm$^2$/s) | 2.20 | 1.9–6.0 | ASTM D6079 |
| Moisture content (%) | <0.001 | <0.03 | ASTM D6751 |
| %FFA (as oleic acid) | 0.21 | 0.25 max | ASTM D664 |
| Acid value (mg KOH/g oil) | 0.42 | 0.5 max | ASTM D664 |
| Iodine value (g $I_2$/100g oil) | 47.20 | 120 max | EN 14214 |
| Peroxide value (meq $O_2$/kg oil) | 73.50 | – | Jishy and Sankar [11] |
| HHV (MJ/kg) | 41.00 | – | Chinyere et al. [7] |
| Cetane number | 64.62 | 51 min | EN 14214 |
| Mean molecular mass | 296.92 | – | Chinyere et al. [7] |
| Diesel index | 75.86 | 40.40 min | ASTM D6751 |
| API | 39.97 | 36.95 min | ASTM D6751 |
| Aniline point | 136.65 | 331.0 max | ASTM D6751 |
| Flash point (°C) | 120.00 | 120 min | EN 14214 |
| Cloud point (°C) | +5 | −3 to −12 | ASTM 2500 |
| Pour point (°C) | +3 | −15 to 16 | ASTM 97 |
| Cold filter plugging point (°C) | +2 | +5 max | ASTM D6371 |
| Oxidative stability (h) | 4 | 3 min | EN 14112 |
| Carbon residue (% m/m) | 0.028 | 0.05 max | ASTM D4530 |
| **Fatty acid properties (%)** | | | |
| Palmitic acid (C16:0) | 22.80 | – | – |
| Palmitoleic acid (C16:1) | 1.90 | – | – |
| Stearic acid (C18:0) | 14.00 | – | – |
| Oleic acid (C18:1) | 45.20 | – | – |
| Linoleic acid (C18:2) | 13.80 | – | – |
| Linolenic (C18:3) | 2.10 | – | – |
| Others | 0.20 | – | – |

specifications are presented in Table 8.9. Observation shows that the properties of produced MOB satisfied the biodiesel specifications standard. Main biodiesel physicochemical properties including density, viscosity, acid value, cetane number, higher heating value, diesel index, and API are within the limits recommended by biodiesel standard. The peroxide value, iodine value, saponification value, flash point, pour point, and cold point in the MOB agreed with the values reported by other researchers [7, 11]. Oxidative stability of biodiesel is one of the most important properties of fatty acid alkyl esters and primarily affects the stability of biodiesel during extended storage, compromises fuel properties, and impairs fuel quality and engine performance. The minimum recommended value is 3 h, but the value of 4 h minimum obtained in this showed the MOB is highly stable within a short period. Cold filter plugging point (CFPP) is the lowest temperature at which a

given volume of diesel type fuel still passes through a standardized filtration device in a specific time when cooled under certain conditions. A high CFPP will clog up vehicle engines in cold temperature regions. The maximum recommended value is 5; the value of 2 obtained for CFPP of biodiesel in this study showed the diesel is of good qualities with low temperature clogging effects. The carbon residue value obtained was within the ASTM D4530 specification. Fatty acid compositions of the MOB showed the presence of saturated and monosaturated fatty acid in the MOB.

## 8.4 Conclusion

The mixture of two oils produced low viscous oil at blend level of 60:40 (v/v) NO:PFO. CaO-based catalyst derived from the mixture of PKSH and FKNH is helpful in successful conversion of the blend oil to biodiesel. The predicted biodiesel yield of 98.05 (wt. %) at different variable conditions was in agreement with the validated average optimum biodiesel of 98.03 (wt. %).

Based on XRD analysis result, CaO is the predominant constituent element present in the mixture of two catalysts, but all other elements constitute to the completion of reaction processes. The qualities of MOB were within the specification limits of biodiesel standard which indicates that the developed catalyst successfully convert the mixed oil with 60:40 ratio of NO:PFO to biodiesel.

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# Chapter 9
# Castor Oil: A Promising Source for the Production of Flavor and Fragrance Through Lipase-Mediated Biotransformation

**Suman Singh, Naziya Syed, Shivani Chaturvedi, Ashween Deepak Nannaware, Prashant Kumar Rout, and Yung-Tse Hung**

## 9.1 Introduction: Why Castor Oil?

Castor plant (*Ricinus communis*) belongs to the family Euphorbiaceae and is commonly known as Arandi in Hindi. Castor plants are cultivated in South America, India, and Africa and are widely grown in tropical regions [1]. Brazil, China, and India are the major castor-producing countries in the world [2, 3]. Over 90% of castor oil is exported by India. On the other hand, about 84% of castor oil is imported by the United States, European Union, and China [4]. The oil obtained from castor beans is nonvolatile fatty oil. It is a pale yellow semi-transparent liquid having a high boiling point and density. Castor oil and its derivatives are generally used in making soaps, paints, surface coating, inks, waxes, cold-resistant plastic, biodiesel, and lubricants. The importance of castor oil in the pharmaceutical industry is growing in terms of its use as an anti-inflammatory and antimicrobial agent. It also has good moisturizing properties, due to which it is regarded as safe for hair and skin [5].

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Furthermore, castor oil is also considered as a major raw material for the industrial sector in the development of various products like perfumes and flavors, receiving increased attention nowadays. Castor oil is recognized as a low-priced, biodegradable, and low-toxic oil compared to other vegetable oils. Due to this reason, it has attained an impetus in the food industry in terms of food additives, mold inhibitors, and flavoring agents [5]. Thus, all these characteristics of castor oil drive it toward high market growth and valued products.

Castor oil, rich in ricinoleic acid (RA), is generally used as a substrate for the production of γ-decalactone through biotransformation using efficient microbial catalysts. RA having a long-chain fatty acid composes around 85% of the fatty acids in castor oil [6, 7]. Consequently, it is promising to use castor oil as an economical way and in copious amounts for industrial production of γ-decalactone [8]. Recently, the use of the biotransformation method to produce aroma compounds has achieved significant global attention. This biotechnological conversion method is of low cost to produce flavor compounds. Before the bioconversion process, castor oil is subjected to the hydrolysis process to produce 85% of pure RA-enriched castor oil [9]. Several microorganisms such as *Candida*, *Yarrowia lypolytica*, *Pseudomonas*, and *Rhodotorula* have been reported to potentially produce aroma compounds [10]. RA enters the mitochondria within the cells of microorganism and degrade to 4-hydroxy-decanoyl-CoA after four β-oxidation cycles, which subsequently cyclizes to γ-decalactone in the biotransformation process [11].

The current review mainly focuses on the properties, market value of castor oil, and biotechnology methods to make value-added products from castor oil.

## 9.2 Geographical Distribution of Castor Plants

Castor is a perennial plant adapted to grow in tropical climates with a height of 10–12 m, whereas in temperate regions it is an annual plant with a height of 1–3 m [12]. Castor plants grow well in low humidity seasons and fertile, slightly acidic, and sandy loam soil. The optimum temperature required to for their growth is about 20–25 °C, but a temperature of above 35 °C can badly affect the seed setting. Cold temperature can trigger chilling injury reducing plant growth [13]. In ancient times, nearly about 6000 years ago, castor plants were cultivated for their oil content in Egypt [14]. Castor plants are native to the Ethiopian region of east African tropical regions and considered to be a weed in the south western United States. In India, it grows abundantly on wastelands [15]. India produced 54% while China 23.4% of castor seeds, followed by Brazil, i.e., 11.9%, in 2000–2009. Other countries that produce castor seeds are Mozambique, Thailand, Paraguay, Cambodia, Ethiopia, Indonesia, Madagascar, Peru, Russia, Philippines, Haiti, South Africa, Tanzania, Vietnam, Uganda, Syria, Pakistan, Ecuador, and Colombia [16]. The highest castor-producing states in India are Gujarat (7.02 lakh ha), Rajasthan (1.54 lakh ha), Andhra Pradesh (0.33 lakh ha), Telangana (0.22 lakh ha), and Odisha (0.04 lakh ha).

Narayanpet (6973 ha), Wanaparthy (5567 ha), Mahabubnagar (5104 ha), and Gadwal (2163 ha) are the major growing districts in Telangana.

## 9.3 Castor Oil Scenario

In 2020, the production of global castor market has reached to 740.5 kilotons (https://www.imarcgroup.com/castor-oil-manufacturing-plant). It is anticipated to show stable growth during the next 5 years. Asia Pacific leads the global market in the growth of castor oil due to the constant expansion in the field of pharmaceutical, surface coatings, and cosmetics industries. The largest market for castor oil has been acquired by China itself because of having large geographical area [17]. In the current pandemic (COVID-19) situation, many castor oil-producing entities have been closed down due to the lockdown imposed by the government of different countries, which has badly affected the market growth.

Due to the rising consumption of castor oil, its demand has increased globally at a rate of 7320 tons per year. Based on the increased demand, it is not sufficient to meet the expected rate of castor oil production for supply in the current pandemic situation. Therefore, as the previous trends have shown that castor oil costs and demand will increase gradually and are predicted to reach 1.81 billion by 2020 in the global market [18]. The increasing use of castor oil can be found due to its application in biopolymer and biofuels industries, cosmetics, and pharma industries. Some companies working in the production of castor oil and its derivatives in the global market are Adani Wilmer Ltd., Gokul Agri international Ltd. (GAIL), Jayant Agro Organics Ltd., Kandla Agro and Chemicals Pvt. Ltd. (KACPL), Hokoku Corporation, Thai Castor Oil Industries Co., Liaoyang Huaxing Chemical Co. Ltd., Bom Brazil, ITOH Oil Chemicals Co. Ltd., and Kanak Castor Products Pvt. Ltd. [19].

The international castor oil market was recorded as $1180 million in 2018 and is projected to reach $1470 million by the end of 2025, rising at a CAGR (compound annual growth rate) of 2.8% between 2019 and 2025, according to global reports. India exports castor oil to Europe, Japan, China, and the USA [20]. Due to the trade war between USA and China, the demand from China has been slowed down. Moreover, due to the shortage in the supply of castor oil in 2019, the export rate is less as compared to 2018, when the country had exported 5.5–6 lakh tons of castor oil. In the local market, its price has grown by 27% to Rs 1150 per 10 kg from Rs 900 a year ago, although the local market is very small compared to overseas demand [21].

The castor seed production increased during 2018–2019 because its prices enhanced up to 30% to Rs 54,750 a quintal. However, due to the COVID-19 pandemic across the globe, the prices have dropped to below Rs. 40,000 a quintal. Since then, the prices have improved after the increase of exportation up to 71,900 tons in May. From the commercial point of view, having hydroxylated fatty acid, castor oil is a vital component for the chemical industry worldwide [22].

## 9.4 Composition, Properties, and Applications of Castor Oil

Different methods have been applied for the extraction of castor oil from castor beans, such as mechanical pressing, solvent extraction, or a combination of both. Depending on the geographical distribution, extraction methods, and varieties, castor seeds contain around 44–55% of oil by weight. The alterations in the castor oil properties can be a result of the extraction methods, different varieties, seasonal variations, and type of soil. For example, low iodine value, low acid value, and high saponification value have been reported in cold-pressed castor oil compared to solvent extracted oil. In addition, the physicochemical property and composition have also been observed to be changed due to seasonal variations [23]. Resembling other vegetable oils, castor oil also contains unsaturated and saturated fatty acids attached to a glycerol [24, 25]. The castor oil mixture has a higher content of fatty acids, ricinoleic acid of around 90%, and other constituents in small amounts, as given in Table 9.1. The commercial value of castor oil has increased due to the presence of the high content of ricinoleic acid (RA), which can be utilized in various applications in the chemical industry.

Castor oil is colorless to pale yellow thick liquid with a unique taste, having a mild odor, and have a boiling point at 586 K [26]. The distinctive property of castor oil is due to the occurrence of the hydroxyl group in ricinoleic acid, which makes it complementary with plasticizers of a broad range of synthetic and natural resins, polymers, waxes, and elastomers [27]. For example, the oil has high specific gravity and viscosity; it has little solubility in aliphatic petroleum solvents while highly soluble in alcohols [28]. The physicochemical properties of castor oil are given in Table 9.2. In comparison to other oils, castor oil has the highest viscosity and is reported to be different in other parameters like cloud point, flash point, etc. As compared to standard engine oil (SAE 40), the values of thermal conductivity, pour point, viscosity, and density of castor oil were found to be higher [32].

Castor oil and its derivatives play a vital role in the production of soaps, lubricating agents, coating, pharma products, paints, plasticizers, food, pesticide, perfumery, purgative, disinfectant, and germicidal agents [33]. Its high ricinoleic acid content allows its ready derivatization through the presence of the hydroxyl group. Biodiesel is also produced through the transesterification process. Due to these multiple applications, it is considered an industrial crop. Industrial uses of castor oil are given in Table 9.3.

## 9.5 Castor Oil Processing Techniques for Aromatic Compounds

As compared to other vegetable oils, castor oil is considered the most suitable raw material for industrial purposes to convert various high value-added products. Due to the presence of the high amount of RA in castor oil along with the occurrence of

**Table 9.1** Chemical composition of castor oil [23]

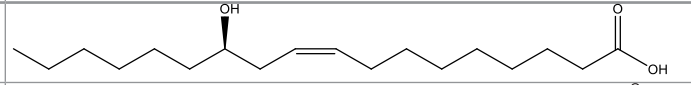
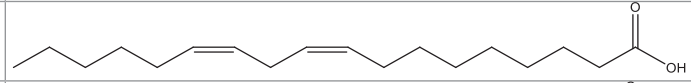
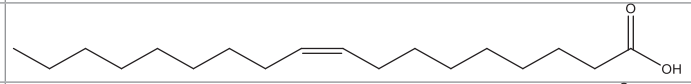
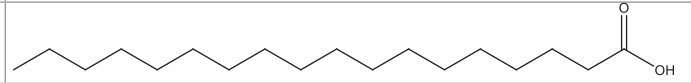
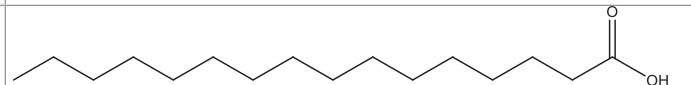
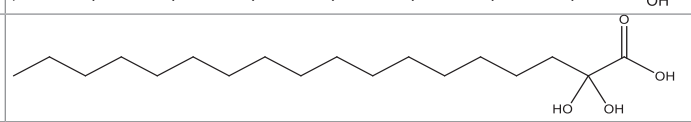
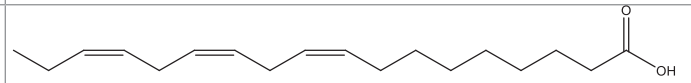
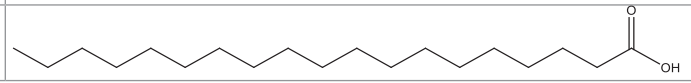
| Composition | Percentage | Chemical formula | Structure |
|---|---|---|---|
| Ricinoleic acid | 89.5 | $C_{18}H_{34}O_3$ |  |
| Linoleic acid | 4.2 | $C_{18}H_{32}O_2$ |  |
| Oleic acid | 3 | $C_{18}H_{34}O_2$ |  |
| Stearic acid | 1 | $C_{18}H_{36}O_2$ |  |
| Palmitic acid | 1 | $C_{16}H_{32}O_2$ |  |
| Dihydroxystearic acid | 0.7 | $C_{18}H_{36}O_4$ |  |
| Linolenic acid | 0.3 | $C_{18}H_{32}O_2$ |  |
| Eicosanoic acid | 0.3 | $C_{20}H_{40}O_2$ |  |

**Table 9.2** Physicochemical properties of castor, jatropha, and pongamia oil

| Physical properties | Castor oil [29] | Jatropha oil [30] | Pongamia oil [31] |
|---|---|---|---|
| Density (Kg/m$^3$) | 899–955 | 889.27–910 | 924 |
| Viscosity kinematic 40 °C (mm$^2$/s) | 15.25 | 33.86 | 40.2 |
| Thermal conductivity (W/m°C) | 4.727 | 0.09 | – |
| Oxidation stability (110 °C, h) | 1.1 | – | – |
| Specific heat (kJ/kg/K) | 0.089 | 4.73 | – |
| Cloud point (°C) | −13.4 | 2 | 3.5 |
| Flash point (°C) | 260 | 144.67–292 | 225 |
| Pour point (°C) | 2.7 | 2.67 | −3 |
| Refractive index | 1.480 | 1.48 | – |
| Lipid content (%) | 43.30 | – | – |
| Moisture content (%) | 0.20–0.30 | – | – |
| Acid value (mg/g) | 2.07–14.80 | 3.38 | 5.40 |
| Iodine value (mg/g) | 58.39–84.50 | 187.3 | 87 |
| % Free fatty acid | 3.4–7.4 | 1.70 | – |
| Saponification value (mg/g) | 175–182 | – | – |
| Peroxide value (mg/kg) | 10–158.6 | – | – |
| Fire point (°C) | 256 | – | – |
| Smoke point (°C) | 215 | – | – |
| pH | 5.8–6.2 | – | – |
| Congealing temperature (°C) | 18 | – | – |

hydroxyl and carboxyl groups and double bonds, it has more potential and versatile applications in various industries [14]. It is also used in the formation of aroma compounds where the oil needs to undergo a biotransformation process to obtain value-added aroma compounds by using microbial catalysts. Before the biotransformation method, the castor oil undergoes chemical hydrolysis to purify the RA for maximum conversion of aroma compounds. Cosmetics and pharmaceuticals accounted for over 25% of total market volume in 2013 and was the largest application segment. Growing demand for bioingredient-based cosmetics would continue to remain a key driver for this segment. Castor oil and its derivatives are used in a number of industries for the production of a wide variety of products. It is utilized as a raw material in the production of a number of chemicals, which are further used in the fabrication of surfactants, soaps, cosmetics, surface coatings, pharmaceuticals, perfumes, plasticizers, greases, lubricants, and rubber (Table 9.3). Its basic derivatives, undecylenic acid and heptaldehyde, are used to produce various perfumery compounds [41]. In pharmaceuticals and cosmetics, it is used as an ingredient in various formulations. The demand of around 4% of flavors and fragrance from castor oil per annum is estimated globally. In Asia/Pacific region, the demand for flavors and fragrances is estimated to be rising at a rate of about 7% per annum through 2008. The current research studies mainly focus on the development of flavor and fragrance from natural sources and have a preference to utilize raw materials that are safe and biodegradable. In light of this fact, there is an excellent possibility for castor oil derivatives in the international market.

**Table 9.3** Applications of castor oil and its derivatives

| Products | Application | References |
|---|---|---|
| Fuel and biodiesel | Fatty acid methyl esters of castor oil when blended with diesel fuel work as biodiesel | Bello and Makanju [34] |
| Polymer materials | Castor oil was cross-linked and polymerized with sulfur or di-isocyanates to produce the vulcanized and urethane derivatives, respectively; plasticizers, coupling agents, plastic films, polyols | Yenwo et al. [35] |
| Lubricants | Castor oil has been utilized to produce soaps, wax, hydraulic fluids, corrosion inhibitors, aircraft lubricants, jet engine and racing car lubricants, and greases in some studies | Burt and Mealy [36]; Lehrer et al. [37]; Dwivedi and Sapre [38] |
| Fertilizers | Mixture of castor cake and castor husks used as fertilizer supported significant plant growth up to the dose of 4.5% of meal | Lima et al. [39] |
| Coatings and paints | Castor oil can be successfully dehydrated by nonconjugated oil–maleic anhydride adducts to provide constructive paint or furniture oil applications; plasticizer for coatings, lacquers, and varnishes | Grummitt and Marsh [40] |
| Textile chemicals | Dyeing support, nylon, synthetic fibers, resins, synthetic detergents, pigment wetting agents | Ibeagha [5] |
| Pharmacological and medicinal use | Act as antidiarrheal agent, antihelmintic, antidandruff, cathartic, emollient, also used a drug delivery vehicle | Ibeagha [5] |
| Cosmetics and perfumery | Used in hair tonic, shampoos, emulsifiers, polishes, deodorants, lipstick | Ibeagha [5] |
| Food | Used as flavorings, surfactants, viscosity reducing additives | Ibeagha [5] |

### *9.5.1 Hydrolysis and Purification of Castor Oil to Form Ricinoleic Acid*

Some chemical and biochemical methods have been performed for the isolation of RA from castor oil. RA and glycerol have been yielded upon hydroxylation of the ester linkages in the triglyceride molecules. Under chemical methods, castor oil hydrolysis reaction is usually performed by the gradual addition of 80% sodium hydroxide in castor oil for 1 h, and fatty acids are formed. The fatty acids are released by adding distilled water and acidified with concentrated hydrochloric acid. Further fatty acids are extracted in ethyl acetate and dried over magnesium sulfate. Clearing up of fatty acids is performed by addition of n-hexane (1:5 w/v) and kept at −4 °C for 3 days in darkness. This reaction produces ricinoleic acid and glycerol. The purity of RA is 88%, and the rest are the palmitic, stearic, oleic, vaccenic, linoleic, and linolenic acids.

Another method to isolate RA from castor oil is the use of biocatalysts such as lipase enzymes. Some lipase-producing microbes such as *Geotrichum candidum*, *Candida rugosa*, and *Pseudomonas cepacia* have been reported to be used for hydrolysis of castor oil [42]. In this study, the hydrolysis of oil was performed in a reaction including oil (100 mg), 0.5 M phosphate buffer (pH 7) (600 μL), and about 2–5 mg of lipase enzyme at 30 °C at 500 rpm for 1–4 h in an incubator shaker. The level of hydrolysis was assessed by titration method against 0.1 N NaOH solutions (pH 12), taking the hydrolysis mixture in diethyl ether:ethanol:water solution (3:3:2). In this reaction, *P. cepacia* lipase showed successful conversion of castor oil to RA up to 27% in comparison to other microbial enzymes like *C. rugosa* and *G. candidum* that are recorded to be 13%. Another study also exhibited that when immobilized *C. rugosa* was applied for the lipolysis of castor oil, the yield of RA was about 20–40% under controlled conditions such as pH, temperature, and the quantity of enzymes and substrate [43]. One more remarkable study was done by Piazza and Farrell [44] on hydrolysis of castor oil using ground oats (*Avena sativa* L.) lipase, which has shown a conversion of 90% of RA.

Another green method using microwave-assisted extraction of RA from castor oil has been described [45] wherein hydrolysis of castor oil was done by ethanolic KOH solution kept in a microwave oven with a few pieces of ice. A water condenser and a magnetic stirrer were connected in the microwave oven and heating for 19 min was given continuously for the reaction, which has yielded 89% of RA. The above-mentioned methods for the separation of RA from castor oil were good for RA yield while using different catalysts and heat sources.

### *9.5.2 Chemical Transformation of Castor Oil*

Due to the presence of long-chain hydrocarbon, OH, and COOH groups and double bonds in RA, there are lots of possibilities of modifications and transformations of the castor oil into a number of value-added products. Some chemical reactions have been performed, for instance, esterification [46] and amidation [24], leading to transformation due to the presence of carboxylic group, while double bond allows the transformation of the oil through a number of reactions like carbonylation [47], hydrogenation [24], and epoxidation [48]. In addition, the presence of OH group also drives the chemical reactions of dehydration [49] to remove the hydroxyl group and acetylation [48] and alkoxylation [50] to enhance the unsaturation of the oil. A new double bond formation occurs in the chain of ricinoleic acid upon catalytic dehydration reaction, which results in good color maintenance, fast drying, and water resistance for protective coatings [49]. Further heating at 249.85 °C in the presence of NaOH, capryl alcohol (2-octanol) and sebacic acid (a 10-carbon dicarboxylic acid) are formed. Both capryl alcohol and sebacic acid have several applications [51]. A study has reported that when a co-solvent system involving ethanol and isopropyl ether (35:65; v/v) applied to castor oil produced a purified RA (about 98.6% purity). The recovery of RA was recorded at about 70%, and the total yield of 55.5 ± 2.5% was obtained [51].

### 9.5.3 *Biotransformation of Castor Oil to Form Enriched Flavored Products*

The synthesis of aroma compounds can be done by the biotechnological methods following two important techniques: one is to use biotransformation method, and another is via de novo synthesis. Complex molecules are synthesized from simple molecules (sugars, amino acids, nitrogen salts, minerals, etc.) using intricate metabolic pathways of organisms, and this is defined as the de novo synthesis method. Biotransformation refers to the formation of value-added products from structurally similar substrates in a single reaction catalyzed enzymatically (microbial cells or pure enzymes) [52]. Biotransformation or bioconversion is considered a greener, cheaper, and commercial approach in comparison to de novo synthesis methods for the production of bioflavors [53]. In 1950, microbial production of blue cheese-note compounds was discovered the first time, and since then, numerous bio-based aroma compounds have been developed during the time [54].

γ-Decalactone is a peach-like aroma compound extensively used in food and beverages, which is the cause of the big interest in its biotechnological production [55]. To enhance the accessibility of ricinoleic acid to cells, castor oil can be hydrolyzed by lipases [56], generating esters such as methyl ricinoleate [55]. The process comprises the breakage of ricinoleic acid into 4-hydroxy-decanoic acid, a precursor of γ-decalactone, which is achieved by the process of peroxisomal *β*-oxidation enzymes [57].

#### 9.5.3.1 Lipase-Mediated Biotransformation

A variety of microbial strains are able to convert substrates into valuable aroma compounds, and the examples of some microbial strains involved in the bioconversion process are given in Table 9.4, which demonstrate the potential of microbes for the synthesis of aroma compounds. Some microorganisms such as *Pseudomonas*, *Candida*, *Rhodotorula*, and *Yarrowia lipolytica* have been reported for higher productivity of aroma compounds through biotransformation [10]. Lactones are well recognized for their wonderful flavor and fragrance (like pineapple, peach, raspberry, apricot, mango, coconut, papaya, grapes), which are produced industrially hundreds of tons yearly [64]. γ- and δ-lactones are the highly valuable five- and six-membered rings, respectively, with 12 carbons equal or less. It comprises compounds like γ-decalactone/4-decanolide (peach-like) [65], 4-dodecanolide (fruity-coconut like) [66], 4-octanolide (sweet herbaceous coconut-like) [67], 5-decanolide/δ-decalactone (creamy-coconut peach-like) [68], 5-dodecanolide (fruit-oily peach-like) [69], and 6-pentyl-α-pyrone (6PP, strong coconut-like) [70]. Although lactone can be synthesized chemically from keto acids, production of δ-decalactone (DDL) and γ-decalactone (GDL) through the biotransformation method is growing currently due to their generally recognized as safe (GRAS) values reaching between US$ 1400 $kg^{-1}$ and US$ 6000 $kg^{-1}$ [71]. However, the prices reduced up to US$ 300

per kilogram due to the growing production of both lactone compounds over the years [65].

Castor oil is generally utilized as a substrate to produce γ-decalactone by yeast cells through β-oxidation cycles of fatty acids recurring in the cells [11, 72]. However, prior to entering the biotransformation process, the castor oil is hydrolyzed to get refined castor oil having 86% of ricinoleic acid as the major component [9]. In the microbial cells, RA inserts inside the microbial cells, and after recurring four β-oxidation cycles, RA breaks into 4-hydroxy-decanoyl-CoA, which subsequently cyclizes to γ-decalactone [11]. In a study, it has been observed under bioconversion reaction that γ-decalactone concentration was 52.9 and 62.4 mg/L from 2.5% castor oil and 1.5% RA, respectively [73].

Currently, nonconventional oleaginous yeast, *Yarrowia lipolytica*, is getting more importance in the bioconversion of castor oil or RA into γ-decalactone. Six-member family of acyl-CoA oxidases (Aox) encoded by POX1 to 6 gene, which plays a vital role in β-oxidation of fatty acids, is present in *Y. lipolytica* [74]. This four-step procedure involves mainly two oxidation steps, one hydration, and another cleavage reaction, which are catalyzed by three enzymes. At each cycle, the compound confers two-carbon shorter metabolite and an acetyl group [75]. One more study has reported about Aox family and revealed that Aox2 is long-chain specific (C18 to C10), whereas Aox3 is short-chain specific (C10 to C4) [74, 76, 77], both showing strong activity [60]. Furthermore, some other studies utilized modified strains of *Y. lipolytica* W29 (POX2 overexpressed and POX3–5 disrupted) and were competent to make more lactone without any degradation [60, 76].

It has been illustrated that extracellular lipases, specifically the endogenous lipase of *Y. lipolytica* (W29) and extracellular lipases (Lipozyme TL IM), were able to release ricinoleic acid from castor oil, and as a result, γ-decalactone produces rapidly [56, 78]. However, this method is not much appropriate as per industrial approaches since it is time- and cost-consuming. Therefore, to fill the gap of this problem, overexpression of the Lip2 enzyme would improve the production rate of γ-decalactone with no additional expenses. This was further investigated by Braga et al. [59] that *Y. lipolytica* (JMY3010) has the ability to synthesize extracellular lipase due to the presence of an additional copy of LIP2 gene necessary for the production of γ-decalactone. From a biotechnological point of view, gene expression studies during bioconversion of RA into γ-decalactone are still very less; here, a few studies involving upregulation and downregulation or disruption of some genes that play an important role in γ-decalactone production are given in Table 9.4.

#### 9.5.3.2 Bioconversion of Ricinoleic Acid (RA) to γ-Decalactone (GDL)

The most essential determining factor to produce γ-decalactone and 3-hydroxy-γ-decalactone is β-oxidation pathway in microbial cells, which was first discovered by Okui et al. [79]. It is four-step reaction sequences, yielding an acyl CoA, which has two carbons less and an acetyl-CoA. This reaction recurred many times until the total breakdown of the compounds. Lactonization can take place at the C10 stage

**Table 9.4** Various genes involved in the improved production of γ-decalactone

| Strain | Substrate | Overexpressed gene | Disrupted/ underexpressed gene | γ-decalactone | Reference |
|---|---|---|---|---|---|
| *Y. lipolytica* MTLY40-2P | Castor oil | Aox2p, Lip2p | POX2–5 | Increased the γ-decalactone production rate | Braga et al. [58, 59] |
| *Y. lipolytica* | Methyl ricinoleate | *POX2* | POX3 | No re-consumption of γ-decalactone | Guo et al. [60] |
| *Yarrowia lipolytica* TA1 | – | *CRF1* | *POX3* | γ-decalactone production | Guo et al. [61] |
| *Escherichia coli* | Peach fruit | PpAAT1 | – | Catalyze the formation of γ-decalactone | Peng et al. [62] |
| Mono disrupted *Yarrowia lipolytica* | Methyl ricinoleate | Pox3 | – | Formation of γ-decalactone | Waché et al. [63] |

resulting in the formation of strong fruity notes [80]. Under anoxic conditions, the activity of 3-hydroxy-acyl-CoA dehydrogenase is minimized, because regeneration of its cofactor ($NAD^+$) is not enough. When the regeneration of NAD is not enough, lactone accumulation takes place because a reduction in the β-oxidation flux decreases the requirement for NAD and thus the cofactor is not necessary any longer, a limiting compound for the pathway. This phenomenon also happens when the aeration of cells is changed, and this accumulation can be a signal of upscaling complexities in industry. 3-Hydroxy lactone is released when aeration is reduced with the declination in the β-oxidation pathway flux, and as a result, NAD is again regenerated in plenty amount and 3-hydroxy lactone does not accumulate [81, 82]. A variety of microbial cells have been isolated from different sources by various researchers for the bioconversion of lactones from different substrates (Table 9.5).

A study on bioconversion of methyl ricinoleate into γ-decalactone was carried out using *Pichiu guilliermondii* through the peroxisomal beta-oxidation pathway [102]. This study showed that enzymes of peroxisomes and peroxisomal β-oxidation, i.e., catalase and acyl-CoA oxidase activities, respectively, were stimulated by methyl ricinoleate. The activity of acyl-CoA oxidase increased approximately 40 times when this yeast was transferred in glucose medium in which fatty acid methyl ester act as the only carbon source. The acetyl-CoA molecules are afterward moved from the peroxisomes to the mitochondria by the acetyl-carnitine-transferase system. It has been reported that peroxisomal β-oxidation has a detoxification role because the peroxisomal reaction occurs in the absence of a mitochondrial respiratory chain involved in fatty acid consumption [103]. On the other hand, due to the accumulation of hydroxy fatty acids in the culture medium, β-oxidation process appears to be obstructed. Current research studies have focused on gene function that encodes acyl-CoA oxidase isozymes, oxygen mass transfer rates, lipid

**Table 9.5** Biotransformation of various substrates using different strains in lactone

| Strain | Substrate | Product | Conversion yield (%) | References |
|---|---|---|---|---|
| *Waltomyces lipofer* | 10-Hydorxystearic acid | γ-Dodecalactone | 76.1 | An et al. [83] |
| *Waltomyces lipofer* | 10-Hydorxystearic acid | γ-decalactone | 46 | An and Oh [84] |
| *Rhodotorula aurantiaca* | Castor oil | γ-Decalactone | 33 | Alchihab et al. [85] |
| *Yarrowia lipolytica* HR145 | Castor oil | γ-Decalactone | 22.7 | Rabenhorst and Gatfield [86] |
| *Saccharomyces cerevisiae* | Grape juice | γ-Butyrolactone | Not reported | Carrau et al. [87] |
| *Yarrowia lipolytica* | Ricinoleic acid (castor oil) | γ-Decalactone | | Pagot et al. [88] |
| *G. fragrans and Geotrichum sp.* | Ricinoleic acid (castor oil) | γ-Decalactone | 600 mg $L^{-1}$ | Neto et al. [89] |
| *Ceratocystis moniliformis* | Olive press cake | δ-Decalactone (coconut), γ-decalactone | Not reported | Lanza et al. [90] |
| *S. salmonicolor* | Castor oil hydrolyzate | γ-Decalactone | 54.6 mg/L | Lee et al. [91] |
| *Sporidiobolus ruinenii* | Ricinoleic acid | γ-Decalactone | 0.39 mg/L/day | Dufosse et al. [92] |
| *Yarrowia lipolytica* | 10-Hydroxystearic acid | γ-Dodecalatone | 24.3 | Farbood et al. [93] |
| *Mortierella isabellina* | Dodecanoic acid | γ-Dodecalatone | 21.4 | Han and Han [94] |
| Baker's yeast | 10-Hydroxystearic acid (0.5) and oleic acid | γ-Dodecalatone | 22.5 | Gocho et al. [95] |
| *Sporobolomyces odorus* | Oleic acid | γ-Dodecalatone | <14.0 | Haffner and Tressl [96] |
| *L. saturnus* CCMA 0243 (pH 5), *Y. lipolytica* CCMA 0242 (pH -6.0) | Castor oil | γ-Decalactone | 512.5 mg/L, 214.8 mg/L | Pereira de Andrade et al. [97] |
| *Saccharomyces cerevisiae* MF013 | Ricinoleic acid | γ-Decalactone | 19.5 | Rong et al. [98] |
| *Candida boidinii* | Safflower oil | γ-Docecelactone | 25 | Jo et al. [99] |
| *Yarrowia lipolytica* | Castor oil | γ-Decalactone | 0.061 | Farbood and Willis [100] |
| *Yarrowia lipolytica* | Castor oil | γ-Decalactone | 0.0613 | Gomes et al. [9] |
| *Geotrichum fragrans* | Castor oil | γ-Decalactone | 0.012 | Neto et al. [89] |
| *Penicillium roqueforti* | Soybean oil | γ-Docecelactone | 0.00036 | Chalier and Crouzet [101] |

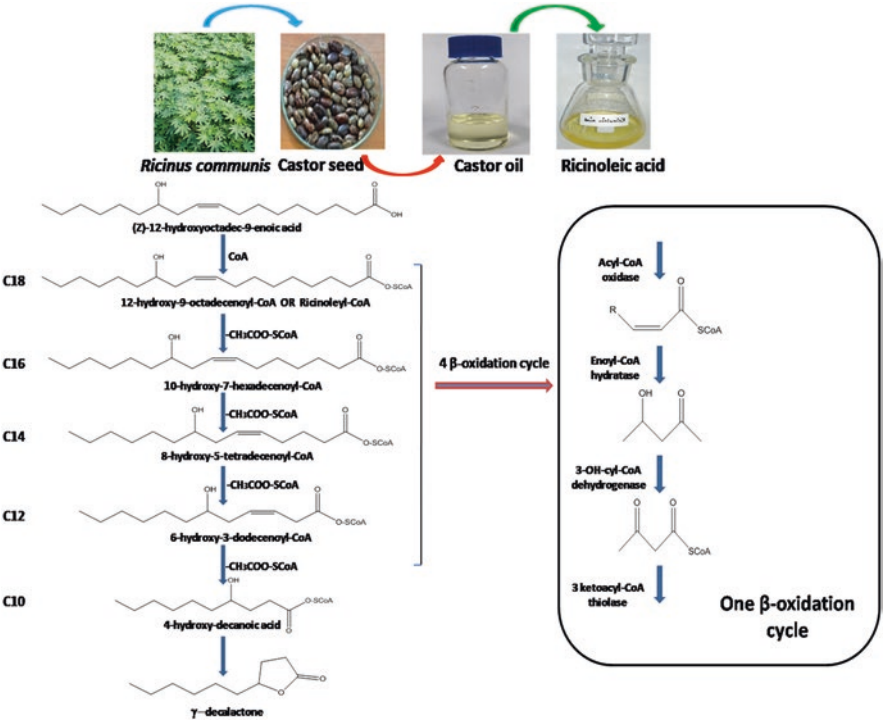



**Fig. 9.1** The biosynthetic pathway of γ-decalactone (C10) from RA (C18) and the enzymes involved in the yeast β-oxidation cycles in the biotransformation process

metabolism, interactions of cell–substrate to increase the production of γ- decalactone, and selection of overproducing mutants. β-Oxidation is a four-reaction cycle that shortens the carbon chain by two after catalyzed in peroxisomes by the following enzymatic activities: acyl-CoA oxidase, 2-enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase, and 3-ketoacyl-CoA thiolase (Fig. 9.1). A 10-carbon compound (4-hydroxydecanoic acid) is obtained from the 18-carbon chain in the last reaction, which is then cyclized to γ-decalactone. The products obtained from biotransformation reaction also fluctuate according to the precursor fatty acid: ricinoleic acid is degraded to an 8-carbon acid, whereas homoricinoleic acid degradation is guided to synthesize an intermediate of 11-carbon. The uncertainty of hydroxyl fatty acid oxidation relies both on the carbon chain length and hydroxy group position [104].

Even though some attempts have been made so far, the products yields are too low; therefore, additional studies are required to overcome the constraints observed at present to make the biotechnological process more useful. The necessary step toward higher production of aroma compounds is mainly done by focusing on the optimization attempts on fermentation technologies and downstream processes.

#### 9.5.3.3 Optimal Conditions for Biotransformation

The biotransformation process is dependent on various parameters such as temperature, pH, time, enzyme amount (microbial cells), substrate concentration, and types of additives for γ-decalactone production. The first parameter is carbon source (substrate such as castor oil or ricinoleic acid) that acts as an activator of enzymes in the pathway of γ-decalactone production. The second parameter is nitrogen sources comprising peptone and yeast extract that are imperative to enhance cell growth and biotransformation process. The last factor is pH, which should be optimized to maintain the highest cell growth and γ-decalactone production.

##### 9.5.3.3.1 Effect of Carbon Source

Synthesis of lipase, which is responsible for the bioconversion of castor oil into lactones, can be induced by the carbon sources in the culture medium. Some examples of carbon sources are carbohydrates, glycerol, organic acids, oils or fats, and other alcohols [105]. Some oils such as soybean oil, olive oil, oleic acid, and tributyrin have been observed to be effective in inducing lipase activity [106, 107]. For instance, the use of oleic acid exhibited increased production of lipase and biomass content in comparison to other fatty acids of different carbonic chains [108]. Another study showed that vegetable oil possessing linoleic and oleic acids was found superlative in lipase production from *Candida* species [109]. Olive oil used as a carbon source was also found to induce lipase activity by *Y. lipolytica* [110].

##### 9.5.3.3.2 Effect of Nitrogen Source

Like carbon source, nitrogen source is also a chief factor influencing the synthesis of lipase enzymes. Both the inorganic and organic nitrogen have an essential role in the synthesis of the enzyme [109, 111]. Yeast extract, malt extract, peptone, amino acids, urea, nitrate and ammonium salts, agroindustrial waste, such as water soy flour and corn, are nitrogenous compounds generally used for lipase production [111, 112]. The selection of nitrogen compounds mostly depends on the microorganism used and the addition of other ingredients in the culture medium [112]. Nitrogen sources are the necessary component for cellular physiology because they provide vitamins and amino acids as enzyme cofactors [113]. For instance, cell growth and lipase synthesis were found to be increased by the addition of yeast extract and tryptone. In a study, various nitrogen sources have been examined on the growth of *Y. lipolytica* for lipase synthesis, and it was observed that mineral nitrogen did not show any significant effect on the growth of yeast possessing lipase activity [114]. Tryptone N1 (a casein hydrolysate) showed maximum production of lipase compared with other nitrogen-containing organic or mineral substrates [105].

#### 9.5.3.3.3 Effects of Temperature

The temperature influences the growth parameters of microbes, such as the specific growth rate and the adaptation time (lag phase), and affects the biosynthesis of primary and secondary metabolites [115]. It has been observed in a study that lipase production by *Y. lipolytica* (681) was most influenced by the temperature [116]. The optimum temperature for significant lipase activity was 29.5 °C.

#### 9.5.3.3.4 Effect of pH

Microbial growth can be affected by the hydrogen ion concentration in a culture medium indirectly, affecting nutrient accessibility or directly acting on cellular surfaces [115]. It is reported that under low pH, the synthesis of lipase by liquid substrate fermentation, with diverse microorganisms, is highest at the end of cultivation [117, 118]. Mooradi et al. [73] have optimized that under acidic pH, nitrogen source, and increased amount of yeast extract and castor oil, the production of γ-decalactone will be enhanced. Other researchers have also observed that acidic pH is more suitable for bioconversion of RA into 4-hydroxydecanoic acid and then γ-decalactone [57, 85, 91, 119].

### 9.5.3.4 Significance of Bioreactors

Bioreactors are devices for biotransformation or any fermentation process in which biological or biochemical reactions are performed under closely monitored and controlled conditions. A number of different types of bioreactors have been used for fermentation methods. Among these, two are commonly categorized as solid-state or stirred-tank bioreactors with two different fermentation methods, such as SSF and SmF, respectively. For research and development, both SmF and SSF have been employed for the production of bioflavor compounds. Approaches for the development of new types of reactors are going on continuously for special purposes [120]. In SmF type of bioreactor, mostly the reactions of microorganisms and substrate are performed in a liquid medium and the products produced by microbial enzymes reaction are recovered from the liquid medium and purified. Gas/air mixture required for fermentation reaction is delivered to a culture medium in sterilized environments. During the bioconversion process in bioreactors, optimized conditions for pH, temperature, aeration, and foam sensors are needed. Mechanical agitation is required for mixing and bubble dispersion. In addition, nutritional factors such as carbon and nitrogen sources are also important for the growth and synthesis of flavor compounds in lab as well as industrial scale [81, 121]. Several researchers have studied the microbial conversion of bioflavor compounds by SmF-type bioreactors [122–126].

De novo synthesis or transformation methods are generally performed in solid-state fermentation (SSF) system for the aroma compound production using various yeasts and fungi such as *Kluyveromyces marxianus*, *Ceratocystis fimbriata*, *Moniliella suaveolens*, *Trichoderma harzianum*, *Pityrosporum ovale*, *Ceratocytis oniliformis*, *Aspergillus niger*, and *Rhizopus oryzae* [127–131]. Furthermore, compatible combinations of yeasts and fungi, which cannot be cultured in submerged fermentation (SmF), can yield many aroma-active compounds in SSF [132]. In SSF, the fermentation process takes place in the absence of water on solid support generating important metabolites in the presence of microbes. SmF is a technique in which microorganisms can grow in liquid broth consuming the nutrients to release the desired metabolites into solution during fermentation. Although SSF has been used since ancient times and has many biotechnological advantages, there have been some limitations involved during scaling up in this technology, usually due to the heterogeneous nature of the mass, substrate, and troubles in heat transfer [127, 132, 133]. Besides the development of digital imaging technologies for assessing the growth kinetics in filamentous fungi in SSF, further improvement in mathematical modeling tools has been achieved to describe the scale-up studies [134].

Braga et al. [58, 59] have used stirred tanks and airlift bioreactors for the production of γ-decalactone using castor oil in batch cultures of *Y. lipolytica* (W29). Airlift bioreactors are generally considered for the developing processes on the basis of aerobic cultures because of the requirement for high oxygen transfer rates. Air was introduced at the bottom of the bioreactor using a five-hole sparger. γ-Decalactone concentration (around 3 g/L) was twofold increased in the airlift compared to the STR (stirred tank reactor). In a study, it has been reported that when castor oil (substrate) was reacted with microbial enzymes, the production of GDL (11 g/L) was recorded in 55 h [65].

For immobilized enzymatic reactions for the production of bioflavor in industry, packed-bed bioreactors, especially operated in continuous mode, and fluidized bed reactors are the most often utilized [127, 135]. In addition, studies have led to designing new reactors such as immersion bioreactors, rotating drum bioreactors, and mixed solid-state bioreactors, which would succeed over the troubles for large-scale productions [10, 131].

Fed-batch strategy is an interesting strategy to avoid degradation of γ-decalactone and cut down the inhibitory impacts of ricinoleic acid on the cells [136]. Using intermittent feed in fed-batch, aroma concentration of 6.7 g $L^{-1}$ was obtained as compared to 1.9 g $L^{-1}$ in batch fermentation and the formation of the side product 3-hydroxy-γ-decalactone augmented concurrently to 10 g $L^{-1}$ in the bioreactor. However, in this system, the maintenance of an emulsion causes several restraints to ensure the supply of fresh medium and remove an emulsion with the same characteristics. Thus, the substrate addition by step-wise fed-batch (pulses) is a method of avoiding this problem. In a step-wise fed-batch method, the productivity of γ-decalactone of 0.043 g $L^{-1}$ $h^{-1}$ has been reported when methyl ricinoleate (30 g $L^{-1}$) was fed twofold to the bioreactor using *Y. lipolytica* W29 [136]. Braga et al. [59] also attempted a step-wise fed-batch method with MTLY40-2P strain, in which castor oil (60 g $L^{-1}$) was supplemented in two pulses, leading to a twofold increase in γ-decalactone synthesis (about 7 g $L^{-1}$) as compared to batch mode.

## 9.6 Castor Oil: Advantages and Disadvantages

### *9.6.1 Advantages*

Castor is regarded as the most important industrial and medicinal plant since thousands of compounds can be extracted from it. These compounds have been reported to have antimicrobial activity against diverse pathogenic bacteria [137, 138]. Castor oil is extensively utilized in the industry with diverse applications [23]. The usage of castor oil is safe from broad historical usage to industrial application. Some ground-breaking technologies have been developed for the production of value-added castor oil chemicals and their derivatives. Food-grade castor oil is utilized in the formation of food additives, flavorings, and mold inhibitors and in packaging [139]. The products obtained by castor oil are not dangerous to the environment. It is biodegradable, nontoxic, and agriculture oriented. The export of castor oil can boost the economy of the country and also encourage the development of the agricultural sector.

Castor cake can be used as organic manure, which prevents soil from exhausting. Castor cake has a high content of N (6.4%), potash (1%), and phosphoric acid (2.55%) and has moisture retention capacity. It also contains crude protein (20%), ash (15%), and sugar (50%) [140]. Castor cake is used to control nematodes in soils [141]. Ricin can remain in the soil for about 2 years after castor harvesting [142]. However, the effect of the residual toxin on flora and fauna in the soil is yet to be determined.

### *9.6.2 Disadvantages*

Castor contains toxic ricin in seeds. However, these toxic compounds can be utilized to formulate drugs to treat many diseases globally [143]. Soil micro flora is one of the most imperative factors that augment soil fertility in various ways. Antimicrobial activity of castor was also observed against soil microbial community, which in turn disturbs soil health and fertility during its cultivation in soil.

It has been observed that microbial diversity declined in soils when castor was cultivated as compared to other plants [144]. There may be possibilities of the occurrence of some residual inhibitors in the soil sensitive to certain fungal and bacterial species affecting the growth of plants and soil health [145]. However, certain microorganisms have the ability to degrade and survive at high concentrations of inhibitors. It has been observed in a study that *Pseudomonas* and *Erwinia* sp. can efficiently degrade the protein in an in vitro assay. Consequently, these bacteria can be utilized as biofertilizers for castor cultivation without damaging microbial diversity in soil enhancing plant growth and soil health [144].

### *9.6.3 Safety Assessment or Toxicity Study of Castor*

The seeds of castor contain two types of proteins, *R. communis* agglutinin (RCA120) and ricin (RCA 60), which are toxic to eukaryotic cells [146]. Ricin is a strong cytotoxin with weak haemagglutinin properties, while RCA120 is less toxic and has powerful haemagglutinin, which functions as an allergen causing a health hazard during harvesting and processing [147]. Ricin remains in the meal after its oil extraction. There are various methods for the detoxification of castor toxins [148, 149]. Ricin can be detoxified with the help of proteolytic enzymes, sodium ricinoleate, $H_2O_2$, $KMnO_4$, autolyzed yeast or *Azotobacter*, halogens, ethanol, high temperature, and UV irradiation. In addition, other methods like steam treatment, NaCl, $Ca(OH)_2$, formaldehyde, $NH_4OH$, $(NH_4)_2SO_4$, $KMnO_4$, or urea can also be employed for detoxification of ricin [150]. US Department of Health and Human Services (NIH) has established safety measures for the usage of castor oil. In 1990, no harmful side effects of castor oil have been noticed in the feed studies on rats or mice. In 90 days, studies on dietary concentrations showed no effect on survival or body weight gains.

## 9.7 Concluding Remarks

Various types of aroma compounds are isolated from natural and synthetic sources to meet the market demand. The aroma industry has a great interest in natural products, especially fragrance and flavor compounds; therefore, an alternative method has been generated in the metabolic engineering field to obtain natural aroma compounds. Microbial biosynthesis and biotransformations are considered safe and most suitable technologies for the production of bioflavors and fragrances. This method has a number of advantages over traditional methods because microbes can be genetically and metabolically altered to improve the production of desired important compounds.

Castor oil is a significant and potential nonedible crop, producing numerous industrially important chemicals and products. Due to its lots of functional value in agriculture, industry, pharmaceutical, and cosmetics sectors, castor oil is proved to be a potential bio-based preliminary material increasing the economy of a country. Castor oil has unique properties of the presence of a double bond, carboxylate group, and hydroxyl group in the ricinoleic acid, which is useful in its easy derivatization into vital industrial raw materials. Due to the manufacture of large-scale end products from castor oil and being a green bioresource for chemical transformations, castor seed has global demand for enhanced production rate.

Castor oil conversion in aroma compound γ-decalactone by the biotransformation method using efficient microorganisms is a striking method, but the production time is high and quantity is very low. Various approaches have been adopted to overcome these problems by applying suitable microbial enzymes for the synthesis

of aroma compounds. Fed-batch fermentation method can be applied, choosing suitable microorganisms for higher yields of lactone. This method is also useful to overcome the inhibitory effect of the substrate in lactone production. Solid-state fermentation is the most appropriate method in producing microbial metabolites in large quantities, which also provides high-quality yields with improved product characteristics accompanied by low economic costs. A much higher research thrust on productivity improvement is needed for mitigating the demand–supply gap of castor seeds. Besides breeding, efforts should be focused on three other prime objectives: (1) improvement of castor oil quantity and quality, (2) development of ricin-free castor, and (3) production of value-added aroma products from castor crop.

Next to these strategies, the application of bioprocess engineering has countless benefits to attain higher yields and product concentrations by a comprehensive understanding of the regulation of different pathways involved in aroma production. However, so far, the products yields are very poor to make the biotechnological method feasible, and more research studies are needed to overcome these limitations. Furthermore, selection of mutant or new potential strains, fermentation methods, downstream processes, and up-scaling from lab to industrial levels need severe studies to maximize the yield as well as lessen the limitations. The continuous development of genetic engineering and systems biology tools, in association with advanced strategies, will allow more bioflavors to be produced in this manner in the future.

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# Chapter 10
# Treatment and Minimization of Waste in Baker's Yeast Industry


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## 10.1 Introduction

Yeasts have been used by humans to produce foods for thousands of years. Nowadays, modern industries require very large amounts of selected yeasts to obtain high-quality reproducible products and to ensure fast, complete fermentations [1]. Today, the overall production of yeast (≈3.5 Mt/year) is placed in around 300 industrial sites across the world [2].

Baker's yeast, which is one of the main products in the preparation of bread making, is manufactured through the aerobic fermentation of the selected strains of *Saccharomyces cerevisiae* according to their special qualities relating to the needs of the baking industry [3]. The water usage in the baker's yeast industry is around 75 $m^3$ water/ton of dry yeast. For industries using large quantities of water such as baker's yeast plants, it is essential to treat and reuse their wastewater. In the baker's yeast industry, the amount of wastewater is around 7.8 $m^3$ wastewater/$m^3$ molasses [4]. The industrial production of baker's yeast results in the discharge of large quantities of dark-colored liquid wastes generally called weak vinasses. These wastes

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have a high organic content, are strongly nitrogenous, sulfate-rich, phosphorous variable, and recalcitrant for biodegradation, and are highly colored due to the presence of melanoidins [3, 5, 6]. The dark brown color from baker's yeast effluents interferes with the absorption of sunlight, which reduces the natural process of photochemical reactions for self-purification of the surface waters [7]. Since the effluent generated from the baker's yeast industry is highly polluted, it needs to be treated before discharging into receiving water bodies in order to prevent important environmental problems. In addition to pollution, increasingly stringent environmental regulations are forcing the manufacturers to improve existing treatment and also explore alternative methods of effluent management [8]. Land disposal options generate problems with ground water pollution and are prohibited in some countries [9].

Industries have traditionally treated the waste products before discharging them to the environment. Because the treatment occurs after the production of waste, this type of treatment is called "end-of-pipe" treatment and this is being seriously questioned. The alternative solution, "waste minimization," aims at reducing the pollution problem by dealing with it during the manufacturing process itself. There are many ways by which waste minimization can be achieved. Improved housekeeping, changing process technology, changing product, changing input material, recycling process chemicals and raw materials, recovering by-product/waste, and reducing input to the process have been proven successful in achieving waste minimization.

Yeast bread fermentation is closely related to alcoholic/brewery fermentation. Therefore, we evaluated all fermentation with *S. cerevisiae* together. In this chapter, we reviewed the processes of yeast production, treatment, disposal, reuse, and minimization of wastewater/waste from the baker's yeast industry. Here, biological, physicochemical, and advanced treatment methods of wastewater are presented. Also, the disposal/reuse options, waste, sludge, and by-products such as vinasses are evaluated.

## 10.2 Baker's Yeast Industry

### *10.2.1 Yeast*

Yeasts belong to the group of living things called fungi. Yeast size can vary greatly depending on the species, typically measuring 3–4 μm in diameter, although some yeasts can reach over 40 μm. Yeasts are facultative anaerobes [10]. Yeast growth is affected by a number of factors. These include the composition of the medium commonly sugar source, aeration (oxygen), agitation of the medium, pH (4.5–5.0) [1], temperature (30 °C) [1], and period of propagation [10]. For their nutrition, yeasts require a source of carbon for growth and energy, a nitrogen source for the synthesis of protein and other nitrogenous materials, inorganic nutrients for the buildup of the normal functioning and structure of the cell, and vitamins. In the absence of oxygen,

they can metabolize sugar into alcohol (ethanol) and carbon dioxide and low biomass. In well-aerated conditions, the cells could be able to get enough energy and convert sugar into carbon dioxide, water, and biomass. Bread, wine, sake, and beer are made with the essential contribution of yeasts, especially from the species *S. cerevisiae* [1]. *S. cerevisiae* is a species of budding yeast. It is the microorganism behind the most common type of fermentation. It reproduces by a division process known as budding [10]. The fermentations focus on a maximum biomass yield with limited ethanol production, favoring the oxidative or aerobic metabolic yeast pathway (Fig. 10.1) [11].

Until now, in the production of baker's yeast, whether continuous, batch, or fed-batch bioreactors, different operating parameters have been controlled using modern control techniques so that maximum yields of production and economic profit can be achieved. One of the most popular control techniques is the self-tuning

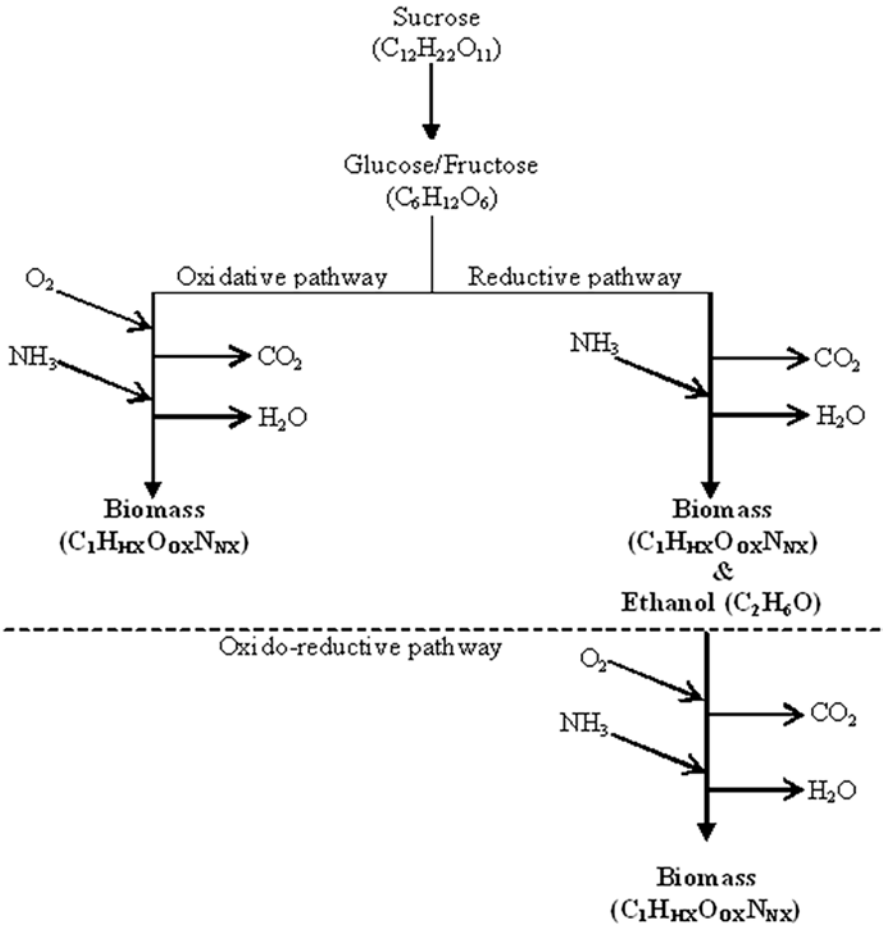



**Fig. 10.1** Simplified growth pathways of *Saccharomyces cerevisia* [11]

method. Generalized minimum variance, pole-placement self-tuning, and generalized predictive control are known as self-tuning control techniques [12]. In the last few decades, the yeast biomass production industry has contributed with many advanced approaches to traditional technological tools with a view to studying the physiology, biochemistry, and gene expression of yeast cells during biomass growth and processing. This has provided a picture of the determinant factors for the commercial product's high yield and fermentative fitness. Cell adaptation to adverse industrial conditions is a key element for good progress to be made in biomass propagation and desiccation and toward the characterization of specific stress responses during industrial processes to clearly indicate the main injuries affecting cell survival and growth. One major aspect of relevance in the complex pattern of molecular responses displayed by yeast cells is an oxidative stress response, a network of mechanisms ensuring cellular redox balance by minimizing structural damages under oxidant insults. Different components of this machinery have been identified as being involved in cellular adaptation to industrial growth and dehydration, including redox protein thioredoxin, redox buffer glutathione, and several detoxifying enzymes such as catalase and superoxide dismutase, plus protective molecules like trehalose, which play a relevant role in dehydration [1].

### *10.2.2 Substrates*

The raw materials used as a substrate for industrial yeast biomass production are usually agricultural, forestry, and food waste by-products [10]. Beet or cane molasses are the main substrates used in yeast production plants. These materials were selected for two main reasons: first, yeasts grow very well using the sugars present in the molasses and second, they are economically interesting since they are a waste product coming from sugar refineries without any other application. Usually, molasses contain approximately 50% sugars, mainly sucrose [13], but the composition is highly variable depending on the sucrose-refining procedure and on the weather conditions of that particular year. Sucrose is extracellularly hydrolyzed by yeasts in two monosaccharides, glucose and fructose, which are transported to and incorporated into the yeast metabolism as carbon sources.

In the last years, the price of molasses has increased because of their use in other industrial applications such as animal feeding or bioethanol production, thus rendering the evaluation of new substrates for yeast biomass propagation a trending topic for biomass producers' research. New assayed substrates include molasses mixtures with corn steep liquor, different agricultural waste products, and other possibilities such as date juice or agricultural waste sources, also called wood molasses, which can be a substrate only for yeast species capable of using xylose as a carbon source [1].

Molasses could not supply all the essential nutrients for yeast growth. Therefore, the addition of supplements such as $(NH_4)_2SO_4$, urea, yeast extract or peptone as

**Table 10.1** Percentage composition of beet and cane molasses [10]

| Constituent | Molasses type | |
|---|---|---|
| | Beet (%) | Cane (%) |
| Total sugar as invert | 48–58 | 50–58 |
| Nitrogen | 0.2–2.8 | 0.08–0.5 |
| Total solid | 78–85 | 78–85 |
| $P_2O_5$ | 0.02–0.07 | 0.009–0.07 |
| MgO | 0.01–0.1 | 0.25–0.5 |
| $K_2O$ | 2.2–4.5 | 0.8–2.2 |
| Carbon | 28–34 | 28–33 |
| $SiO_2$ | 0.1–0.5 | 0.05–0.3 |
| $Al_2O_3$ | 0.0005–0.06 | 0.01–0.04 |
| $Fe_2O_3$ | 0.001–0.02 | 0.001–0.01 |
| Total ash | 4.0–8.0 | 3.5–7.5 |

nitrogen source, $KH_2PO_4$, $H_3PO_4$ as phosphorus source, other macro elements such as calcium in the form of calcium salts, magnesium in the form of magnesium salts, and microelements such as iron, zinc, copper, and manganese is necessary for maximizing biomass yield of *S. cerevisiae* or any other types of yeasts. Vitamins are also required for yeast growth [10]. The aromatic nature of molasses is the result of phenolic compounds [14, 15]. Table 10.1 shows the percentage composition of both beet and cane molasses [10].

Aransiola et al. [16] investigated the bioconversion of raw cassava starch into value-added products. This involved hydrolysis of extracted starch from freshly harvested cassava tubers using three different methods. The results show that the hydrolysates obtained by hydrolysis of indigenous raw cassava starch via acid, acid–enzyme, and enzyme–enzyme methods were all able to support the growth of *S. cerevisiae.* The highest Dextrose Equivalent of 34 with a starch conversion efficiency of 87.3% was achieved by the enzyme–enzyme hydrolysis method [16].

Sugar beets contain no coloring materials, but they do contain color-forming substances. Color compounds in molasses are predominantly melanoidins (sugar–nitrogen complexes), caramel substances, polyphenol–iron compounds, and, to a lesser extent, plant pigments. The humic non-sugars (or polymerized forms of melanoidins) are claimed to be particularly relevant to the color of yeast produced. The color of sugar solutions varies with pH, with the darker colors occurring at higher pH levels [14, 15]. It is of great significance that baker's yeast is not contaminated with toxic substances like heavy metals, during its production or through contact with unsuitable packaging materials. Contamination of baker's yeast with lead (II) may originate from molasses produced from sugar beet or sugarcane crops irrigated

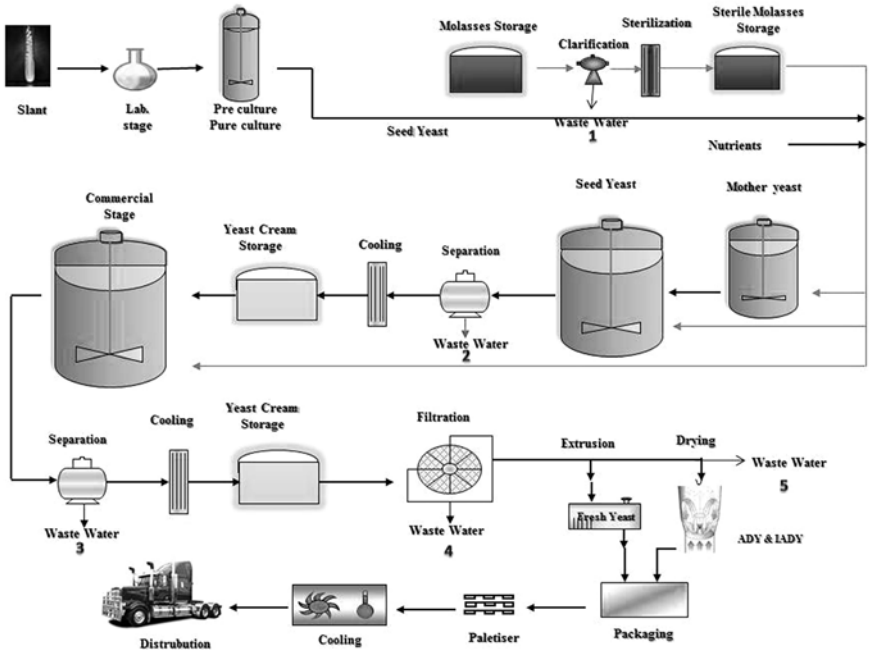
with contaminated water or grown in areas adjacent to high lead (II) emission industries [17].

### *10.2.3 Production of Baker's Yeast*

The baker's yeast production process flow can be divided into four basic steps, namely molasses and other raw material preparation, culture or seed yeast preparation, fermentation and harvesting, and filtration and packaging [18]. Nowadays, biomass propagation of wine, distiller's, and brewer's yeasts is usually achieved using baker's yeast plants. The procedure is designed as a multistage-based fermentation, previously defined for the production of baker's yeast using supplemented molasses as growth media [1]. The flowchart of the baker's yeast process is shown in Fig. 10.2 [19]. Also, the source, distribution, and characteristics of generated wastewater are shown in the process flow diagram.

The first stage is initiated with a flask culture containing molasses inoculated with the selected yeast strain [1]. Yeast can grow in the presence or absence of air. In bread dough, yeast grows very little under anaerobic conditions; instead, the sugar that can sustain either fermentation or growth is used, mainly producing alcohol and carbon dioxide. This means that the baker who is interested in the leaving action of the carbon dioxide works under conditions that minimize the presence of dissolved oxygen. In contrast, under aerobic conditions, in the presence of a sufficient quantity of dissolved oxygen, yeast grows by using most of the available sugar for growth and producing only negligible quantities of alcohol. So, a yeast manufacturer that wants to produce more yeast cell mass works under aerobic conditions by bubbling air through the solution in which the yeast is grown. The problem to the yeast manufacturer, however, is not just as simple as adding air during the fermentation process. If the concentration of sugar in the fermentation growth media is greater than a very small amount, the yeast will produce some alcohol even if the supply of oxygen is adequate or even in abundance. Adding the sugar solution slowly to the yeast throughout the fermentation process can solve this problem. The rate of addition of the sugar solution must be such that the yeast uses the sugar fast enough so that the sugar concentration at any one time is practically zero. This type of fermentation is referred to as fed-batch fermentation [18].

At the end of the fermentation stage, the yeast is present as a suspension of cells in a dark brown liquid containing the residues of the molasses. The yeast is removed from the fermentation liquid by a process of washing and separating in centrifugal separators, signaling the end of the fermentation and beginning of the downstream processing stage. Downstream processing can be defined as the stages of processing that take place after the fermentation or bioconversion stage. The yeast broth produced by fermentation, containing approximately 5% solids, can be manipulated into two main types of baker's yeast product and an additional intermediate saleable product. These are cake yeast, granular yeast, and cream yeast, each of which requires the downstream process to arrive at the desired product. At the end of the




| Parameter | Unit | Wastewater Source | Wastewater Source | Wastewater Source | Wastewater Source | Wastewater Source |
|---|---|---|---|---|---|---|
| Wastewater Source | | **1** | **2** | **3** | **4** | **5** |
| Contribution | % | 10 | 20 | 50 | 15 | 5 |
| Total COD | mg/L | 100000 | 45000 | 45000 | 15000 | 6090 |
| Soluble COD | mg/L | 90000 | 40000 | 40000 | 13000 | 4980 |
| $BOD_5$ | mg/L | 72000 | 35000 | 35000 | 11000 | 3780 |
| TKN | mg/L | 2300 | 2000 | 2000 | 750 | 274 |
| $NH_4^+$-N | mg/L | - | - | - | 35 | 132 |
| $SO_4^{2-}$ | mg/L | - | 3000 | 3000 | 1100 | 484 |
| pH | | 4.5 | 5.5 | 5.5 | 5.5 | 5.5 |
| Total solids | mg/L | - | - | - | | 40 |
| Suspended solids (SS) | mg/L | - | 3000 | 3000 | 1000 | 500 |

**Fig. 10.2** Schematic representation of baker's yeast production [19]

fermentation, the fermenter/yeast broth is concentrated using a series of combined centrifugation and washing steps into a yeast cream with a solids concentration of approximately 20%. The yeast is then cooled to approximately 4 °C, an ideal temperature to restrict the growth of any contaminating mesophilic microorganism. Cream yeast is basically the liquid product and can therefore be transferred into sterile tanks/containers and distributed to bakeries, where it is used to produce yeast-based products. The other pathway further manipulates the yeast cream into compressed or dried yeast. Granular yeast, also known as "Instant Dried Yeast," is a

**Table 10.2** Chemical composition of baker's yeast [20, 21]

| Composition | Chemical composition (% as dry matter) | |
|---|---|---|
| | [20] | [21] |
| Dry materials | 30–33 | – |
| Nitrogen | 6.5–9.3 | – |
| Proteins | 40.6–58 | 42–46 |
| Carbohydrates | 35.0–45.0 | 30–37 |
| Lipids | 4.0–6.0 | 4–7 |
| Minerals and various amounts of vitamins | 5.0–7.5 | 7–8 |
| Nucleic acid | – | 6–8 |
| Moisture | – | 2–5 |
| Ash | – | N/A |

form of compressed yeast. Stored cream/liquid yeast is passed through a filter, usually a filter press or rotary vacuum filter, which removes water, increasing its solids content to approximately 30%. Salt may also be added to the cream yeast prior to filtration to aid the removal of water. The filtered yeast is then dried using fluid-bed dryers. The yeast is dry and generally does not require refrigeration as the low water content reduces the risk of microbial contamination. Emulsifiers and oils can be added at this point to texturize the yeast and aid the cutting process. The filtered and the dried yeast can alternatively be used to make cake yeast. Cake yeast is another form of compressed yeast and can be categorized as active dried yeast. It differs from granular yeast in that rather than granulation, the dried yeast is extruded or cut into blocks/cakes. Similar to granular yeast, cake yeast also contains about 30% solids. Both types of compressed yeast are then packaged, typically vacuum packed to reduce the risk of contamination by aerobic bacteria, and distributed to wholesalers or traders [11]. The quality of baker's yeast is often discussed in terms of microbiological purity and gas-producing activity [18]. The chemical composition of baker's yeast is shown in Table 10.2 [20, 21].

## 10.3 Wastewater Management

### *10.3.1 Wastewater Sources*

Wastewater is generated in large quantities in the yeast industry. The wastewater originated from the baker's yeast industry can be classified into two groups: high strength process wastewater and low-medium strength process wastewater. The former one is generated from the yeast separators and processes such as centrifuges and rotary vacuum filters, whereas the latter one mainly constitutes the floor washing and equipment cleaning water and water from cooling or packaging [22–24] The generated wastewater from both the yeast washing stages and separators

accounts almost 85% of the total wastewater [19]. A major part of the non-sugar substances in the molasses is not assimilable by the yeast and is released unchanged to the processing wastewater. These compounds represent the principal waste from the yeast production process. Besides, the chemicals added during fermentation (e.g., various salt antifoams, propionic acids, brine, etc.), yeast metabolites, and residual yeast cells are in the wastewater [15, 25, 26].

### *10.3.2 Wastewater Characterization*

Yeast production wastewater is a complex mixture. Most of the contaminants in the wastewater are due to the use of molasses as a main raw material [27]. Molasses spentwash contains nearly 2% of a dark brown recalcitrant pigment called melanoidin formed due to Maillard amino-carbonyl reaction [28].

Melanoidins are acidic, high molecular weight polymers with a complex structure and behave as anionic hydrophilic polymers [29]. The formation of melanoidins comprises a set of consecutive and parallel chemical reactions between amino compounds and carbohydrates during a nonenzymatic browning reaction [29–31]. The empirical formula of melanoidin is $C_{17\text{-}18}H_{26\text{-}27}O_{10}N$. It is a product of nonenzymatic reaction between sugars and amino compounds. The molecular weight distribution is between 5000 and 40,000 [28, 32].

Effluents generated from baker's yeast industries are characterized by the low levels of readily degradable sugars, acids, and high concentrations of biochemical oxygen demand ($BOD_5$), chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN), total dissolved solids (TDS), trimethylglycine, phenol, and sulfate. The other major characteristics of this industry are the generation of wastewater with a dark brown color in large quantities [33–36]. The average concentration of organic pollutants by total COD is 25,000 mg/L, of which up to 33% is accounted for betaine [36]. The wastewater characterization from baker's yeast industries that has been reported in the literature is shown in Table 10.3 [14, 25, 27, 35, 37–42].

### *10.3.3 Wastewater Treatment Processes*

The processes that are applied in the treatment of baker's yeast industry effluent are the following:

1. Biological treatment (combined anaerobic–aerobic system with nitrification and denitrification)
2. Physicochemical treatment (coagulation/flocculation, adsorption, evaporation)
3. Advanced treatment (membrane processes, advanced oxidation processes (AOPs), electrochemical processes, wet-air oxidation, ultrasound process)

**Table 10.3** Characterization of baker's yeast wastewater

| Reference | | [37] | [38] | [35] | [27] | [39] | [25] | [40] | [14] | [41] | [35] | [42] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Parameter | Unit | Molasses wastewater | Baker's yeast effluent | High-strength wastewater of yeast plant | Yeast effluent | Yeast effluent | Yeast effluent | Baker's yeast effluent | Baker's yeast effluent | Baker's yeast effluent | Baker's yeast effluent | Baker's yeast effluent |
| Total COD | mg/L | 105,851 | 6090 | 20,000–24,000 | 28,194 | 14,400–25,700 | 25,020 | 15,848 | 20,760 | 10,000–30,000 | 25,000 | 70,000–100,000 |
| Soluble COD | mg/L | – | 4980 | – | – | – | 23,420 | 15,193 | – | – | – | – |
| $BOD_5$ | mg/L | 22,985 | 3780 | 8000 | – | – | – | – | 14,974 | 7000–21,000 | 12,000 | 18,340–27,200 |
| TKN | mg/L | 2326 | 274 | 2000 | – | – | – | 1196 | – | – | – | 1610–2060 |
| $NH_4^+$-N | mg/L | 120.5 | 132 | – | – | – | – | 206 | – | – | – | – |
| Total-N | mg/L | – | – | – | 2106 | 250–350 | 1470 | – | – | 250–1200 | 1500 | – |
| Total-P | mg/L | – | 3 | 30 | 55 | 17.3–48.2 | 100 | 20.1 | – | – | 100 | 108 |
| $PO_4^-$-P | mg/L | – | 2.28 | – | – | – | – | 6.6 | – | 27 | – | – |
| $SO_4^{2-}$ | mg/L | – | 484 | 4000 | 2449 | 3500–5300 | 2940 | – | – | 100–2700 | 2900–5700 | 1700–3200 |
| pH | | 4.6–5.5 | 6.45 | 4–5 | – | – | – | 6.2 | 6.0 | 6–8 | – | 4–5 |
| VFA | mg/L | – | – | – | – | | | – | 3917 | – | – | – |
| Conductivity | µS/cm | – | – | – | – | – | – | – | – | – | – | – |
| Total solids | mg/L | – | 35,510 | – | – | – | – | – | – | – | – | – |
| Suspended solids (SS) | mg/L | – | 585 | 500 | 2654 | – | – | 835 | – | 50–1200 | – | 3800–7300 |
| Volatile suspended solids (VSS) | mg/L | – | 475 | – | – | – | – | 810 | 1545 | – | – | – |

| Reference | | [37] | [38] | [35] | [27] | [39] | [25] | [40] | [14] | [41] | [35] | [42] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Parameter | Unit | Molasses wastewater | Baker's yeast effluent | High-strength wastewater of yeast plant | Yeast effluent | Yeast effluent | Yeast effluent | Baker's yeast effluent | Baker's yeast effluent | Baker's yeast effluent | Baker's yeast effluent | Baker's yeast effluent |
| Alkalinity | mg $CaCO_3$/L | – | 1475 | – | – | – | – | 2349 | – | – | – | – |
| Trimethylglycine | mg/L | – | – | – | – | 3700–4000 | – | – | – | – | – | – |
| Color | Pt-Co 475 nm | 38 | – | – | – | – | – | 14,000 | – | 11,000 | – | – |
| Magnesium | mg/L | – | – | – | – | – | – | 30.7 | – | – | – | – |
| Ferrous | mg/L | – | – | – | – | – | – | 4.9 | – | – | – | – |
| Oil and grease | mg/L | – | – | – | – | – | – | – | 10 | – | – | – |
| Temperature | °C | 78–84 | – | – | – | – | – | – | – | 20–28 | – | – |

Studies on the water quality of a river contaminated with distillery effluent displayed high $BOD_5$ values of 1600–21,000 mg/L within an 8-km radius. Adequate treatment is therefore imperative before the effluent is discharged. Land disposal of distillery effluent can lead to groundwater contamination. Deep well disposal is another option but limited underground storage and specific geological location limit this alternative. Constructed wetland was also applied as the tertiary treatment step to treat the effluent from the conventional anaerobic–aerobic treatment system. Sohsalam and Sirianuntapiboon [43] worked on the treatment by surface flow constructed wetland system of anaerobically treated molasses wastewater. The results show that both removal efficiency and plant growth rate were increased with the decrease of the organic loading rate.

Decolorization of effluent is the main problem in baker's yeast industries. Physicochemical treatment, adsorption, coagulation/flocculation, oxidation processes, and membrane treatment have been examined with particular emphasis on effluent decolorization. Though these techniques are effective for both color removal and reduction in organic loading, sludge generation and disposal is a constraint in coagulation/flocculation and adsorption [8]. The other treatment methods like the evaporation of spentwash to produce animal feed have also been practiced.

#### 10.3.3.1 Biological Treatment Processes

A medium-sized fermentation factory, which uses about 50 metric tons of molasses per day, is estimated to generate about 1.5 tons of COD daily [13, 24]. Yeast processing wastewater contains melanoidins, which are known to be lethal to many microorganisms because of their antioxidant properties. Generally, biotreatment of melanoidins containing wastewater has not given impressive results, and post-treatment is necessary [44].

Biological treatment, typically the combination of anaerobic and aerobic processes, is normally effective in removing BOD from molasses wastewater. Removals of COD range from 55% to 75% by anaerobic pretreatment. However, the brown color remains due to the presence of melanoidin pigment [22, 24]. Conventional anaerobic–aerobic treatment processes can accomplish the degradation of melanoidins only up to 6 or 7% [8, 30]. Phenolic compounds responsible for beet molasses wastewater color are partly removed (63% removal) during the aerobic–anoxic treatment process, but the color removal accounts for only 8–23% [26].

The conventional treatment method is the anaerobic pretreatment stage with upflow sludge blanket (UASB) reactors followed by an aerobic treatment with activated sludge (Fig. 10.3) [35].

Zub et al. [35] investigated that the application of a combined anaerobic/anoxic system to a full-scale treatment plant supported biogas production up to 1300 $m^3$/day (Fig. 10.4). Baker's yeast wastewater was treated in a mesophilic anaerobic/anoxic continuous stirred tank reactor (CSTR) system. The average removal efficiency in this system was 79% by total COD and 100% by $SO_4^{2-}$ in which the concentration of sulfides in the effluent did not exceed 50 mg/L [35].

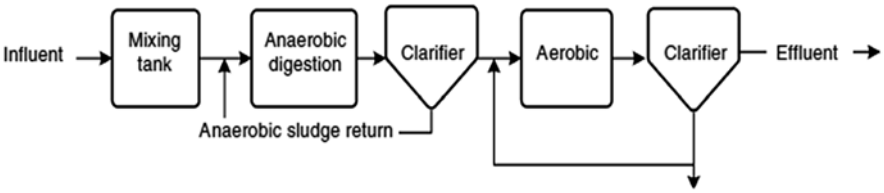



**Fig. 10.3** The conventional treatment method is: anaerobic pretreatment stage with UASB reactors followed by an aerobic treatment with activated sludge [35]

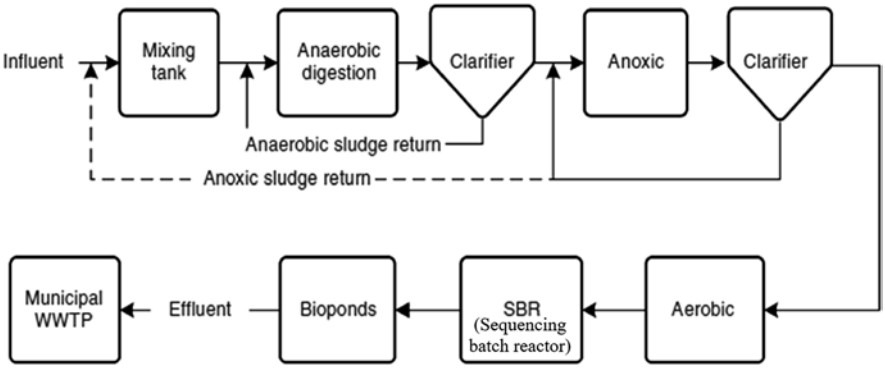



**Fig. 10.4** The application of combined anerobic/anoxic system to a full-scale wastewater treatment plant (WWTP) [35]

The characteristics of biologically pretreated baker's yeast wastewaters are summarized in Table 10.4 [14, 40, 41, 45].

There are lots of studies on removing the color from molasses' effluents. In general, microbial decolorization is an environment-friendly and low-cost alternative to chemical decomposition process. Nevertheless, the problem still persists because several organisms that have been shown to degrade melanoidin are not best suited for treating molasses-containing wastewater [46]. The studies so far can be seen as an early step toward solving the problem. Moreover, most of the microbial decolorization studies required effluent dilution for optimal activity. While using microorganisms, the use of media supplement creates extra load on the overall effluent treatment process [8].

Biological treatments using specific microorganisms such as *Coriolus* [8, 47–50], *Aspergillus* [50–52], *Phanerochaete* [53–55], *Bacillus* [37, 56–58], and *Lactobacillus* [59] have been reported. A detailed list of microorganisms studied by different researchers for decolorization of melanoidins is given in Table 10.5 [6, 8, 49–52, 55, 57–64].

Miyata et al. [65] investigated the microbial decolorization of melanoidin-containing wastewaters using activated sludge and the fungus *Coriolus hirsutus*.

**Table 10.4** Characterization of biologically pretreated baker's yeast effluents

| Parameter | Unit | [45] | [14][a] | [40][b] | [41] |
|---|---|---|---|---|---|
| COD | mg/L | 3700 ± 100 | 731 | 529–2006 | 2000–2500 |
| Soluble COD | mg/L | – | – | 490–1052 | – |
| TOC | mg/L | 1000 ± 70 | – | – | – |
| $BOD_5$ | mg/L | 300 ± 100 | 119 | – | – |
| TKN | mg/L | – | – | 171–432 | – |
| $NH_4^+$-N | mg/L | – | – | 152–418 | 550–600 |
| Ammonium | mg/L | 1000 ± 30 | – | – | – |
| SS | mg/L | – | – | 20–50 | – |
| VSS | mg/L | – | 39 | – | – |
| Color | Pt-Co | – | – | 915–3740 | 3500–4000 |
| 400 nm | Abs | – | – | 0.515–1.995 | – |
| 455 nm | Abs | – | – | 0.253–1.995 | – |
| 475 nm | Abs | 2.4 ± 0.1 | – | – | – |
| Total-P | mg/L | – | – | 2.2–6.8 | – |
| pH | – | 8.7 ± 0.1 | 8 | 8.1–8.2 | 8.5 |
| Alkalinity | mg/L $CaCO_3$ | 9000 ± 200 | – | 3450–8850 | – |
| Sulfate | mg/L | 700 ± 80 | – | – | – |
| Chloride | mg/L | 800 ± 50 | – | – | – |
| Oil and grease | mg/L | – | 8 | – | – |
| VFA | mg/L | – | 10 | – | – |
| Conductivity | µS/cm | – | – | – | 3200–3500 |

[a] Average of 27 values
[b] Three different samples

The results showed that the increase in manganese-independent peroxides and manganese peroxides activities were considered to play an important role in the enhanced ability of *C. hirsutus* [65].

In the case of pure culture experiments, *Candida utilis* and *Trichoderma viridiae* each showed less than 65% reduction in COD, whereas *C. utilis* and *A. niger* together resulted in 89% COD removal. This reduction was from sugarcane stillage-based media with an initial COD of 40–75 g/kg [8].

Treatment of fermentation wastewater by the use of *Pseudomonas putida* followed by *Aeromonas* sp. in a two-stage bioreactor resulted in COD as well as color reduction. *P. putida* produces hydrogen peroxide, which is a strong decolorizing agent. Since the organism cannot use spentwash as a source of carbon, some glucose supplement was provided [8].

Many effluents contain nitrogen in much higher concentrations than municipal wastewaters. In some industrial wastewaters, nitrogen can be in the form of organic nitrogen (urea, proteins, etc.) like in food industry effluents [66]. Wastewaters from baker's yeast industry effluent have a high organic contamination. The classical biological treatments under aerobic and anaerobic conditions lead to a good efficiency in removing the organic carbon, but nitrogen efficiency is variable. The

**Table 10.5** Microorganisms employed for the decolorization of baker's yeast effluent

| Name | Comments | Color removal (%) | Reference |
|---|---|---|---|
| *Coriolus versicolor* | Two types of enzymes, sugar-dependent and sugar-independent, were found to be responsible for melanoidin decolorizing activity | 80 | [60] |
| *Aspergillus oryzae* | The thermophilic strain adsorbed lower molecular weight fractions of melanoidin and required sugars for growth | 75 | [50] |
| *Bacillus thuringiensis* | Addition of 1% glucose as a supplementary carbon source was necessary | 22 | [57] |
| *Acetobacter acetii* | The organism required sugar, especially glucose and fructose for decolorization of MWWs | 76.4 | [58] |
| *Lactobacillus hilgardii* | Immobilized cells of the heterofermentative lactic acid bacterium decolorized 40% of the melanoidins solution within 4 days aerobically | 40 | [59] |
| *Phanerochaete chrysosporium* | Diluted anaerobically digested spentwash | 80 | [55] |
| *Coriolus hirsutus* | Decolorization of melanoidin pigment was reported by extracellular peroxidase produced by *C. hirsutus* | 71–75 | [8] |
| *Aspergillus niveus* | With a reduction of 75% COD | 60 | [51] |
| *Aspergillus niger* | With a reduction of 95% COD | 69 | [51] |
| *Geotrichum candidum* | Fungus immobilized on polyurethane foam showed stable decolorization of diluted molasses solution | 80 | [8] |
| *Citeromyces* | The yeast *Citeromyces* resulted in high and stable removal efficiency in both color intensity and organic matter (BOD 76%) for diluted spentwash | 75 | [61] |
| *Aeromonas formicans* | 57% COD reduction was observed after 72 h on the pre-digested distillery effluent | 55 | [6] |
| *Xanthomonas fragariae* *Bacillus megaterium* *Bacillus cereus* | Three different bacterial strains were used both in free form as well as after immobilization on calcium alginate beads for the treatment of 33% predigested fermentation effluent | 75(with *B. cereus)* | [49] |
| TA2 and TA4 | When used together to the treatment of anaerobically treated distillery effluent, the reduction in BOD was found to be higher, 80% | 76 | [62] |
| *P. putida* | With a reduction of 44% COD in spentwash | 60 | [63] |
| *Aspergillus niger* | With diluted synthetic melanoidin<br>50% biodigested effluent | 72<br>80 | [52] |
| *Lactobacillus plantarum* | Decolorization of molasses wastewater was achieved under both anaerobic and facultative conditions | 68.12 | [64] |

biological processes involved in nitrogen removal from wastewaters of the baker's yeast industry are also dependent on physical and chemical conditions in which the activated sludge microbiota work to mineralize to organic compounds or to

bio-convert them into gases. Nitrogen removal through biological nitrification and denitrification processes is generally classified as an advanced treatment process [67].

Baker's yeast industry produces a great amount of nitrogen compounds as a result of the use of water in biotechnological processing. Removal of nitrogen from baker's yeast wastewater is a complex process, even for large wastewater treatment plants. Conventional nitrification–denitrification systems can be applied to remove nitrogen from the baker's yeast industry effluents. When an anaerobic pretreatment is operated, the anaerobic effluent has a low COD/N ratio, which can lead to incomplete denitrification. It is possible either to operate conventional nitrification–denitrification process with the addition of an external carbon source or to combine the anaerobic digestion process with an aerobic nitrifying reactor and to recycle the nitrified effluent in the digestor. If nitrification and denitrification must be performed in the aerobic stage, it is necessary to run a bypass (fresh or acidified wastewater) around the methane reactor to provide sufficient biodegradable COD for denitrification. The biological processes involved in nitrogen removal from wastewaters of the baker's yeast industry are also dependent on physical and chemical conditions in which the activated sludge microbiota work to mineralize the organic compounds or to bio-convert them into gases [67].

The biotreated effluent of molasses wastewaters still contains high levels of refractory organic matter, color, and inorganic salts. Ozonation can decolorize molasses secondary effluent ($\gg$95%) and does not lead to toxic by-products; however, it does not remove much COD (35% maximum). Refractory organics and color in secondary effluent are removable by activated carbon adsorption, but this needs a relatively high carbon usage. COD and color removal efficiency were obtained to be 97% and 91% by adsorption, respectively [68].

Current biological treatment of baker's yeast effluent involves combinations of anaerobic digestion and aerobic systems that successfully reduced BOD to acceptable limits but does not deal effectively with either the dark color or the associated COD that remains and limits the reuse/recycling of the process water [7]. The color-imparting melanoidins are barely affected by conventional biological treatment such as methane fermentation and the activated sludge process [8].

Pirsaheb et al. [69] used integrated anaerobic baffled reactor granular activated carbon for pretreatment of baker's yeast wastewater to discharging sewerage or aerobic treating processes. Also, 94.6% COD, 93.7% $BOD_5$, and 54% color removal efficiencies were obtained with a loading COD of 15,000 mg/L. They reported that the optimal operating condition of the reactor was 4 d for HRT and 50% of granular activated carbon filling ratio. They also investigated methane production. The methane-yielding rate was calculated between 0.31 and 0.44 L/g COD removed, and specific methanogenic activity was between 0.13 and 0.38 g COD/g VSS [69].

#### 10.3.3.2 Physicochemical Treatment Processes

Sugarcane molasses spentwash after biological treatment by both anaerobic and aerobic methods can still have a BOD of 250–500 mg/L [70] and a dark color [3]. The color-imparting melanoidins are barely affected by conventional biological treatment such as methane fermentation and the activated sludge process [71]. The shortcomings of multistage biological treatment include operational difficulty and occasional formation of hazardous by-products/secondary pollutants and intensification of the color due to re-polymerization of colored compounds [24, 30, 72].

In this context, various physicochemical treatment options have been explored. Physicochemical processes such as coagulation/flocculation, carbon adsorption, and chemical oxidation have been applied for the decolorization of molasses wastewater.

##### 10.3.3.2.1 Coagulation/Flocculation

Coagulation has remained the most widely practiced method of removing particulate and organic matter in wastewater treatment. Conventional coagulants in wastewater treatment are alum ($Al_2(SO_4)_3.14H_2O$), ferric chloride ($FeCl_3.6H_2O$), sodium aluminate, aluminum chloride, and ferric sulfate. Conventional coagulants are basically salts of a strong acid (e.g., HCl or $H_2SO_4$) and a weak base ($Al(OH)_3$ or $Fe(OH)_3$); thus, they are a mixture of a cation (from a base) and an anion (from an acid) [28]. Chemical coagulants can destabilize particulates by four distinct mechanisms: double layer compression, charge neutralization, enmeshment in a metal hydroxide precipitate, and inter-particle bridging [30].

Experimental results indicate that ferric chloride was the most effective among the conventional coagulant, achieving 89% COD and 98% color eliminations, while aluminum sulfate was the least effective, giving COD and color reductions of 66% and 86%, respectively. In addition to metal cations, counter-ions significantly influence the coagulation performance since $Cl^-$-based metal salts attained better removal efficiency than $SO_4^{2-}$-based ones at the optimal coagulant dosage. Coagulation of molasses effluent is a highly pH-dependent process, with better removal efficiency achieved at lower pH levels. Rapid mixing intensity, rather than rapid mixing time, has a relatively strong influence on the settling characteristics of flocs formed. Lowering mixing intensity resulted in increasing settling rate but the accumulation of floating flocs. When used as coagulant aids, synthetic polyelectrolytes showed little effect on the improvement in organic removal. On the other hand, cationic polyacrylamide was observed to substantially enhance the settleability of flocs as compared to anionic polyacrylamide [24].

Liang et al. [31] studied coagulation for removal of color and COD from molasses effluent using ferric chloride. Under the optimum conditions, up to 86 and 96% of COD and color removal efficiencies were achieved. Residual turbidity in the supernatant was less than 5 NTU, and $Fe^{3+}$ concentration was negligible because of effective destabilization and subsequent sedimentation. The low-molecular-weight

fraction of melanoidins is more reactive than the high-molecular-weight fraction, and an increase in the concentration of the lowest molecular weight organic group is related to the capacity of charge neutralization. Charge neutralization and co-precipitation are proposed as predominant coagulation mechanisms under the optimum conditions [31].

Zhou et al. [30] showed that when alum is applied in post-treatment of molasses wastewater, the initial pH value should be above 7 to guarantee coagulation efficiency. Charge neutralization is the predominant mechanism in the coagulation of molasses effluent with alum. The residual turbidity can be drastically reduced, and the percentage of COD and color removal further improved. The mixing condition also has some effect on coagulation performance, and the optimal mixing rate during the rapid mixing stage is 500 rpm when the residual turbidity is reduced to 14 NTU [30].

Inanc et al. [3] reported that coagulation with alum and iron salts was not effective for color removal. They explored lime and ozone treatment to anaerobically digested effluent. The optimum dosage of lime was found to be 10 g/L resulting in 82% COD removal and 67% reduction in color in a 30-min period. These findings are in disagreement with those of Migo et al. [71] who used a commercial inorganic flocculant, a polymer of ferric hydroxysulfate with a chemical formula $[Fe_2(OH)_n(SO_4)_3]$, for the treatment of molasses wastewater. The treatment resulted in about 87% decolorization for biodigested effluents; however, an excess of flocculant hindered the process due to an increase in turbidity and TOC content.

$FeCl_3$ and $AlCl_3$ were also tested for decolorization of biodigested effluent and showed similar removal efficiencies. About 93% reduction in color and 76% reduction in TOC were achieved when either $FeCl_3$ or $AlCl_3$ was used alone. The process was independent of chloride and sulfate ion concentration but was adversely affected by high fluoride concentration. However, in the presence of high flocculant concentration (40 g/L), the addition of 30 g/L CaO enhanced the decolorization process resulting in 93% color removal. This was attributed to the ability of calcium ions to destabilize the negatively charged melanoidins; furthermore, the formation of calcium fluoride ($CaF_2$) also precipitates the fluoride ions. Almost complete color removal (98%) of biologically treated distillery effluent has been reported with conventional coagulants such as ferrous sulfate, ferric sulfate, and alum under alkaline conditions [73].

The best results were obtained using Percol 47, a commercial organic anionic polyelectrolyte, in combination with ferrous sulfate and lime. The combination resulted in 99% reduction in color and 87 and 92% reduction in COD and BOD, respectively. Similar findings have also been reported by Mandal et al. [74].

Coagulation studies on spentwash after anaerobic–aerobic treatment have also been conducted using bleaching powder followed by aluminum sulfate. The optimum dosage was 5 g/L bleaching powder followed by 3 g/L of aluminum sulfate that resulted in 96% removal in color, accompanied by up to 97% reduction in BOD and COD. Nonconventional coagulants, namely wastewater from an iron pickling industry rich in iron and chloride ions and titanium ore processing industry containing significant amounts of iron and sulfate ions, have also been examined [73]. The

iron pickling wastewater gave better results with 92% COD removal, combined with over 98% color removal.

Prasad [28] studied the color removal from distillery spentwash through coagulation using *Moringa oleifera* seeds. In the study, the effects of dosage, pH, and concentration of salts (NaCl and KCl) were investigated for an optimized condition of color removal. The actual color removal at optimum conditions was found to be 53% and 64%, respectively, for NaCl and KCl salts [28].

#### 10.3.3.2.2 Adsorption

Adsorption is the process of accumulating substances that are in solution on a suitable interface. Adsorption is a mass transfer operation in that a constituent in the liquid phase is transferred to the solid phase. The adsorption process takes place in four more or less definable steps: (1) bulk solution transport, (2) film diffusion transport, (3) pore transport, and (4) adsorption (or sorption). Because the adsorption process occurs in a series of steps, the slowest step in the series is identified as the rate-limiting step. The principal types of adsorbents include activated carbon, synthetic polymeric, and silica-based adsorbents, although synthetic polymeric and silica-based adsorbents are seldom used for wastewater adsorption because of their high cost. Economical application of activated carbon depends on an efficient means of regenerating reactivating the carbon after its adsorptive capacity has been reached [75].

Among the physicochemical treatment methods, adsorption on activated carbon is widely employed for the removal of color from organic compounds from wastewater, molasses wastewater, melanoidin, and other specific organic pollutants. Activated carbon is a well-known adsorbent due to its extended surface area, microporous structure, high adsorption capacity, and high degree of surface reactivity [76, 77]. Adsorption of organic compounds is controlled by physical and chemical interactions [78]. It was recently demonstrated that an activated carbon with a significant distribution of both micropores and mesopores and a significant amount of macropores that are assumed to act as conduits providing access to micro- and mesopores have a good adsorption efficiency for melanoidins and colored compounds of molasses spentwash after anaerobic digestion [79]. Decolorization of synthetic melanoidin using commercially available activated carbon as well as activated carbon produced from sugarcane bagasse was investigated by Bernardo et al. [80].

Significant decolorization was observed in packed bed studies on anaerobically treated spentwash using commercial activated charcoal with a surface area of 1400 $m^2/g$ [81]. Nearly complete decolorization (>99%) was obtained with 70% of the eluted sample, which also displayed over 90% BOD and COD removal. In contrast, other workers have reported adsorption by activated carbon to be ineffective in the treatment of distillery effluent [74, 82]. Adsorption by commercially available powdered activated carbons resulted in only 18% color removal; however, combined treatment using coagulation–flocculation with polyelectrolyte followed by adsorption resulted in almost complete decolorization [82].

Low-cost adsorbents such as pyorchar (activated carbon both in granular and powdered form, manufactured from paper mill sludge) and bagasse flyash have also been studied for application. Ramteke et al. [83] reported color removal up to 98% with pyorchar. However, to achieve the same level of color removal, larger doses of the indigenously prepared powdered and granular pyorchar were required in comparison to commercial activated carbon [83].

Graphene oxide nanosheets could be a cost-effective, efficient, and potential adsorbent for melanoidin removal. The adsorption capacity of graphene oxide nanosheets as an adsorbent for melanoidin removal from industrial molasses wastewater is 18.31 g/g [84].

Mall and Kumar [70] compared the color removal using commercial activated carbon and bagasse flyash, and 58% color removal was reported with 30 g/L of bagasse flyash and 80.7% with 20 g/L of commercial activated carbon. Since the bagasse flyash has high carbon content and the adsorbed organic material further increases its heating value, the spent adsorbent can be used for making fire briquettes [70].

Yet another adsorbent that has been examined is the natural carbohydrate polymer chitosan derived from the exoskeleton of crustaceans. Lalov et al. [85] studied the treatment of fermentation wastewater using chitosan as an anion exchanger. At an optimum dosage of 10 g/L and 30 min contact time, 98% color and 99% COD removal were observed.

#### 10.3.3.2.3 Evaporation Systems

The high-strength wastewater coming from the yeast separators is treated by the evaporation process to reduce COD and nitrogen loads from the industry [38]. An evaporator in a chemical plant or a fermentation operation is a highly engineered piece of processing equipment in which evaporation takes place. All evaporators are fundamentally heat exchangers because thermal energy must be added to the process, usually across a metallic barrier or heat transfer surface, in order for evaporation to take place. Since the purpose of an evaporator is to concentrate a dilute feed stream and to recover a relatively pure solvent, this separation step must be defined. Figure 10.5 is a scheme for any evaporator.

The properties of the liquid feed and the concentrate are important factors to consider in the engineering and design of an evaporation system. The heating surface of an evaporator represents the largest portion of the evaporator cost; heat transfer is the most important single factor in the design of an evaporation system.

Most evaporators consist of three main elements or parts: a heating unit (calandria), a region for liquid–vapor separation (sometimes called a vapor head, flash chamber, or settling zone), and a structural body to house these elements and to separate the process and heating fluids. The simple largest variable cost factor in making separation by evaporation is the cost energy. If crude oil is the ultimate source of energy, the cost of over \$126.67 per $m^3$ (\$20 per barrel) is equivalent to more than \$3.33 for one million kJ. Water has a latent heat of 480 kJ/kg at

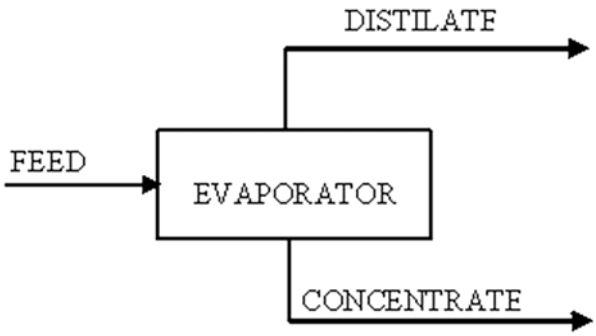



**Fig. 10.5** Scheme of evaporator

760 mmHg, absolute, so the energy required to evaporate 1 kg of water exceeds 0.16 cents. Therefore, the efficient utilization of energy is the most important consideration in evaluating which type of evaporation system should be selected.

From the process viewpoint, the two parameters that should be regulated are the concentration and flow rate of the bottoms product. If the composition of the feed stream is constant, good control of the feed rate and the evaporation rate will give the desired concentrated product at the proper production rate. Of course, the method of control can depend upon the evaporator type and method of operation. Flow rates are the largest single group of process measurements used for control, and flow is the only process variable for which significant energy may be required by the measuring device. Energy economy and evaporative capacity are the major measures of evaporator performance. Other performance variables to be considered include product quality, product losses, and decrease in performance as scaling, salting, or fouling occurs [86].

At the end of the fermentation stage, the resulting broth contains 5–6% yeast cells. The cells are separated by large-scale continuous-type separators and collected as yeast cream liquid, while the cell-free fermentation broth (mainly weak vinasse) is sent for evaporation. The cell-free fermentation broth (wash) is preheated to about 90 °C by heat exchange with the effluent ("spentwash") and then sent to the evaporators. Here, the liquor is heated by live steam and fractionated to give about 60–55% dry matter. The condensed water from this stage, known as "spentlees," is usually pumped back to the wastewater system. The photographs of weak vinasse and evaporations outlet products are shown in Fig. 10.6 [19].

Economic considerations frequently suggest the simplest answers to the question of how to utilize stillage produced, specifically as either fodder or fertilizer. Such uses, however, do not resolve the problem because of the large scale of yeast production. Molasses-based vinasse is characterized by high potassium content and therefore limits its potential as farm animal fodder. Another use, particularly in the case of vinasse, consists in classifying stillage as wastewater and making it subject to anaerobic biodegradation.

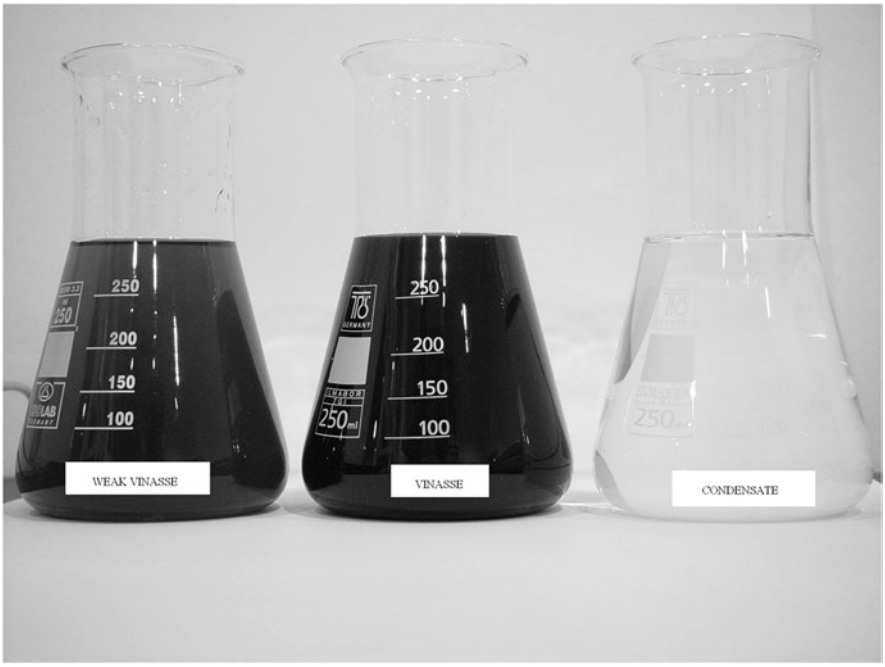



**Fig. 10.6** The photographs of weak vinasse and evaporations outlet products [19]

### 10.3.3.3 Advanced Treatment Processes

Biological treatment of wastewaters eliminates important compounds of the organics in the waste. However, biochemical decomposition by conventional treatment methods may not be enough to satisfy the discharge limits if these pollutants are in the form of refractory organics. Thus, it can be necessary to use more effective processes for the destruction of such contaminants [40].

The main problems in the treatment of yeast wastewater are the high concentration of COD in the effluent, color, odor, sulfate, and a high amount of excess sludge generated in the wastewater treatment process. If a higher degree of purification is required, biological treatment can be used in combination with other processes such as advanced treatment processes, and these processes are detailed in the following section.

#### 10.3.3.3.1 Membrane Process

Membrane processes have found wide applications for the treatment of aqueous-based systems involving material recovery, reuse, and for pollution prevention [87]. The role of the membrane, as shown in Fig. 10.7, is to serve as a selective barrier that will allow the passage of certain constituents and will retain other constituents

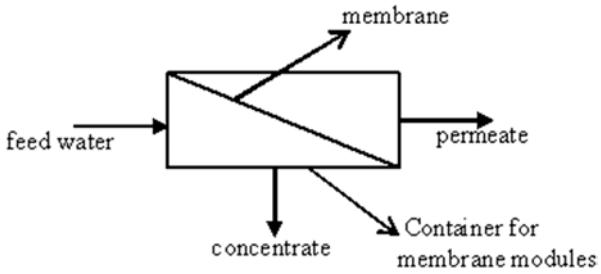



**Fig. 10.7** Definition sketch for a membrane process [75]

found in the liquid. The influent to the membrane module is known as the feed stream (also known as feedwater). The liquid that passes through the semi-permeable membrane is known as permeate (also known as the product stream or permeating stream), and the liquid containing the retained constituents is known as the concentrate (also known as the retentate, reject, retained phase, or waste stream). The rate at which the permeate flows through the membrane is known as the rate of flux, typically expressed as kg/m$^2$ day [76].

Based on the different driving forces applied, the range of separations can be divided into various filtration processes (microfiltration, ultrafiltration, nanofiltration, and reverse osmosis), gas and vapor separation, pervaporation, and electromembrane processes (including electrodialysis, membrane electrolysis, and bipolar membrane processes). Additionally, based on preferential wetting properties, porous membranes have been used as a support for liquid membranes and for various contactor applications (including membrane-based solvent extraction and gas absorption). These processes usually focus on the desired separation of a gas or liquid mixture [87]. Membrane distillation (MD) is a thermally driven membrane process in which a hydrophobic microporous membrane separates a hot and cold stream of water [88].

The ability of the membrane depends on the size of pores, types of materials, types of wastewater, solubility, and retention time. Permeability, flux, transmembrane pressure (TMP), and resistance are the parameters that also need to be considered. Membrane structure plays an important role in transporting mechanism whether the structure is parallel or in series. The types of membranes used are different depending on the size of contaminants contacting during the treatment process [89]. Microfiltration membranes having the largest pore size (0.1–0.2 μm) among the categories can separate microbial cells for their subsequent recycling to the bioreactor to ensure high cell concentration and thus high productivity. Ultrafiltration membranes with an average pore size much less than that of the microfiltration membranes can retain cells and proteins. Separation by microfiltration and ultrafiltration membranes is based on size exclusion, and for effective cell harvesting, at least 100–300 kDa molecular weight cutoff (MWCO) value should be ensured for this. Nanofiltration membranes between reverse osmosis and ultrafiltration membranes with an average pore size of 1 nm can separate cells, proteins,

nutrients, salts, and unconverted carbon sources from lactic acids. Reverse osmosis normally known as nonporous membrane, where separation is based on solution diffusion mechanism, can separate the same components as nanofiltration membranes do but at much higher operating pressure than what is needed in nanofiltration from fermentation [90].

Considering the large diversity of membranes suited for technical applications, it will be useful to introduce the membrane classifications as membrane materials, membrane cross-section, preparation method, and the membrane shape [91].

Two types of materials that are commonly used to construct membranes are polymeric and ceramic. The ceramic membrane is usually used by industrial wastewater, which has a good performance in filtration compared to polymer due to its high chemical resistance, inertness, and ease to clean. The polymer membrane (porous membrane) has its own weakness that it can foul easily because of its hydrophobic characteristic. However, the hydrophobic membrane weakness can be improved by coating the membrane using hydrophilic polymer. There are two types of operation: dead-end and cross-flow operation. Cross-flow is liquid flow parallel toward a filter surface and transports suspended particles of membrane surface by permeating flow due to pressure drop. Basically, this type of filtration is carried out by using hollow fiber (HF), flat sheet (FS), or multi tubular (MT). Cross-flow filtration can reduce the formatting of the cake layer on the surface of the membrane [89]. A membrane can be homogeneous or heterogeneous, symmetric or asymmetric in structure, and solid or liquid; it can carry a positive or negative charge or be neutral or bipolar. Transport through a membrane can be affected by convection or by diffusion of individual molecules, induced by an electric field or concentration, pressure, or temperature gradient. The membrane thickness may vary from as small as 10 μm to a few hundred micrometers [92].

After membrane treatment, membrane concentrates containing high amounts of recalcitrant organics that must be disposed of. To avoid this problem, the concentrates are recycled back to the biological treatment plant influent, but this would cause a continuous increase of recalcitrant and toxic or inhibitory pollutants in the biological treatment system.

In order to overcome such a drawback, it has been proposed that, before being recycled back, membrane concentrates can be treated by ozone. After ozone treatment, recalcitrant pollutants are transformed to more easily biodegradable pollutants as well as degradation of toxic pollutants that inhibit biomass activity. Membrane treatment systems with UF and/or RO for biologically pretreated high-strength fermentation industry effluents are very effective in the removal of COD, color, $NH_3$-N, and conductivity. The final disposals of the concentrates from the membrane systems are major problems in these types of applications. Koyuncu et al. [41] stated that the biodegradability of the concentrates from the RO systems could be increased significantly by applying advanced oxidation with ozone and ozone + $H_2O_2$. Additional $BOD_5$ in the ozonated concentrates can be treated by the existing aerobic biological treatment facilities [41].

There have been many applications of membrane processes for the treatment of color and COD from the baker's yeast wastewater. Mutlu et al. 2002 studied the

decolorization of baker's yeast wastewater with membrane processes. Maximum rejections obtained were 94%, 89%, and 72% for optical density, color, and COD, respectively, when 0.8-μm microfiltration membranes and 400-D nanofiltration membranes were used in series [93].

Pretreatment of spentwash with ceramic membranes prior to anaerobic digestion is reported to have the COD from 36.000 to 18.000 mg/L [94]. The total membrane area was 0.2 $m^2$, and the system was operated at a fluid velocity of 6.08 m/s and 0.5 bar transmembrane pressure. In addition to COD reduction, the pretreatment also improved the efficiency of the anaerobic process, possibly due to the removal of inhibiting substances.

Kumaresan et al. [95] employed the emulsion liquid membrane technique in a batch process for spentwash treatment. Water–oil–water type of emulsion was used to separate and concentrate the solutes resulting in an 86 and 97% decrease in COD and BOD, respectively.

Electrodialysis has been explored for desalting spentwash using cation and anion exchange membranes resulting in 50–60% reduction in potassium content [96]. In another study, Vlyssides et al. [97] reported the treatment of vinasse from beet molasses by electrodialysis using a stainless steel cathode, titanium alloy anode, and 4% w/v NaCl as an electrolytic agent. Up to 88% COD reduction at pH 9.5 was obtained; however, the COD removal percentage decreased at higher wastewater feeding rates. In addition, reverse osmosis (RO) has also been employed for fermentation wastewater treatment. A processing effluent was obtained after anaerobic digestion, followed by a hold-up in a tank maintained under aerobic conditions in a RO system. Furthermore, 290 $m^3$/day of RO treated effluent is mixed with 300 $m^3$/day of freshwater and used in the process for various operations like molasses dilution, make-up water for cooling tower, fermenter washing, etc. Another unit employs disc and tube RO modules for direct treatment of the anaerobically digested spentwash. Permeate is discharged while the concentrate is used for biocomposting with sugarcane pressmud. In the other study, Decloux et al. [98] have studied electrodialysis to reduce the potassium concentration to prevent the crystallization and even increase the final dissolved solids of the concentrated vinasses and have found the process successful and the results encouraging.

Nataraj et al. [99] reported pilot trials on fermentation spentwash using a hybrid nanofiltration (NF) and RO process. Both the NF and RO stages employed thin-film composite membranes in spiral wound configuration. NF was primarily effective in removing the color and colloidal particles accompanied by 80%, 95%, and 45% reduction in total dissolved solids (TDS), conductivity, and chloride concentration, respectively, at an optimum feed pressure of 30–50 bar. The subsequent RO operation at a feed pressure of 50 bar resulted in 99% reduction each in COD, potassium, and residual TDS.

#### 10.3.3.3.2 Oxidation Process

The goal of any AOP design is to generate and use hydroxyl free radical ($HO^{\bullet}$) as a strong oxidant to destroy compounds that cannot be oxidized by conventional oxidants. Although AOPs have many advantages, one common problem in all AOPs is the high electrical energy demand for UV lamps, which causes high operational costs [22]. Advantages of ozonation in treatment applications are removal of toxicity, destruction of organic matter, and enhancement of the biodegradability of recalcitrant wastewaters [25].

Chemical oxidation, especially ozonation, has already been demonstrated to be an effective means of removing refractory and/or toxic chemicals from water and wastewater. Ozonation reduces color as well as the organic matter content due to its ability to react with unsaturated bonds (olefins-aromatics) that are responsible for the coloration of wastewaters [45]. Ozone reacts with aqueous organic pollutants found in water and wastewater via two different pathways, namely direct molecular ($pH \leq 2$) and indirect ($pH \geq 7$) radical chain-type reactions. The ozonation reaction pathway strongly depends on the characteristics of the wastewater to be treated, i.e., pH, concentration of ozone decomposition initiators, promoters, and scavengers in the reacting medium.

However, ozonation alone still has low TOC and COD removal due to some refractory small molecular organic compounds produced in an aqueous solution. This fact has led to the research on how to enhance the efficiency of ozonation for various applications, and many advanced oxidation processes (AOPs), such as $O_3$/$H_2O_2$, UV/$O_3$, and catalytic ozonation, have been developed.

Homogeneous oxidation with the Fenton reagent occurs in the presence of ferrous or ferric ions with hydrogen peroxide via a free radical chain reaction that produces hydroxyl radicals. It is considered to be a metal-catalyzed oxidation reaction, in which iron acts as the catalyst. Process efficiency is closely related to the solution pH whose optimal values are between 2 and 4 as well as the COD:$H_2O_2$:catalyst ratio in the feed. Moreover, efficiency can be enhanced in the presence of UV irradiation as more hydroxyl radicals are produced in the so-called photo-Fenton reaction.

Heterogeneous semi-conductor photocatalysis using $TiO_2$ as the photocatalyst is an emerging technology with key advantages including operation at ambient conditions as well as the fact that the catalyst itself is inexpensive, commercially available at various crystalline forms and particle characteristics, nontoxic, and photochemically stable. From a mechanistic point of view, illumination of an aqueous $TiO_2$ suspension with irradiation with energy greater than the band gap energy of the semiconductor generates valence band holes and conduction band electrons. Holes and electrons may either undesirably recombine liberating heat or make their separate ways to the surface of $TiO_2$, where they can react with species adsorbed on the catalyst surface. Valence band holes can react with water and the hydroxide ion to generate hydroxyl radicals, while electrons can react with adsorbed molecular oxygen reducing it to superoxide radical anion, which, in turn, reacts with protons to

form peroxide radicals. Besides $TiO_2$, ZnO and CdS have also been employed as photocatalysts in water treatment [100].

Çatalkaya and Şengül [22] investigated decolorization and mineralization of baker's yeast industry effluents by photochemical advanced oxidation processes (AOPs) utilized UV with $H_2O_2$ and photo-Fenton. The optimum $H_2O_2$ concentration and irradiation time were found to be 5 mM and 50 min at pH 3 for UV/$H_2O_2$ processes. In the photo-Fenton process application, maximum decolorization efficiency (96.4%) was obtained at the optimum reaction conditions that were 100 mM $H_2O_2$ and 1 mM $Fe^{2+}$ doses at pH 3 and 10 min of irradiation time [22].

Altınbaş et al. [40] studied the effectiveness of chemical oxidation by applying ozonation, ozonation with hydrogen peroxide, and Fenton's processes for decolorization and residual COD removal of biological pretreated baker's yeast industry effluents. In Fenton's oxidation studies, the removal efficiencies of COD and color for a reaction time of 30 min for three different types of baker's yeast industry effluents were found about 86 and 92%, respectively. Unit costs of Fenton oxidation for the post-treatment of baker's yeast industry effluents are in the range of $0.6–1.9 per cubic meter depending on the initial COD levels of the waste [40].

Ozone destroys hazardous organic contaminants and has been applied for the treatment of dyes, phenolics, pesticides, etc. [72]. Oxidation by ozone could achieve 80% decolorization for biologically treated spentwash with simultaneous 15–25% COD reduction. It also resulted in improved biodegradability of the effluent. However, ozone only transforms the chromophore groups but does not degrade the dark-colored polymeric compounds in the effluent [72, 101]. Similarly, oxidation of the effluent with chlorine resulted in 47% color removal, but the color reappeared after a few days [74]. Ozone in combination with UV radiation enhanced spentwash degradation in terms of COD; however, ozone with hydrogen peroxide showed only marginal reduction even on a very dilute effluent [102].

Peña et al. [45] showed that continuous ozonation was effective for the decolorization of molasses wastewater. Operating with a hydraulic residence time of 45 min and an applied ozone mass flow of 1.7 g/h, color and COD reductions were about 80% and 14%, respectively The results showed that color reduction was mainly due to direct oxidation reactions between ozone and chromophore groups, whereas the indirect reaction pathway contributed to the reduction of the organic matter content [45].

Altınbaş et al. [40] cited that the best COD and color removals at an ozonation time of 80 min were observed as 43 and 96%, respectively, in ozonation processes. Ozonation always requires a significantly high initial investment cost compared to Fenton's oxidation [40].

Blonskaja et al. [25] studied the treatment of yeast industry wastewaters using ozone. They showed that the post-ozonation of biological treatment resulted in the reduction of 30–49% COD and consumed ozone dosage ranged from 1.2 to 2.5 mg ozone/mg removal COD [25].

Blonskaja and Zub [15] studied post-treatment of biologically treated wastewater from yeast factories. They reported that coagulants and ozone could be used in the process of the post-treatment of effluents of yeast industry for the purpose of

decreasing the color and general concentration of pollutants, but these processes are very expensive [15].

Inanç et al. [103] studied color removal from biological treatment effluent of baker's yeast industry with massive lime and ozone treatment. The optimum lime dose for reducing the color to values around 1000 Pt-Co was found as 10.0 g/L, while 0.9 g/L ozone was necessary to obtain the same residual color. Economical evaluation has indicated that the cost of lime treatment was 1.3–1.4 US$/m$^3$ while it was 2.5 USD/m$^3$ for ozone treatment. Color, COD, total phosphorus, and ammonia removed were 84.3%, 89.5%, 99.9%, and 31.1% with 20 g/L lime, respectively. It was possible to remove all the apparent colors and produce clear effluent at prolonged contact periods [103].

The industrial applications of ozonation are limited by its high costs and low ozone utilization caused by the mass transfer rate of ozone [104].

Pala and Erden [14] studied Fenton oxidation using biologically pretreated baker's yeast effluent. The best $Fe^{2+}/H_2O_2$ dosage was 1200 mg/L $Fe^{2+}$/800 mg/L $H_2O_2$ at pH 4 and in a reaction time of 20 min for mineralization of dissolved organic carbon (DOC) and COD. For these conditions, the maximum color removal efficiency was obtained as 99%, and maximum DOC and COD removal efficiencies were obtained as 90% and 88%, respectively [14].

Another option is photocatalytic oxidation, which has been studied using solar radiation and $TiO_2$ as the photocatalyst [105]. The use of $TiO_2$ was found to be highly effective as the destructive oxidation process leads to the complete mineralization of effluent to $CO_2$ and $H_2O$. Up to 97% degradation of organic contaminants was achieved in 90 min. Pikaev et al. [106] studied combined electron beam and coagulation treatment of distillery slops from distilleries processing grain, potato, beet, and some other plant materials. Humic compounds and lignin derivatives constitute the major portion of this dark brown wastewater. The distillery wastewater was diluted with municipal wastewater in the ratio of 3:4, irradiated with an electron beam, and then coagulated with $Fe_2(SO_4)_3$. The optical absorption in the UV region was decreased by 65–70% after this treatment. The cost was found to be less than the existing method wherein the effluent was transported about 20 km via pipeline to a facility for biological treatment followed by sedimentation. The treatment cost was 0.45–0.65 US$/m$^3$, which dropped to 0.25 US$/m$^3$ using the combined electronic-beam and coagulation method.

Can and Genç [107] studied the pretreatment of fermentation wastewater by photocatalytic oxidation. They showed the effects of photocatalytic oxidation process on color removal and improvement in biodegradability by observing the BOD/COD ratio. It was seen that the ratio increased from 0.11 to 0.18 at the end of the irradiation time of 120 min [107].

The advanced treatment of biologically treated baker's yeast wastewater for the purpose of irrigation reuse was studied by Balcıoğlu et al. Baker's yeast wastewater was treated using combined ozonation with the membrane process. The wastewater was treated with NF90 and BW 30 membranes after ozonation, and 96–98% color and 56% COD removals were obtained with ozonation at pH 7.5 and 25 °C. The treated wastewater was classified as class B considering pH, $BOD_5$, SS, and fecal

coliform parameters. It was met the criteria of II class in terms of SAR parameter. They reported that the concentrate obtained from the membrane process could be circulated back to the existent unit of the primary sedimentation tank in the baker's yeast mill [108].

#### 10.3.3.3.3 Electrochemical Processes

Electrochemical oxidation processes are environmentally friendly emerging methods for the decontamination of wastewaters contaminated with toxic and persistent herbicides, pesticides, chlorophenols, nitrophenols, polychlorinated biphenyls, pharmaceuticals, etc. Electrochemical reactions are heterogeneous ion transfer reactions that ionic compounds left from the electrolyte to be oxidized or reduced over anode or cathode.

Electrocoagulation (EC) involves the generation of coagulants in situ by dissolving electrically either aluminum or iron ions from respectively aluminum or iron electrodes. The metal ion generation takes place at the anode, and hydrogen gas is released from the cathode. The hydrogen gas would also help float the flocculated particles out of the water. The electrodes can be arranged in a mono-polar or bi-polar mode. The materials can be aluminum or iron in plate form or packed form of scraps such as steel turnings, millings, etc. The nascent $Al^{3+}$ or $Fe^{2+}$ ions are very efficient coagulants for particulates flocculating. The advantages of electrocoagulation include high particulate removal efficiency, compact treatment facility, relatively low cost, and possibility of complete automation [109].

The supply of current to the electrocoagulation system determines the amount of $Al^{3+}$ or $Fe^{2+}$ ions released from the respective electrodes. The quality of the treated water depends on the amount of ions produced (mg) or charge loading, the product of current, and time (Ah). For aluminum, the electrochemical equivalent mass is 335.6 mg/(Ah). For iron, the value is 1041 mg/Ah [109]. The value of the required $Al^{3+}$ power consumption for removing color is 0.04–0.1 mg $Al^{3+}$ and 10–40 Wh/$m^3$ and for preliminary purification is 0.1–0.2 mg $Al^{3+}$ and 40–80 W h/$m^3$, respectively [110].

Electroflotation (EF) is a simple process that floats pollutants to the surface of a water body by tiny bubbles of hydrogen and oxygen gases generated from water electrolysis. Therefore, the electrochemical reactions at the cathode and anode are hydrogen evolution and oxygen evolution reactions, respectively. The pollutant removal efficiency is largely dependent on the size of the bubbles formed. In an EF reactor, usually, an anode is installed at the bottom, while a stainless steel screen cathode is fixed at 10–50 mm above the anode. Such an electrode arrangement cannot ensure quick dispersion of the oxygen bubbles generated at the bottom anode into wastewater flow, affecting flotation efficiency [109].

In the literature, there are a lot of studies interested in electrochemical technologies, including EC and EF; however, there are only a few studies on the electrochemical treatment of fermentation industry wastewaters.

In a study by Vlyssides et al. [97], a number of experiments have been conducted in a laboratory-scale pilot plant using $Pt/TiO_2$ anode in the presence of sodium chloride as a supporting electrolyte. Influent COD of 72,000 mg/L has been reduced to 8000 mg/L in effluent with an 89% COD removal. According to the authors, treatment efficiency depends on the catalytic activity of the anodes used, the COD loading rates, and the pH of the solution.

Manisankar et al. [111] have researched the effect of halides in the electrochemical treatment of distillery effluent using anodized graphite plate anodes and graphite cathodes. The effect of pH and current density on the treatment has been studied. Sodium fluoride, sodium chloride, and sodium bromide have been chosen as electrolytes, and their influence has been studied by the authors. Complete decolorization has been reached in all cases. A maximum of 93% of BOD reduction, 85% of COD reduction, and 98% absorbance reduction have been obtained in the presence of sodium chloride as a supporting electrolyte.

Electrochemical oxidation over anodes made of graphite, Pt, $TiO_2$, $IrO_2$, $PbO_2$, several Ti-based alloys, and, more recently, boron-doped diamond electrodes in the presence of a suitable electrolyte (typically NaCl) has been employed for the decontamination of various organic-containing effluents. Two mechanisms are responsible for organic matter electrochemical degradation, namely (a) direct anodic oxidation where the pollutants are adsorbed on the anode surface and destroyed by the anodic electron transfer reaction and (b) indirect oxidation in the liquid bulk, which is mediated by the oxidants that are formed electrochemically; such oxidants include chlorine, hypochlorite, hydroxyl radicals, ozone, and hydrogen peroxide [100].

Gengec et al. studied electrocoagulation for removals of color, COD, and TOC from baker's yeast effluent in a batch electrocoagulation reactor using aluminum electrodes. The maximum color, COD, and TOC were 88%, 48%, and 49% at 80 A/$m^2$, $pH_i$ 4, and 30 min for anaerobic effluents and 86%, 49%, and 43% at 12.5 A/$m^2$, $pH_i$ 5, and 30 min for anaerobic–aerobic effluents, respectively. The operating costs for anaerobic and anaerobic–aerobic effluents at the optimized conditions were 0.418 €/$m^3$ and 0.076 €/$m^3$, respectively [7].

#### 10.3.3.3.4 Wet-Air Oxidation Process

Wet-air oxidation (WAO) is a technology used to treat the waste streams that are too dilute to incinerate and too concentrated for biological treatment. It can be defined as the oxidation of organic and inorganic substances in an aqueous solution or suspension by means of oxygen or air at elevated temperatures and pressures either in the presence or absence of catalysts. According to this method, the dissolved or suspended organic matter is oxidized in the liquid phase by some gaseous source of oxygen, which may be either pure oxygen or air. Typical conditions for WAO are 150–320 °C for temperature, 2–15 MPa for pressure, and 15–120 min for residence time; the preferred COD load ranges from 10 to 80 kg/$m^3$. WAO destroys toxics in industrial wastewater by breaking down complex molecular structures into simpler

components such as water and carbon dioxide, without emissions of $NO_x$ $SO_2$, HCl, dioxins, furans, and fly ash. It is reported that the WAO process is capable of a high degree of conversion of toxic organics with more than 99% destruction rate; however, some materials are not oxidized completely to carbon dioxide and water; instead, some intermediate compounds are formed, which represent a quarter of the original mass of organic matter [110].

By using homogeneous catalysts (Cu, Fe, Cu-Mn-Fe, Fe/$H_2O_2$, etc.) and heterogeneous catalysts (Co/Bi, Sn/Bi, Zn/Bi, Ni/Bi, $Bi(OH)_3$ or Ru-Rh, Pt-Pd, Ru, Mn-Zn-Cr, etc., and rare metals attached on alumina (Pt/$Al_2O_3$, Pd/$Al_2O_3$, Ce/$Al_2O_3$)), it is possible to reach higher reaction rates at lower temperature and pressure. Complete oxidation of organic materials can be achieved by applying severe operating conditions; however, these conditions necessitate high operating costs. Operating costs can be decreased appreciably by using the process as a step of pretreatment where partial oxidation is achieved at moderate conditions prior to conventional biological treatment. When this process is considered a pretreatment, the inhibition–toxic effect of degradation by-products to the biological system has to be recognized. The aqueous phase formed as a consequence of the wet oxidation process contains low-molecular-weight organic compounds, inorganic acids, and inorganic salts. The residual of most of the pollutants in the aqueous phase is an advantage of this process [112].

A combination of WAO and adsorption has been successfully used to demonstrate the removal of sulfates from distillery wastewater. Studies were done in a counter-current reactor containing 25-cm base of small crushed stones supporting a 20-cm column of bagasse ash as an adsorbent [113]. The wastewater was applied from the top of the reactor, and air was supplied at the rate of 1.0 L/min. The treatment removed 57% COD, 72% BOD, 83% TOC, and 94% sulphates. WAO has been recommended as part of a combined process scheme for treating anaerobically digested spentwash [114]. The post-anaerobic effluent was thermally pretreated at 150 °C under pressure in the absence of air. This was followed by soda-lime treatment, after which the effluent underwent a 2-h wet oxidation at 225 °C. Also, 95% color removal was obtained in this scheme.

#### 10.3.3.3.5 Ultrasound Process

Various researchers have reported several biological, physicochemical, and phytoremediation approaches for the treatment of yeast industry wastewater [115]. However, all these studies have only concentrated on the removal of color/melanoidin from the yeast industry and not on the holistic issue of biodegradability enhancement. A new technology such as cavitation, which has found several applications [116–118], can be effectively utilized as a pretreatment option for refractory/recalcitrant wastes. Cavitation is defined as the formation, growth, and subsequent collapse of microbubbles or cavities in extremely little time intervals as micro to milliseconds, releasing large magnitudes of energy over a small region but at multiple spots in the reactor [119]. Although ultrasonic cavitation is a costly option

owing to its ineffective spatial distribution of cavities on a large scale and less effective transducer outputs at higher operating frequencies [115], an alternative cavitation phenomenon generated by manipulating the liquid flow pattern termed hydrodynamic cavitation is reported to be more energy efficient over acoustic cavitation for some application and even can find large-scale applications [115, 120].

In hydrodynamic cavitation, cavities are formed by passing the liquid through the constriction/geometry provided in lines such as venturi and orifice plate. When the pressure at the throat or vena contracta of the constriction falls below the vapor pressure of the liquid, the liquid flashes, creating a number of vaporous cavities that subsequently collapse when the pressure recovers downstream of the mechanical constriction. The effects of cavity collapse are in terms of the creation of hot spots, releasing highly reactive free radicals, surface cleaning and/or erosion, and enhancement in local transport (heat, mass, and momentum) rates. The collapse of bubbles generates localized "hot spots" with a transient temperature of the order of 10.000 K and pressures of about 1000 atm. Under such extreme conditions, water molecules are dissociated into $OH^{\bullet}$ and $H^{\bullet}$ radicals. These $OH^{\bullet}$ radicals then diffuse into the bulk liquid medium, where they react with organic pollutants and oxidize/mineralize them. The two main mechanisms for the degradation of pollutants using hydrodynamic cavitation are the thermal decomposition/pyrolysis of the volatile pollutant molecules entrapped inside the cavity during the collapse of the cavity and secondly, the reaction of OH• radicals with the pollutant occurring at the cavity–water interface. In the case of nonvolatile pollutants, the main mechanism for the degradation of pollutants will be the attack of hydroxyl radicals on the pollutant molecules at the cavity–water interface and in the bulk fluid medium. The mechanical effects are also significant. In some cases, the intensity of shockwaves generated by the collapsing cavity can break molecular bonds, especially the complex large molecular weight compounds. The broken-down intermediates are more amenable to $OH^{\bullet}$ attack as well as biological oxidation, which can further enhance the rate of oxidation/mineralization of the pollutants in subsequent biological treatment [117].

The literature reports on the application of hydrodynamic cavitation are very scanty and limited only to synthetic wastewater containing only specific pollutants such as textile dyes, pharmaceutical drugs, and pesticides [115, 121]. The use of hydrodynamic cavitation reactors in real industrial wastewater treatment applications has been rarely investigated. Chakinala et al. [115] have tried hydrodynamic cavitation (HC) in conjunction with the advanced Fenton process for the treatment of real industrial wastewater and reported that hydrodynamic cavitation is very effective as a pretreatment to biological oxidation for the effluent samples considered in their work.

The best and most practical utilization of cavitation in an energy efficient manner can be made by using it as a pretreatment in combination with any other advanced oxidation system or conventional biological treatment systems. Hydrodynamic cavitation (HC) was evaluated as a pretreatment for complex wastewater such as biomethanated distillery wastewater. The biomethanated distillery wastewater was subjected to an HC reactor, and the effect of various process parameters was assessed and optimized for maximizing COD/TOC reduction and enhancing the

biodegradability index of the wastewater. The associated color reduction has also been achieved.

In another study, Sangave and Pandit [122] employed sonication of distillery wastewater as a pretreatment step to convert complex molecules into a more utilizable form by cavitation. Samples exposed to 2 h ultrasound pretreatment displayed 44% COD removal after 72 h of aerobic oxidation compared to 25% COD reduction shown by untreated samples. These results are contrary to those of Mandal et al. [74], who concluded ultrasonic treatment to be ineffective for distillery spentwash treatment.

### *10.3.4 Case Studies: Wastewater Treatment Plants and Related Regulations*

Combined anaerobic and aerobic treatment processes are commonly applied to the baker's yeast industry. Anaerobic first stage is also a two-phase system: acid production phase and methane production phase. The anaerobic treatment system has several units including a buffer tank, an effluent pumping station, an acid reactor, two methane reactors, vacuum degasifiers, lamella separators, a gas storage tank, a boiler system, and a flare of emergency. Anaerobic reactors are constructed as UASB reactors. Lamella separators and vacuum degasifiers are added to prevent the washout of the biomass from the system. The aerobic second stage is designed and operated as an extended aeration-activated sludge system with a special selector unit at the beginning. The main treatment units of the aerobic stage are the selector compartment, the aeration basin with four equal aerated cells connected in series, and the final sedimentation tank with a sludge recirculation facility. Surface aerators are used for aeration. In these processes, high-strength wastewaters are fed to the anaerobic reactors, while low-strength wastewaters are directly fed to the aerobic process. The major factor affecting COD removals is high initial and microbial inert COD [3]. The typical wastewater treatment plant scheme of the baker's yeast industry applied to Turkey is given in Fig. 10.8.

The "İzmit Industrial and Domestic Wastewater Treatment Plant" is a plant where raw domestic wastewaters and pretreated industrial wastewaters of various sectors such as tire, drug, chemical, and yeast industries that have been discharged to collectors are treated biologically [123]. Wastewater discharge limits for this collector are shown in Table 10.6 [124]. Wastewater discharge limits are provided by such yeast industry with conventional biological treatment. In order to improve the performance of the treatment plant, studies on the addition of evaporator and membrane units are carried out.

In Turkey, industries are obliged to treat their effluent to the standards specified for the category where they belong to the environmental legislation and as described in the Water Pollution Control Regulations (12.31.2004/25687), before being discharged into the receiving medium. Discharge standards for the yeast industry are given in Table 10.7 [125]. It is difficult to ensure the standard of color parameter given in

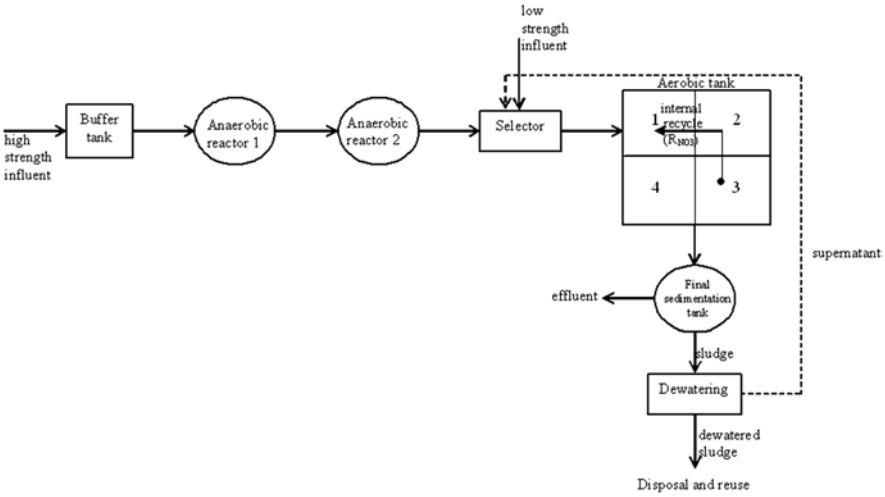



**Fig. 10.8** The another typical wastewater treatment plant scheme of baker's yeast industry [11]

**Table 10.6** Pretreatment limit values for Kocaeli Municipal Wastewater Treatment Plant [124]

| Parameters | Limit values | Parameters | Limit values |
|---|---|---|---|
| BOD (mg/L) | 250 | Copper (mg/L) | 2 |
| COD (mg/L) | 800 | Lead (mg/L) | 3 |
| SS (mg/L) | 350 | Nickel (mg/L) | 5 |
| Total nitrogen (mg/L) | 40 | Zinc (mg/L) | 5 |
| Total phosphate (mg/L) | 10 | Mercury (mg/L) | 0.5 |
| Detergent (mg/L) | 5 | Silver (mg/L) | 5 |
| Arsenic (mg/L) | 10 | Cyanide (mg/L) | 10 |
| Antimony (mg/L) | 3 | Phenol (mg/L) | 10 |
| Tin (mg/L) | 5 | Sulfur (mg/L) | 2 |
| Barium (mg/L) | 3 | Temperature | 40 °C |
| Cadmium (mg/L) | 2 | pH | 6–9 |
| Chrome | 5 | – | – |

**Table 10.7** Effluent standards of baker's yeast industry in Turkey [125]

| | | Table 5.2: Sector: Food industry (yeast production) | |
|---|---|---|---|
| Parameter | Unit | Composite sample (2 h) | Composite sample (24 h) |
| Chemical oxygen demand (COD) | (mg/L) | 1200 | 1000 |
| Suspended solids (SS) | (mg/L) | 200 | 100 |
| Oil and grease | (mg/L) | 60 | 30 |
| pH | | 6–9 | 6-9 |
| Color | (Pt-Co) | 280 | 260 |

regulation with conventional treatment process at the baker's yeast industry. It is reached to this limit with applying advanced treatment process as post-treatment.

Another full-scale wastewater treatment plant, RomPak Baker's Yeast Company, Pascani, Romania, was investigated by Ifrim et al. [67]. The maximum flow of the WWTP is ~4000 $m^3$/day. The effluent of the baker's yeast wastewater treatment plant meets the requirements of NTPA 002/2002 normative being sent to the municipal plant. The researchers determined a strategy for nitrogen removal from baker's yeast wastewater in order to increase the yield and rate of nitrogen compound bioconversion. According to this strategy, the effluent of the baker's yeast wastewater is mixed with the recycled sludge from the clarifier in the selector (Fig. 10.9). Then, nitrification and denitrification exist in the tank, which is an assembly of concentrically tanks. In the middle, there is the denitrification zone, and in the outside, there is the nitrification area. The volume of these areas is 960 $m^3$ for the denitrification zone and 2880 $m^3$ for the nitrification zone. The best efficiency, almost 90%, is obtained on nitrification–denitrification processes, and they have stated that the advanced biological treatment is completely ecological, and it does not need chemicals for pretreatment processes [67].

Because of the high amount of wastewater pollutants, which should be discharged into the sewerage network, a pretreatment is necessary for clearing partially industrial effluents so treated waters comply with either standard conditions for direct wastewater discharge into the local sewerage networks and wastewater treatment plants (NTPA 002/2005) or standard load limits of pollutants from industrial wastewater discharge into the natural receptors (NTPA 001/2005). Nevertheless, by the usage of wastewater treatment into a single anaerobic stage, the purified wastewaters are not able to comply with standard discharges requirements for baker's yeast industries. For these reasons, the effluents of the anaerobic treatment process

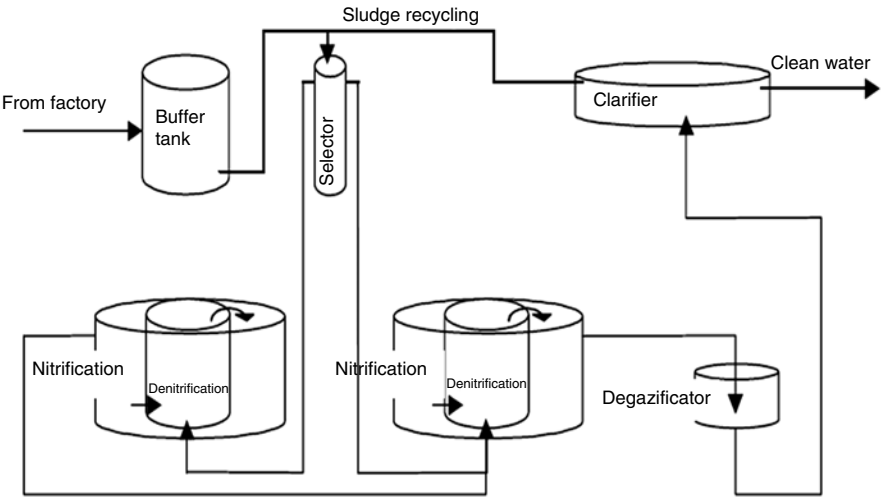



**Fig. 10.9** Biological treatment plant of baker's yeast wastewater [67]

should be further treated by the other treatment technology in order to fulfill the NTPA 002/2005 requirements [33].

Industrial fermentation plays an important role in Taiwan, where sugarcane is one of the most important crops. Water quality items and limits for effluent standards for enterprises, sewage systems, and building sewage treatment facilities are given in Table 10.8 for Taiwan [126].

## 10.4 Sludge Management

Wastewater treatment generates sludge, which in turn must be either disposed of or used. Sludge management begins with sludge generation and continues through sludge processing and ultimate disposal [127]. Sludge treatment and disposal operations on a local or regional basis need careful planning to ensure that the strategy undertaken is environmentally acceptable, reliable, and cost-effective [128].

The product-specific waste from the food industry is characterized by its high proportion of organic material. The disposing of this waste can be difficult for biological stability and the potential growth of pathogens, high water content, rapid autoxidation, and changes due to enzymatic activity [129].

Generally, the baker's yeast industry is equipped with a full wastewater treatment plant comprising an anaerobic pretreatment phase and an aerobic post-treatment phase. The anaerobic phase of the treatment generates very little excess sludge. The aerobic treatment, however, results in the generation of relatively large amounts of excess sludge. The physical and chemical characteristics of a typical baker's yeast industry sludge are provided in Table 10.9 [130].

Generally, the heavy metal content of baker's yeast industry sludge is lower than the maximum allowable heavy metal content for agricultural use. The agricultural use of such sludges with organic carbon and high nitrogen content is preferred environmentally and ecologically [131].

Anaerobic sludge is a potential source of organic matter, nutrients, and minerals and may be useful as an agricultural soil supplement. Freshly digested sludge is

**Table 10.8** Effluent characteristics and limits in Taiwan [126]

| Applicable scope | Effluent characteristics | Effluent limits |
|---|---|---|
| Fermentation industries (brewing industry; MSG production industry, wine or liquor, alcohol and vinegar production industries; soy sauce production industry; and antibiotic and organic solvent manufacturing industries) | Biological oxygen demand (BOD) (mg/L) | 50 |
| | Chemical oxygen demand (COD) (mg/L) | 150 |
| | Suspended solids (mg/L) | 50 |
| | True color | 550 |

**Table 10.9** Physical and chemical characteristics of a typical baker's yeast industry sludge [130]

| Parameter | Typical value (*) |
|---|---|
| pH | 8–8.5 |
| Total moisture content (%) | 78–84 |
| Total volatile solids (%) | 8–11 |
| Total nitrogen (%) | 6.5–7 |
| Total phosphorus, as $P_2O_5$ (%) | 3–3.5 |
| Lead (mg/kg) | 47 |
| Cadmium (mg/kg) | 11 |
| Chromium (mg/kg) | 18 |
| Copper (mg/kg) | 61 |
| Nickel (mg/kg) | 45 |
| Mercury (mg/kg) | 0.4 |
| Zinc (mg/kg) | 56 |

(*) The metal concentrations are based on the dry weight

unstable under normal environmental conditions as it is biodegradable, has an unpleasant odor, and contains various noxious or corrosive gases such as $NH_3$ and $H_2S$. Therefore, it must be stabilized before it can be adequately disposed of in the natural environment. All of these problems can be overcome by composting, which is an obvious solution to this problem, where all unwanted by-products can be reduced to an acceptable level.

There are a few management alternatives for the direct disposal of digested sludge. With a lack of other options, mechanically dehydrated sludge can be dried to 90% with the use of biogas and utilized as an alternative solid fuel in various industrial kilns using various methods of energy production. The net calorific value of dry sludge is approximately 10–12 MJ/kg, with an ash content of approx. 35–45% [132].

The excess sludge withdrawn from a biological treatment system usually contains 93–97% water. In order to significantly reduce the amount of sludge to be handled, the sludge is dewatered using equipment such as thickeners, centrifuges, belt filter press, and filter press. Dewatered sludges usually contain 20–30% dry matter. In some cases, sludge can be applied directly on agricultural land for the purpose of irrigation and also to provide nutrients such as nitrogen and phosphorus. When the sludge cannot be directly applied onto land, it must be treated to stabilize it and then properly disposed of either on plant property or off-site at an appropriate landfill, or composted for the purpose of being used as a soil conditioner.

In proper waste management, reuse/recovery is considered to be very high in the hierarchy and composting is regarded as an appropriate management method that results in a beneficial product where nutrients such as nitrogen and phosphorus are returned to the soil for uptake rather than being unnecessarily burnt in an incinerator or disposed at a landfill [130].

### *10.4.1 Description of Composting Operation*

High-moisture (greater than 60%) organic wastes represent a rather unique management problem. Direct application to land is possible, but such practice is usually limited to rural areas where sufficient land is available. Composting can be particularly effective in converting wet materials to a more usable or easily disposable form. At the same time, composting can stabilize putrescible organics, destroy pathogenic organisms, and provide significant drying of the wet substrate. All of these advantages are obtained with minimal outside energy input: the major energy resource being the substrate organics themselves. Furthermore, composting is a flexible process: it can be viewed as a conversion process to produce a material suitable for reuse or simply as a stabilization and drying process to provide easier disposal. Composting is also compatible with a wide variety of feedstocks [133]. Most of the compost was produced from bio-waste (4.8 Mt), green waste (5.7 Mt), the rest from sewage sludge (1.4 Mt), and mixed waste (1.4 Mt) [134].

Composting is the biological decomposition of organic material. The process can be represented in general terms as follows:

$$\text{Fresh organic waste} + O_2 \rightarrow \text{stabilized organic residue} + CO_2 + H_2O + \text{heat} \quad (10.1)$$

The extent of reaction is synonymous with the extent of decomposition (degree of stabilization), and it is a determinant of product quality [135].

Aerobic composting is the decomposition of organic substrates in the presence of oxygen (air). Anaerobic composting is the biological decomposition of organic substrates in the absence of oxygen. The principal differences between these two processes are summarized in Table 10.10 [136].

**Table 10.10** Comparison of aerobic and anaerobic composting processes [136]

| Characteristic | Aerobic process | Anaerobic process |
|---|---|---|
| Energy use | Net energy consumer | Net energy producer |
| End products | Humus, $CO_2$, $H_2O$ | Sludge, $CO_2$, $CH_4$ |
| Volume reduction | Up to 50% | Up to 50% |
| Processing time | 21–30 days | 20–40 days |
| Primary goal | Volume reduction | Energy production |
| Secondary goal | Compost production, waste stabilization | Volume reduction, waste stabilization |

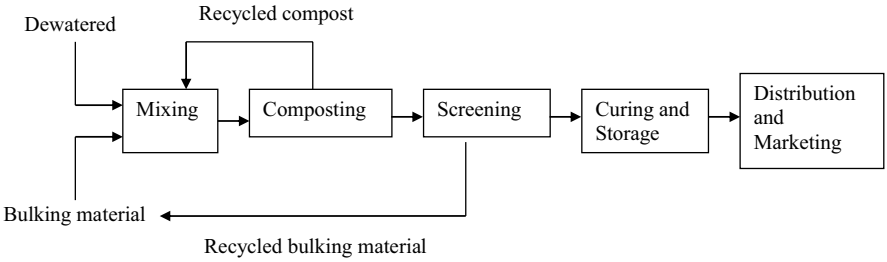



**Fig. 10.10** The basic stages in composting of dewatered sludge [137]

Most sewage sludge composting operations are composed of two sources (sludge and bulking agent), one process (composting), and one sink (utilization or disposal of the end product). They represent the four main issues that must be considered in any compost plant. The basic stages in any composting operation may include dewatering, mixing, composting, curing, and screening (Fig. 10.10). Sludge must be dewatered prior to composting, so it can be handled as a semisolid and not as a liquid. The sludge is dewatered to approximately 25% solids and 75% moisture content. In the mixing stage, dewatered sludge is mixed with the bulking agent. The objective is to avoid sludge compactness by creating spaces for air circulation and, if needed, to provide enough extra carbon for a suitable C/N ratios [137].

Dewatered sludge cake is not a bulky material, and it lacks the porosity of materials such as straw or refuse. Because of its plastic nature, sludge also tends to compact under its own weight, which further reduces the void volume [133]. The mixture of bulking agents and sludge is composted with three techniques. After 21 days of active composting, the material is further stabilized at a 30-day curing period. The purpose of screening is to separate the finished compost from the bulking agent [137].

Wastewater treatment plant sludge hass high water content, low organic contant, low porosity, and high viscosity. Therefore, for effective composting process, wastewater treatment plant sludge must be mixed with leaves, sawdust, wood, straw, compost amendment, or with the city life rubbish compost. Aerobic composting processes of sewage sludge are mainly controlled by factors such as feed moisture content, ventilation system, temperature, pH value, C/N, and conditioner. The requirements for mixing composting sludge with raw material are moisture content of 50–60%, pH 5–9, C/N 25–35/1, organic matter content of 20–80%, and 12–60 mm size [138].

The integration of some organic wastes of the food industry in the composting of sewage sludge might enhance the composting process and provide more aromatic moieties for humic acid synthesis. In addition, the ratio of sludge:bulking agent:organic food waste (i.e., initial C:N ratio) and the texture of the bulking agent influence the composting reaction rate and the final compost quality [139].

The aeration provides oxygen, which is essential to avoid anaerobic conditions and to carry out the aerobic decomposition of the organic matter. Because of that, the aeration affects microbial activity, substrate degradation rate, and temperature variation of the process. In general, the main aeration methods providing oxygen during composting are physical turning of the mass, natural convection, and forced aeration [140]. Using the forced-air (positive pressure) aeration that blows air from the bottom of the composting container to diffuse air upward for aeration will maintain a favorable composting environment to reduce the composting time to about 28–35 days. A major disadvantage of this method is the emission of odorous gas to the environment causing serious pollution. The negative-pressure aeration uses perforated pipes placed near the bottom of the composting reactor and connected to a blower. When the blower is turned on to withdraw air from the reactor, negative pressure is created in the reactor [141].

Oxygen feedback controllers have been used to enhance the composting process. Several ranges of oxygen concentration in the exhaust gases have also been recommended. It has been stated that oxygen levels between 5 and 20% (v/v) result in an optimum range, a minimum of not less than 8% [140]. The oxygen uptake rate controller is recommended for the airflow regulation in composting systems with automatic control [137, 139].

Temperature controllers are based on the removal of excess heat accumulated in the composting matrix. In most cases, by satisfying the needs of the temperature criteria, the oxygen requirement is also met [140]. If the thermophilic phase period of the composting process (T > 65 °C) has persisted for more than a few days, the compost produced may be considered sanitized and free of pathogens [132]. Biodegradability of waste sludge from the yeast industry in in-vessel aerobic composting was investigated [142]. The maximum temperature reached was 60 °C in in-vessel composting (C/N ratio of the raw sludge at approximately 12.5).

Stabilization is an oxidation process of the sludge to a resistant form, not causing any adverse environmental effect after the disposal of the organic fraction of sludge. Composting is a stabilization process. The stabilization degree of treatment sludges is not defined completely yet, but various stabilization indicators are given in the literature. Some indicators that can be used in biological stabilization under aerobic conditions are decreases in temperature, in organic content, and in oxygen uptake rate, presence of nitrate, absence of ammonia and starch, odor reduction, and increase of redox potential [123, 133, 143].

Degremont [144] describe the criteria that can be used for assessing stabilization as follows: sludge respiration rate determines the stabilization degree (the limit is 0.10–0.15 $kgO_2$/kg organic matter/day in endogenous phase); during aerobic stabilization, dissolved oxygen in the sludge liquid should be 2 mg/L after 120 h continuous aeration and loss dry matter should not exceed 10% by weight.

### *10.4.2 Composting Techniques*

Composting is a bioremediation technique for the food industry waste such as fruit and vegetable processing industry, meat and poultry, and fermentation industry. There are several composting types with the same general stages but differ in capital and operating costs and in the ways that they use to achieve the proper conditions for bacterial growth and in the time required for completing their task. The methods employed may be classified into three general categories [144, 145].

1. Windrow: In a windrow system, a mixture of dewatered sludge and the bulking agent is constructed from 1 to 2 m high and 2 to 4.3 m at the base. The rows are turned and mixed periodically during the composting period. Under typical operating conditions, the windrows are turned a minimum of five times while the temperature is maintained at or above 55 °C [146]. The conventional windrow and aerated windrow processes are viable sludge-disposal options [147].
2. Aerated static pile: The aerated static pile system consists of a grid of aeration or exhaust piping over which a mixture of dewatered sludge and the bulking agent is placed. Material mixed of dewatered sludge and the bulking agent is composted for 21–28 days and is typically cured for another 30 days or longer. Typical pile heights are about 2–2.5 m. A layer of screened compost is often placed on top of the pile for insulation. Perforated pipe is commonly used for air supply [146]. Feed mixture could be composted by either natural ventilation or intermittent aeration [148]. Screening of the cured compost is usually done to recover the bulking agent.
3. In-vessel: In-vessel composting is accomplished inside an enclosed container or vessel. Mechanical systems are designed to minimize odors and process time by controlling environmental conditions such as airflow, temperature, and oxygen concentration [146]. Drying and destruction of volatile solids were highest with high aeration rates and low process temperature. The highest composting rates were achieved when temperatures were kept below 60 °C by high airflow rates [149].

The characteristics of each method are given synoptically in Table 10.11 [145].

Different composting technologies are applied in industrial facilities. The method selection is dependent on the investment and operation cost, the time required to reach compost stability and maturity, the availability of land, and the origin of raw materials. Among the available methods for composting, closed vessel technologies, such as the tunnel technology, can be defined as the most sophisticated and environmentally controlled systems and are successfully implemented as high capacity facilities in populated areas. In contrast, the open-air pile system is the simplest and requires the lowest investment [140].

**Table 10.11** Composting methods [145]

| Method | Composting time | Cost | Usage | Disadvantages |
|---|---|---|---|---|
| Windrow | 2–6 months for municipal solid waste | Low | Used mainly in combination with in-vessel technology for curing the compost | Difficult control of conditions, temperature, water concentration odor |
| Aerated piles | 6–12 weeks | Medium | Used for sewage sludges, municipal solid waste, yard wastes, and industrial organic wastes | Continued electrical costs |
| In-vessel | Less than a week to 2 weeks | High due to installation costs | All types of waste | High cost, intense and skillful management |

**Table 10.12** Maturity indices [154]

| Respirometry rates | Very mature | Mature | Immature |
|---|---|---|---|
| mg $CO_2$-C/gVS/day | <2 | 2–8 | >8 |

### *10.4.3 Compost Quality*

The compost quality depends on the content of pollutants such as heavy metals, persistent organic pollutants, pathogenic bacteria, and inert matter in the mature compost. The properties of the standard compost leachate must also be considered. Heavy metals and persistent organic pollutants accumulate during the composting process and may cause problems upon utilization. The content of heavy metals and persistent organic pollutants is determined by the quality of the input material, which should be carefully controlled in sewage sludge or in the raw wastewater, and by additional treatment and reduction at the source [132].

Nutrient loss, specifically N, can be a major problem in composting. The lower initial C:N ratio had a major effect on N loss. Apart from N loss, P, K, and Na loss can also be significant [150].

Fresh organic waste materials cannot be applied to the soil until they have been sufficiently biostabilized because the application of immature organic materials to soil may affect plant growth due to nitrogen starvation and production of toxic metabolites [151]. During composting, germination and plant growth bioassays showed the toxicity of immature compost. The reason for immature compost toxicity suggested in the literature includes high concentrations of volatile organic acids, high concentration of $NH_4^+$-N, oxygen depletion, or the presence of heavy metals [152]. Methods such as chemical and physical analyses, microbiological assays, plant bioassays, spectroscopy, and degree of humification were developed to assess compost maturity [153]. The maturity of compost was evaluated with the maturity indices (Table 10.12) [154].

**Table 10.13** Physical and chemical characteristics of a typical compost material [130]

| Parameter | Typical value |
|---|---|
| pH | 7.2 |
| Total moisture content (%) | 60 |
| Total nitrogen (%) | 3 |
| Total phosphorus, as $P_2O_5$ (%) | 4 |

The degree of humification can be determined with spectrophotometric ratios. The low values of spectrophotometric ratio reflect a higher degree of aromatic condensation and indicate a higher level of organic matter humification. The slight increase observed in spectrophotometric ratios indicates a relative increase in compounds with phenolic and benzene-carboxylic groups in the structure of the humic substances [139].

Several important soil microbiological properties, such as substrate-induced respiration, potential ammonium oxidation, and nitrogen mineralization, were improved after the application of both leftover material from biogas production and composting. The genetic structure of the soil bacterial community appeared to resist changes caused by the addition of organic waste [155].

Waste sludge obtained from the baker's yeast industry is composted by using the static aerated pile method. Composting was conducted under experimental conditions using wood chips as a bulking agent, a sludge:bulking agent ratio of 1:1.5, and an amount of air supply of 30 $m^3$/h-ton dry sludge. The physical and chemical characteristics of a typical compost material are presented in Table 10.13 [130].

Eriçyel [156] investigated the composting process to treat biosolid waste sludge from the baker's yeast industry. Two types of bulking agents (hazelnut husk and shredded cornstalk) were used to lower the moisture content of the biosolids to increase porosity and to add a source of carbon. At the end of the study, it was proven that hazelnut husk and shredded cornstalk can be considered as a good bulking agent for the composting of biosolids. The optimal ratio for composting biosolids from the yeast industry was 1:1:1 (biosolid:shredded cornstalk:hazelnut husk). Furthermore, it was obviously shown that when compost was applied at high ratios (10%), additional fertilizer was not needed [156].

Treatments frequently digest or compost their sewage sludge to reduce the level of pathogens and odors. The degree to which a sludge is processed is very important when placing sewage sludge in monofills or on surface disposal sites in order to eliminate the spread of pathogenic diseases [127].

The degradation rate of aerobic digestion was higher than that of composting. Genç et al. [131] stated that at the 13th day of the composting specific organic

nitrogen removal rate was 0.85 mg organic N/g VS/day while at the same time 3.54 mg organic N/g VS/day was measured in digestion. The specific organic carbon removal rate was determined to be 9.19 mg organic C/g VS/day on 13th day of composting while 20 mg organic C/g VS/day in digestion [131].

In the composting facilities, exhaustive controls of the biological risks should be carried out. These controls should include measurements in the compost (in its different phases from elaboration) and in the air (different zones of the facility) of the concentrations of bioaerosols, paying special attention to the Gram-negative bacteria and the fungus *A. fumigatus*. Moreover, the environmental concentrations of VOCs should also be determined, and the personnel should be biologically monitored with certain regularity [157]. Unpleasant odors may be released only during the mechanical turning of the material. For the aerated piles, an air collection system, fitted with a biofilter, is planned. Dust emissions from periodical mechanical turnover are not considered critical when the compost is properly wetted; noise emissions can be controlled by the selection of appropriate low noise equipment [132].

### *10.4.4 Control of Nuisance Conditions*

It is important to engineer appropriate systems for the control of nuisance conditions during the design of a compost facility. Potential nuisances most often associated with composting are odors and dusts [133].

#### 10.4.4.1 Control of Dust Formation

Dust control in a compost facility can be a major problem, particularly in semiarid climates. Municipal sludge organics are characterized by a small-sized particle distribution. When sludge-based compost exceeds about 65–75% solids, the small-sized particles can be easily airborne if agitated. At higher moisture contents, dust is generally not a significant problem. Therefore, dust conditions can potentially occur any time dry compost is handled. This would include turning or agitation of dry windrows, screening dry compost from bulking particles, loading of trucks, and so on. Another potential source of dust is fugitive emissions that may occur from the operation of mobile equipment on dry, unpaved surfaces [133].

The presence of bacteria and fungi is fundamental to the composting process. Agitation of the composting material through shredding, turning, and screening results in the liberation of these organisms into the air, commonly termed a bioaerosol. The concern regarding bioaerosols from composting activities arises because of their potential to cause adverse health effects in employees and the public living in close proximity to such facilities [158]. The airborne release appeared to be related to the amount of microorganisms in the wastes, the design of the site, and the operational procedures used in the composting plants. Airborne microorganisms were

monitored at different composting facilities. *A. fumigatus* and mesophilic bacteria were used as the principal monitoring parameters. The composting plants all showed levels of both airborne microorganisms in the $10^3$–$10^5$ cfu/$m^3$ range in the operating area, making it advisable for the staff to use protective masks [159].

Most of the control measures to avoid excessive dust formation are reasonably straightforward and include the following [133]:

1. For open systems, turning or agitation should be discontinued when the material exhibits noticeable dust formation, which should begin at about 65–70% solids.
2. Controlled aeration systems can be used to reduce the turning frequency.
3. Paved surfaces should be used whenever possible.
4. Mixing of wet cake and compost product in the open should be avoided whenever possible.
5. Operations with a high dust potential, such as mixing and screening, should be conducted in an enclosed reactor or building.
6. Compost can be pelleted before drying or storage to increase particle size.

#### 10.4.4.2 Odor Control

Control of odors is one of the most difficult problems in current sanitary engineering practice. Both inorganic and organic compounds can be malodorous. Hydrogen sulfide ($H_2S$) and ammonia ($NH_3$) are the most common inorganic odorants. Organic sources generally come from low-molecular-weight, more volatile organic compounds (VOCs) [133]. The VOCs produced from composting typically include nitrogen-based compounds, sulfur-based compounds, volatile fatty acids, hydrocarbons, trepans, esters, ethers, alcohols, and aldehydes/ketones [133, 159, 160]. Compost-generated VOCs can be evaluated in terms of two processes: the production within the pile due to organic matter degradation and emission at the pile surface after gas convection within the pile. VOC production within the pile was different from emission at the pile surface. The total mass of VOC production was 1.09 g C/kg dry matter, which was 2.3 times as high as the total mass of emission [160].

The main sources for odorous emissions occur during material handling and during processes where the material is moved. These include delivery of the biowaste, pre-processing such as shredding and screening, and the composting process itself, especially during turning and the final screening. A large portion of odorous emissions can be avoided by ensuring optimal composting conditions. If the biowaste is too wet and not well aerated, malodors often occur as a result of partly anaerobic processes [162].

In a windrow system, frequent turning can help prevent anaerobic pockets in the material. The traditional method to aerate a pile of compost depends on mechanical equipment to turn over the material twice daily to once every other day. The odorous emission is difficult to control with mechanical aeration [141]. If odor persists in an aerated system despite adequate aeration, this may be due to compaction of the

materials and channeling of the air. A turn to remix the compost may solve the problem. In sludge composting, the choice of bulking agent might also be important, as a range of particle sizes will help air movement through the heap [133].

Off-site emissions seem to be the main problem at open outdoor facilities. To avoid these emissions, one option is to enclose at least parts of the process. The aeration process provides operations to reduce emissions. Forced aeration would increase fugitive emissions, whereas if vacuum-induced (negative-pressure) aeration is practiced, the waste gas can be captured and treated. At enclosed composting plants, off-gases are captured and treated [162].

A number of techniques are available to reduce the odor concentration in exhaust gases collected during composting. Techniques such as absorption by scrubbing or condensation, bioscrubbers, adsorption, oxidation by thermal, chemical, or biological means, and use of masking agents are available to the designer [133, 161]. The most relevant systems are bioscrubbers and biofilters [162].

Most exhaust gases collected during composting will be saturated with water vapor (or nearly so) at temperatures above ambient. As these gases cool, condensation of water vapor will occur. Because of this, it is necessary to provide water traps in ducting used to transport such gases. As condensation occurs, it is likely that water-soluble species in the gas will be absorbed into the condensate.

Gas absorption is a unit operation in which one or more soluble components of a gas mixture are dissolved in a liquid. Scrubbing with water or chemical solutions ($KMnO_4$, NaOCl, alkalies, or various acids) can affect a reasonably consistent 45–60% reduction in odor concentration [133]. Bioscrubbing is a process of biological waste gas treatment in which exhaust air is "washed" in an absorber with a scrubbing liquid. The scrubbing liquid is subsequently drawn off and transferred to an activation tank in which the constituents absorbed into the liquid are degraded by microorganisms. The liquid is continuously cycled through the process [162]. A biofilter is a fixed-bed reactor filled with biologically active packing material. Microorganisms settled on the media feed on the organic compounds that are contained in the waste gas. Biofilters usually are combined with wet scrubbers. The scrubbers are used to humidify the air passing to the filter in order to avoid drying of the filter material. Frequently used biofilter media are compost, peat, root wood, bark, wood chips (normally used as a bulking agent), and various combinations [163]. One important property of the media is the ability to store water [161, 163]. Adsorption involves contacting a fluid phase (gas or liquid) with a particulate phase that has the property of selectively taking up and storing one or more solute species from the fluid. Compost deodorization piles have accomplished measured odor reductions averaging 50%, but with wide variations in removal efficiency [133]. The produced unseparated compost is used as a biofilter to capture and biologically oxidize the landfill gas (methane) and other organic compounds found in the landfill gas [132]. In this system, a moistened-bulk solid medium, such as soil or composted sludge, provides the contact surfaces for microbiological reactions to oxidize odorants [146]. The air is passed through a heap of mature compost, which acts as a biological filter. Odorous molecules dissolve in the film of water on the compost particles, and residual micro-organisms from the composting process then break

down the compounds, effectively removing the odor problem [164]. A pilot-scale active aeration reactor was studied for composting food wastes in an open-top container aerated with negative pressure by vacuum. A biological filter bed filled with mature compost was used to remove $NH_3$ from the emission. Using the biological filter to remove $NH_3$, the emission contains less than 1 ppm of $NH_3$ [141]. Oxidation processes result in the oxidation of organic species to carbon dioxide and water, or partial oxidation to other intermediate compounds. In masking agents, perfume scents can be added to the gas stream to mask or combine with an objectionable odor. Masking agents have limited application in compost facilities since more effective techniques are available [133, 145].

The process of waste decomposition in composting facilities releases a variety of odors, airborne particles, and bioaerosols. They cause infections or irritations to humans, especially to sensitive or sick people. Park et al. [165] studied the simultaneous removal of odors, airborne particles, and bioaerosols in a composting facility by dielectric barrier discharge. The removal efficiency of contaminants in the air increased as the specific energy densities (SED) increased, with removal efficiencies of up to 80% and 76% being achieved for ammonia and amines, respectively. The removal efficiency of the overall airborne particles was 75% when 113 J/L of SED was employed, while the bioaerosols removal efficiency was 89% when 38 J/L of SED was used [165].

A complete odor control system consists not only of the collection and treatment of odorous gases but also their proper dispersal into the atmosphere. Design of systems for treatment and dispersal must be coordinated to assure that ground-level ambient odor standards are not violated [133].

### *10.4.5 Land Application of Sludge*

Food industry waste sludge is commonly applied to the land in a number of ways. Applications of sludge to agricultural and nonagricultural lands are the following:

1. Land application to agricultural lands: The method of applying sludge to agricultural land depends on the physical characteristics of the sludge and soil and the crops grown. Liquid sludge may be applied with tractors, tank wagons, irrigation systems, or special application vehicles. Liquid sludge may also be injected under the surface layer of the soil. Dewatered sludge, on the other hand, is typically applied to cropland by equipment similar to that used for applying limestone, animal manures, or commercial chemical fertilizer. Generally, the dewatered sludge is applied to the land surface and then incorporated by plowing or disking. When applied to pasture land, sludge is usually not incorporated into the soil [127].
2. Land application to nonagricultural lands: Sludge is most often sprayed from mobile equipment into established forest stands as a partially dewatered, but still liquid, material. Other types of nonagricultural land application include sewage

sludge applied to public contact sites (e.g., parks, cemeteries, golf courses) and reclamation sites. When sewage sludge is used to stabilize and re-vegetate land at reclamation sites, typically large amounts of sludge are applied on a one-time basis. This large amount is necessary to ensure that sufficient organic matter and nutrients are introduced into the soil to support vegetation until a self-sustaining ecosystem is established [127].

Physical site characteristics of concern include topography, soil permeability, site drainage, depth to groundwater, subsurface geology, proximity to critical areas, and accessibility [146].

Landfilling and land application of the sewage sludge are suggested to be the most economical sludge disposal methods. Sludge amendment to the soil modifies its physicochemical and biological properties. Land application of the sewage sludge is becoming more popular due to the possibility of recycling valuable components such as organic matter, N, P, and other plant nutrients. Organic matter added to the soil as sewage sludge composts improved the soil properties, such as bulk density, porosity, and water holding capacity. The chemical properties of sludge–soil mixture not only depend on the properties of the soil and sludge and the application rates of the mixtures, but also depend on their interaction and soil pH. Sewage sludge amendment increased the soil microbial activity, soil respiration, and soil enzymes activities. In general, it has been shown that the addition of sludge to agricultural land increases the growth and production of crop plants. The increase of crop yield as a result of sludge application often exceeded that of well-managed fertilized controls [166].

Prior to applying sludge to cropland, the following tests should be run: nitrogen content and forms, phosphorus, potassium, heavy metals, percent solids, and tests required by local health agencies. Prior to applying sludge to a particular site, the soil should be tested for pH, cation exchange capacity, and phosphorus and potassium availability. Crop selection would have to be consistent with local farming practices and locally marketable. Site work includes drainage control, monitoring wells, pH adjustment by lime addition, and field preparation. The application rate calculation is frequently based mainly on the nitrogen requirements of the crop to be grown. The computed rate is then adjusted, if necessary, to prevent excessive heavy metal or phosphorus buildup [167].

Plant availability of heavy metals differed widely among the crop species. The accumulation of Cd, Ni, and Zn in the plants showed the greatest increases compared to their background levels. The Cu and Pb accumulation in the plants grown on sludge-amended soils showed only small increases compared to those grown on uncontaminated soil. Multiple regression analyses of various soil properties showed that the surest way to control the accumulation of metals in food plants is by controlling their concentrations in the soil [39].

The aim of Council Directive 86/278/EEC of the European Communities is to regulate the use of sewage sludge in agriculture in such a way as to prevent harmful effects on soil, vegetation, animals, and human beings. Values for concentrations of heavy metals in soil to which sludge is applied, concentrations of heavy metals in sludge, and the maximum annual quantities of such heavy metals that may be introduced into soil intended for agriculture are given in Table 10.14 [168].

**Table 10.14** Limit values for concentrations of heavy metals in soil in sludge for use in agriculture and the maximum annual quantities of heavy metals that may be introduced into the soil [168]

| **Annex 1A** | |
|---|---|
| Limit values for concentrations of heavy metals in soil (mg/kg of dry matter in a representative sample of soil with a pH of 6–7) | |
| Parameters | Limit values[a] |
| Cadmium | 1–3 |
| Copper[b] | 50–140 |
| Nickel[b] | 30–75 |
| Lead | 50–300 |
| Zinc[b] | 150–300 |
| Mercury | 1–1.5 |
| Chromium[c] | – |
| **Annex 1B** | |
| Limit values for heavy-metal concentrations in sludge for use in agriculture (mg/kg of dry matter) | |
| Parameters | Limit values |
| Cadmium | 20–40 |
| Copper | 1000–1750 |
| Nickel | 300–400 |
| Lead | 750–1200 |
| Zinc | 2500–4000 |
| Mercury | 16–25 |
| Chromium[c] | – |
| **Annex 1C** | |
| Limit values for the amount of heavy metals that may be added annually to agricultural land, based on a 10-year average (kg/ha/year) | |
| Parameters | Limit values[a] |
| Cadmium | 0.15 |
| Copper | 12 |
| Nickel | 3 |
| Lead | 15 |
| Zinc | 30 |
| Mercury | 0.1 |
| Chromium[c] | – |

[a] Member States may permit the limit values they fix to be exceeded in the case of the use of sludge on land, which at the time of notification of this Directive is dedicated to the disposal of sludge but on which commercial food crops are being grown exclusively for animal consumption. Member States must inform the Commission of the number and type of sites concerned. They must also seek to ensure that there is no resulting hazard to human health or the environment

[b] Member States may permit the limit values they fix to be exceeded in respect of these parameters on soil with a pH consistently higher than 7. The maximum authorized concentrations of these heavy metals must in no case exceed those values by more than 50%. Member States must also seek to ensure that there is no resulting hazard to human health or the environment and in particular to groundwater

[c] It is not possible at this stage to fix limit values for chromium. The Council will fix these limit values later on the basis of proposals to be submitted by the Commission, within one year following notification of this Directive

Trace metals are trapped in the soil matrix, and nutrients are taken up by plants and converted into useful biomass. The principal metal of concern is cadmium because it can accumulate in plants to levels that are toxic to humans and animals but below levels that are toxic to plants (phytotoxic) [146].

The soil conditioning properties of sludge are more significant than the nutrient additions. Mismanagement of a land application system can result in public health problems, odor nuisance, and/or soil destruction from excessive heavy metal buildup. A land application system must be designed to provide maximum benefits from the sludge without creating problems. The design should therefore include rate determination schedules and methods to be used by operators [167].

The land application systems for sludges should be designed properly not to exceed the maximum assimilation capacity of the land and the product growing on it. Some factors like the scarcity or absence of suitable land area to apply sludge directly and adverse climatic conditions for sludge application make the composting process suitable for obtaining a stabilized product that can be applied to land easily in the sludge assessment [130].

## 10.5 Specific Subjects

### *10.5.1 Control of $H_2S$ in Biogas Generating from Anaerobic Treatment of Fermentation Industry*

Wastewaters containing organic matter and sulfate are generated by many industrial processes that use sulfuric acid (food and fermentation industry) or sulfate-rich feedstocks (sea food-processing industry). Also, the use of less oxidized sulfurous compounds in industrial processes results in the generation of sulfate-rich wastewaters. Sulfate concentration in the molasses fermentation industry is relatively high, approximately 4000 mg $SO_4^{2-}$/L. Although the COD/$SO_4^{2-}$ ratio is not very high, inhibition problems in the anaerobic treatment due to $H_2S$ toxicity are manifested [66].

Many processes have been proposed and employed to remove sulfide from gas streams and sulfide-rich wastewaters. Can Doğan (2008) studied the effectiveness of the control of $H_2S$ gas formed in biogas together with methane as a result of anaerobic treatment of fermentation industry wastewater with the autotrophic denitrification process. High removal efficiencies obtained with low hydraulic retention times revealed sulfide oxidation with the autotrophic denitrification process in which nitrate or nitrite is used as an electron acceptor. In this study, loading rate and molar loading ratios ($NO_3^-/S^{2-}$ and $NO_2^-/S^{2-}$) did not affect the sulfide removal efficiency. However, elemental sulfur formation as a by-product or sulfate formation as an end product as a result of denitrification were closely related to the loading ratio [4].

Removals of sulfide from wastewater of the fermentation industry were applied by using continuous flow stirred tank reactor under anoxic conditions. The stoichiometry of sulfide oxidation with nitrate is calculated assuming different end-products based on the thermodynamic approach and compared with experimental yield values. The calculated maximum volumetric and specific sulfide oxidation rates reached 0.076 kg $S^{2-}/m^3$ h and 0.11 kg $S^{2-}$/kg VSS h, respectively [169]. Nitrite can be used instead of nitrate as the electron acceptor to remove sulfide in the autotrophic denitrification process. When the nitrite to sulfide ratio was above 1.48, the end product was mainly sulfate. Otherwise, as the value of the ratio was under 1.48, the distribution of end products was shifted to the mixture of sulfate and elemental sulfur [170]. The results are obtained at industrially relevant conditions and can be easily adapted to either the biogas cleaning process or to sulfide-containing effluent streams.

Krapivina et al. [171] studied anaerobic mesophilic fermentation of sulfate-containing yeast industry wastewaters at the laboratory scale with anaerobic sequencing batch reactors (ASBR). COD removal of 75–82% was achieved at volume loading rates up to 7.7–8.0 kg COD/$m^3$day and at a COD/$SO_4^{2-}$ ratio of 8.0. Also, the best results for sulfate removal (99%) were achieved in the CSRB, with the concentration of sulfide in the reactor effluent being about 10 mg/L. In the large-scale experiments, the instability of the processes could create significant difficulties in applying the ASBR technology for the treatment of yeast wastewaters. In addition to the inhibition of the process, sulfide formation also costs major malodor problems and corrosion of equipment during the experiment. Accumulation of sulfides was an indication that competition between methanogenous and SRB was won by the latter [171].

### *10.5.2 Vinasse*

The main discharge of yeast fermentation is called vinasses, and it consists of different organic compounds such as betaine, glycerol, and various reducing sugars. Vinasse is a substance by fermentation of molasses. The characteristics of vinasses depend mainly on the raw material used. Vinasse with high concentrations of soluble solids can be obtained when sugarcane, sugar beet, grape, avages, or sweet sorghum are used [172]. Dominant colored compounds are in molasses from the degradation of sugars. Under conditions of high temperature and acidic or alkaline pH, the nonenzymatic reactions of the hexoses may produce melanoidins, invert alkaline degradation products, and caramels. The Maillard reaction occurs in the presence of amine compounds and monosaccharides; brown products are created. During the heating of the sugar with ammonium compounds, a series of heterocyclic compounds are formed. In recent years, the toxicological profile of these compounds has been examined. The type, quantity, and characteristics of toxic colored

compounds of vinasse result in the potential utilization of this by-product being highly limited [173].

Major organic components of sugarcane vinasse are glycerol, lactic acid, ethanol, and acetic acid, whereas sugar beet vinasses also contain glycerol and their main compound is nitrogen-rich betaine, and cellulose and hemicellulose. Both dilute and concentrated vinasse can be spread on agricultural fields or used as organic fertilizer [174]. Vinasses can be used as fertilizer due to their nutrient content, mainly calcium and potassium, and their high organic material content. Vinasses contain phytotoxic, antibacterial, and recalcitrant compounds. Highly colored compounds lead to reduced sunlight penetration in rivers and lakes. Reductions in soil alkalinity, manganese availability, and seed germination inhibition have been reported by the use of these wastes in agriculture. Previous treatment of these wastes is therefore needed before their final disposal. There are many proposals for physicochemical and biological treatments for vinasses. The combination of different processes where the first step was an anaerobic treatment followed by an aerobic or physicochemical process gives better results in the removal of organic load and color. Treated vinasses can be used in agriculture without the risk of polluting soil, underground water, or crops [172].

The composition of compost derived from urban waste is often far from ideal for plant nutrition, and vinasses applied to fields can be easily washed away. However, the addition of vinasses to solid urban wastes before the fermentation steps can both improve the compost formation process and allow problems associated with the separate materials to be overcome [175].

Vinasses from sugarcane molasses were evaluated as a new source of sugarcane wax. Nuissier et al. [176] showed that 1 L of alcohol produced in rum processing, thus 20 L of vinasses, could lead to the recovery of approximately 3.4 g of crude wax.

Vinasse is a suitable feedstuff in the feeding of ruminants since most of its crude proteins comprise amide-substance and are palatable to ruminants. The nutritional value of vinasse as ruminant feed was reported. The use of vinasse as a feed additive in poultry and pigs and the dosage used in ruminant diets were reported to show its influence on animal performance [177]. Despite being a highly polluting effluent, vinasse could be used in the production of single-cell proteins for feed supplementation due to its high carbon content. The generated microbial biomass by biological treatment of vinasse with yeast presented a low anti-nutritional value and an average protein content of 46.85% [178].

Molasses vinasses always have a high level of organic content such as crude proteins, lactic acid, glycerol, cholesterol, amino acid, and reducing sugars (COD in the range of 80,000–100,000 mg/L and BOD in the range of 40,000–50,000 mg/L) and have strong odor and dark brown color, and they also contain nutrients in the form of nitrogen (1660–4200 mg/L), phosphorus (225–3038 mg/L), and potassium (9600–17,475 mg/L). Recently, many research teams focused on the study of preparing nutriment fodder using molasses vinasses. However, the dosage of molasses vinasses was also limited because unexpectedly high potassium content in molasses vinasse could lead to diarrhea in bulls and pigs. Various techniques have been proposed to reduce the potassium level in molasses vinasses [179].

Another alternative for vinasse consumption is combustion. Molasses spentwash containing 4% solids can be concentrated to a maximum of 40% solids in a quintuple-effect evaporation system with thermal vapor recompression [178, 179]. The condensate with a COD of 280 mg/L can be used in fermenters. The concentrated other liquor is spray dried using hot air at 180 °C to obtain a desiccated powder with a calorific value of around 3200 kcal/kg. The powder is typically mixed with 20% agricultural waste and burnt in a boiler. The use of recirculating fluidized bed (RCFB) incinerator is recommended to overcome the constraints due to the stickiness of spentwash and its high sulfate content [180]. Combustion is also an effective method of on-site vinasse disposal as it is accompanied by the production of potassium-rich ash [181] that can be used for land application. Experience with the treatment of vinasse indicates the following trends:

1. Anaerobic digestion, complemented by oxidative chemical treatments (e.g., ozonation), is usually placed as a pretreatment.
2. Aerobic treatment alone and combined with ozonation, which have been directed to remove phenolic compounds and color, have been successfully applied.
3. Physicochemical treatments such as Fenton, electro-oxidation, oxidants, and so on., which are now mostly at the lab-scale stage, have demonstrated a significant removal of recalcitrant organic compounds.
4. Fungal pretreatment with chemical treatment followed by oxidative ($O_3$) or anaerobic digestion seems to give attractive results.
5. Vinasses may be co-composted with solid organic wastes, particularly with those from agricultural activities and agro-industry.

Unicellular protein, biohydrogen, and enzyme production from vinasses could be promising ways to improve the economic feasibility of vinasses treatment. Raw vinasses can be filtered to give two main streams: vinasses and solids rich in yeast and microbial biomass. The latter can be processed to generate an alternative protein source for animal feed or other uses. Filtered vinasses could be subjected to a variety of biotechnological processes in order to give diverse added-value bioproducts: special bioproducts, enzymes, protein (yeast, algal, bacterial, fungal), and soil amenders by co-composting vinasses with agro-industrial and other wastes. In parallel, filtered vinasses could be used for methane and biohydrogen production either as separate stages or as a series of biohydrogen-methane processes. Treated vinasses from bioenergy stages can be directed to co-composting of agro-industrial and other wastes or post-treated with AOP or biological processes before discharge or reuse in irrigation in such a way to close the environmental circle [182].

### *10.5.3 VOC Emissions*

The VOC emissions are generated as by-product of the fermentation process. The two major by-products are ethanol, which is formed from acetaldehyde, and carbon dioxide. These by-products form as a result of excess sugar present in the fermenter

or an insufficient oxygen supply to the fermenter. Under these conditions, anaerobic fermentation occurs and results in the excess sugar being broken down to form alcohols and carbon dioxide. When anaerobic fermentation occurs, 2 moles of ethanol and 2 moles of carbon dioxide are formed from 1 mole of glucose [183].

The two types of control measures that are currently employed at yeast manufacturing facilities are (1) process control and (2) add-on controls [181, 182].

The ethanol production rate is a function of the yeast growth rate, and both of these parameters are related to the residual sugar concentration. By continuously adding only the exact amount of molasses required by the fermentation, conditions of excess sugar are eliminated, thus minimizing ethanol formation. The most common add-on control devices for controlling VOC emissions are wet scrubbers, carbon adsorbers, incinerators, condensers, and biological filtration. A combination system could result in lower control costs and relatively equivalent emission reductions [183]. At facilities manufacturing dry yeast, VOCs may also be emitted from the yeast dryers used to dry the yeast [184].

### *10.5.4 Biogas Production*

Industrialization exerts considerable pressure upon natural resources, along with an increased demand for energy. In addition, the waste generated by the industries is a major environmental concern. Thus, it is imperative for highly polluting industries to adopt a suitable waste treatment process for the clean disposal of high-strength wastewater. Anaerobic digestion is one such technology, which is gaining wider acceptance in the present scenario over aerobic treatment due to the production of biogas, which can be further used for meeting a part of energy demand [185].

Anaerobic digestion is the most common method for the treatment of high-strength wastewater and stabilization of waste sludge from baker's yeast manufacturing facilities.

The following four stages comprise the digestion process: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Various microorganisms are involved in each step. During the first stage, a group of microorganisms secretes enzymes that hydrolyze polymers to monomers to convert particulate materials into dissolved materials. Subsequently, the acidogenic phase includes the action of a large and diverse group of fermentative bacteria. These bacteria hydrolyze and ferment organic materials and produce organic acids, $CO_2$, and $H_2$. In the third phase, acetogenic bacteria convert these monomers to acetic acids. The final step in biogas production is performed by acetoclastic methanogens [186].

One factor influencing the efficiency of waste-activated sludge anaerobic digestion is access to cell-bound organic matter. The hydrolysis of the organic matter is the rate-limiting stage of the anaerobic process. In order to improve hydrolysis and anaerobic digestion performance, several pretreatments have been examined, which cause the lysis or disintegration of sludge cells. In pilot-scale experiments, process performance and biogas production of a cascade of two methanogenic continuously

stirred tank (CSTR) reactors connected in series were compared to a conventional one-step CSTR reactor treating sewage sludge. Results showed that the serial configuration could improve biogas production by 9.5–40.1%. Biogas selectivity was estimated to be 0.49 ± 0.06 $m^3_{biogas}$/kg degraded total volatile solids for the cascade and 0.44 ± 0.02 $m^3_{biogas}$/kg degraded total volatile solids for the one-step process. The process performance and biogas production from sewage sludge can be optimized through serial digestion [187].

Among the microorganisms involved in digestion, methanogens are the major microbiological group responsible for methane production. To study the microbiological equilibrium in an anaerobic reactor, Traversi et al. [186] detected the methanogen concentration during anaerobic digestion as an indicator of biogas production capacity. A positive and significant correlation between the biogas production rate and methanogen abundance was observed. The applied real-time quantitative PCR method is suitable to describe microbiome into the anaerobic reactor; moreover, methanogen concentration may have the potential for use as a digestion optimization tool [186].

The treatment process of wastewater requires large amounts of energy. The major energy consumption process in wastewater treatment plants is sludge stabilization. Besides having high energy consumption, sludge can be used as a renewable energy source. As a result of the anaerobic digestion process, it is degraded to produce biogas, which consists of 65–70% $CH_4$, 30–35% $CO_2$, 1–5% $H_2$, and 0.3–3%$N_2$ with various minor impurities, notably $NH_3$, $H_2S$, and halides [188]. Substances such as $H_2S$ and $NH_3$ may inhibit the anaerobic digestion process or cause corrosion problems in pipelines of plants or in the distribution network [189].

The collected gas from digesters is cleaned of $H_2S$ and $H_2O$ before it is combusted to generate steam or electricity. Typical digester gas is about 70% $CH_4$, with most of the rest of the gas being $CO_2$. Since the energy content of pure methane is 35,800 kJ/$m^3$ at standard temperature and pressure, the digester gas has a net heat value of about 25,000 kJ/$m^3$. The heat value of methane is roughly the same as natural gas, which has a heat content of approximately 37,000 kJ/$m^3$ [190].

If the intended use is for power generation, the biogas must be scrubbed to remove a number of impurities. After conditioning, the biogas can be used for onsite power generation to heat homes or can be added to the national natural gas grid. Biomethane produced from biogas generated by anaerobic digestion of organic matter is an alternative gas source to that of natural gas. Of particular interest is the possibility to inject biomethane, refined biogas with quality comparable to that of natural gas ($CH_4$ concentration greater than 95%), which can be used in place of fossil fuels in all its network applications and in transportation. To produce pipeline-quality biomethane starting from the biogas generated by the anaerobic digestion process, it is necessary to remove water, sulfur compounds, halogenated organic molecules, carbon dioxide, oxygen, and metals [189].

The total energy generation potential from the anaerobic digestion of industrial wastewater is estimated to be 2963 $GWh_e$/a equivalent electric energy. This is equivalent to a 565 MW power plant installation. Distilleries and sugar industries are

potential areas with a combined capacity of 832 $GWh_e$/a equivalent electric energy per annum [185].

The developed thermoeconomic analysis procedure and formulations based on the specific exergy costing method are applied to an existing municipal wastewater treatment plant using actual operational plant data by Abusoglu et al. [188]. Thermoeconomic analysis helps allocate the costs to the plants' main streams and subcomponents. Activated sludge is digested in the anaerobic digestion reactors to produce biogas with a 60% methane content. For each 1 $m^3$ biogas produced in the wastewater treatment plant, 68.26 kg of sludge with a dry matter content of 5.0% is digested. The total exergy rate of the biogas produced with a mass flow rate of 0.212 kg/s is obtained as 6653 kW. The actual exergetic efficiency of the wastewater treatment plant is determined to be 34%, which indicates that 66% of the total exergy input to the plant, mainly by sewage and power consumptions, is destroyed.

A lab-scale anaerobic hybrid (combining sludge blanket and filter) reactor was operated to study anaerobic biodegradation of distillery spentwash [191]. The model of Karhadkar et al. [192], as given by Eq. (1), was employed for the prediction of biogas yield from the reactor. Accordingly, the biogas yield can be expressed as follows:

$$Q_{\text{gas}} = GQ\left(S_0 - S\right) \tag{10.2}$$

where $Q$ is the flow rate of influent, $G$ is the conversion factor, $S_o$ is the influent COD concentration, $S$ is the effluent COD concentration, and $Q_{gas}$ is the biogas yield. The value of $G$ was determined as 0.52 $m^3$/kg COD experimentally. The model predicts gas yield within ±5% of the experimental value.

Anaerobic digestion can convert a significant portion (>50%) of the COD to biogas, which may be used as an in-plant fuel, and also saves the energy that would be required for aeration using aerobic treatment. Sirbu and Begea [33] treated distillery wastewater by full-scale thermophilic (50–55 °C) anaerobic digestion. In this study, more than 60% removal of COD was achieved with 76% of biogas comprising methane, thus making it a valuable fuel [33].

Bioremediation of distillery spentwash by anaerobic digestion is an attractive primary treatment due to its reputation as a low cost, environment friendly, and socioeconomically acceptable technology. Anaerobic digestion of wastewater from a distillery industry was studied in an upflow anaerobic fixed-film bioreactor using different support materials. Among the various support materials studied, the reactor having coconut coir could treat distillery spentwash at 8 d hydraulic retention time with an organic loading rate of 23.25 kg COD/$m^3$ day leading to 64% COD reduction with a biogas production of 7.2 $m^3/m^3$ day having high methane yield without any pretreatment or neutralization of the distillery spentwash [193].

In the UASB reactor, due to the excellent settling characteristics of this granular biomass and the presence of a specially designed three-phase (biogas, water, and biomass) separator device in the upper part of the UASB reactor, an excellent sludge

retention is assured in this reactor system. Performance of full-scale UASB reactors treating distillery spentwash was evaluated. The plant was designed to handle 650 $m^3$/day of distillery spentwash, having an average COD concentration of 112,400 mg/L with an HRT of 6 day. The biogas production was stabilized to the range of 48,290–135,115 $m^3$/week with 60% methane content [194].

The startup performance of upflow anaerobic hybrid reactors (UAHRs) and upflow anaerobic sludge blanket (UASB) reactors in the anaerobic treatment of distillery spentwash has been studied. The maximum volumetric gas production of 149 L/$m^3$ produced more in UAHR than in the UASB reactor during steady-state conditions [195].

The carbon and hydrogen fixed in sludge can be converted into a clean, high calorific value energy source as hydrogen through a cost-efficient bioprocess. Biohydrogen production by dark fermentation of the waste biological sludge obtained from the sedimentation unit of the aerobic wastewater treatment plants of yeast industry showed that the maximum $H_2$ yields 41 mmol/g total sugar consumed [196].

## 10.6 Approaches on Management of Sources in Baker's Yeast Industry

The yeast industry is facing environmental challenges, such as increasing costs of production and non-value adding expenses of waste effluent and process water charges. A satisfactory solution to these multilayered challenges calls for an integrated approach in order to accomplish effective waste management and water conservation.

### *10.6.1 Water Conservation*

Freshwater conservation is another concern in the yeast industry, and it is no longer recognized as a free commodity. Innovative technologies, along with modifications of existing technologies, are commercially available to reduce water consumption. In general, reduction in industrial water and wastewater can be achieved through one or a combination of the following measures:

1. Process modification or change in raw materials to reduce water consumption
2. Direct reuse of wastewater
3. In-plant reuse of recovered wastewater
4. Use of treated wastewater for nonindustrial purposes

In this perspective, improved water use in yeast factories can contribute to better operational efficiency, improved economic competitiveness through reduced water

demand combined with savings in water and wastewater treatment costs, and low environmental impact due to a decrease in surface and subsurface withdrawals as well as less groundwater contaminant intrusion [197].

A step-by-step study of the water cycle in a company allows significantly reducing water consumption. Making the water balance is useful to detect unknown water consumption and is the basis for further optimization. Water pinch or water scan methods allow deciding which streams can be reused, directly or after regeneration. Finally, the remaining wastewater can be included in an overall process scheme aiming at zero discharge of wastewater. For yeast factories, all three steps have been proven to be useful, although a zero discharge is not entirely obtained.

A systematic approach to water management helps keep the water issue under control. This can be done by controlling water input, consumption, and output and by gearing all water-related activities to one another. At the input side, the emphasis is on searching alternative sources for process water. Surface water, if available, can be an option for some companies; brackish water is a possible source on the condition that an adequate pretreatment method is used. Wet countries might investigate the possibility of using rainwater, although this is usually only feasible when small quantities are needed for low-quality purposes, such as sanitary water. A further option is to regenerate wastewater and recycle it as process water. Wastewater often requires an extensive treatment before it can be discharged. Further purification in order to obtain a water quality fit for reuse is often a realistic option in terms of additional treatment cost versus the benefits of using recycled water [198].

Three advantages are to be taken into account. The first one is recycled water, which is a supplementary and reliable source of freshwater and can add to existing sources or replace them. The second advantage is that the net volume of water consumed decreases drastically, and the last one is that the volume of wastewater to be discharged decreases.

Here we use three steps to optimize the water balance in a yeast plant. The first step is to investigate the current water balance in detail. This seems to be very straightforward but usually leads to complex measurements and calculations. Information of how much water is consumed in which processes allows us to take simple measures to save water. The second step is to combine water-consuming processes and reuse water where possible for other purposes requiring a lower water quality. Typical examples of possible candidates are cooling processes and tank cleaning. The water pinch method offers a theoretical approach to do this; however, practical impediments often need to be challenged. The final step is to regenerate partial waste streams and re-introduce them into the process cycle. Because of the complexity of most wastewaters to be treated, this requires one or more membrane separations, often using pressure-driven processes such as ultrafiltration and nanofiltration.

Water balance is a numerical account of how much water enters and leaves a plant and where it is used within the plant. It should contain detailed information about the amount of water used by each process. The water balance is a crucial instrument to understand and manage water flows throughout the plant, to identify equipment with water-saving opportunities, and to detect leaks. The setup of a water

balance requires a preliminary survey of existing data, an assessment of major gaps in the available information, and a decision on how detailed the water balance should be. This leads to choices concerning the amount of work and the technical resources that have to be invested in the project.

In a typical yeast plant, the main source of process water is well water. This water is brought from a depth of 60–360 m at a constant temperature of 17 °C. At peak moments, more water is needed than the well water pumps can provide. Therefore, the company will supplement its water needs with tap water. Before being used in the production process, the water is treated in order to obtain the quality that is needed for each specific application in the plant. On arrival in the yeast factory, all water is first chlorinated and then stored in a reservoir. Also, 80% of the stored water is directly used from this reservoir, without further treatment. This is the "cleaning water." It is mainly used for CIP (cleaning-in-place) cleaning procedures. About 20% of the available water is demineralized. To this end, the water first passes an activated carbon column for dichlorination and removal of organic matter. Afterward, the water is deionized by passage through anion and cation columns. Thus, process water or deminwater is produced mainly for use in the fermentation process. About 8% of the deminwater is further treated by UV irradiation to obtain the sterile and "ultraclean water," which is mainly used in critical yeast processes.

In order to obtain more detailed information on the individual water-consuming processes, supply pipes need to be traced and schemes need to be drawn to understand groupings and connections of equipment in the water network. In many cases, water is taken from existing pipes left from an earlier installation or simply tapped from the water supply of a completely another facility. Thus, for constructing a rough internal water balance, existing water meters provided only partial information. Additional flow measurements need to be carried out for all subunits, including CIP stations, and noncontinuous operations such as cleaning of tanks.

Reusing wastewater streams as an input for less demanding installations is a second step toward a zero-discharge system. The reuse of wastewater is well documented in the literature. Two theoretical approaches can be considered: water pinch techniques and water scan techniques, which provide a more spontaneous approach. In order to apply these concepts, a water balance is essential. Both qualitative and quantitative requirements should be specified for each individual water input at an installation. As for the qualitative requirements, it is important that the minimum quality specifications for each water flow should be stated. The water pinch method implies the determination of the minimal water use by an installation, which is needed to evacuate contaminants from a process, given an initial contamination load. The combination of all these minimal water quantities, effluent characteristics, and maximum allowed contamination loads for all processes in the plant then determines the overall minimal water use. This calculation is executed three times under different assumptions:

1. Existing allowed contamination loads for water inputs are not changed.
2. More effluents become available for reuse if allowed contamination loads for selected processes are relaxed.

3. All or selected effluents are regenerated before being used as an input. In this way, several effluents are theoretically eligible for reuse, but significant investments are required. The analysis of the results also reveals which process is a bottleneck for further reducing water consumption in the plant. Improving the water use characteristics of this process will result in the most cost-effective impact on the water use for the company as a whole [199].

The water pinch method requires that effluents of installations are redirected, serving as an input for another process. New water treatment equipment may have to be added. Frequently, individual effluent streams have to be mixed together in order to obtain the required input characteristics. This typically requires extra piping, pumps, valves, etc. More practical, common sense methods were developed to help reduce the water consumption in a production plant. These methods can be called water scan techniques; they are not based on a theoretical framework but all start from a water balance of the plant, upon which ideas are gathered on how to manage the water system more efficiently, concentrating on reducing costs.

Good housekeeping and opportunities for simple direct reuse are the main attention points at this stage. Application of new techniques for water treatment or investments in less water-consuming technologies is considered. Opportunities for water-saving practices are then classified based on their financial and technical consequences. Taking available resources into account, the most promising projects are chosen and a planning for their implementation is proposed.

It is obvious that regeneration of partial wastewater streams only decreases the water volumes needed for the yeast production activities, but a zero discharge is not yet feasible. The reason for this is that wastewater treatment generates side streams such as sludge volumes, which would need to be reused in the strict sense of zero discharge. Furthermore, yeast production is particular in the sense that materials are brought into contact with food (yeast), which requires special safety regulations, especially in terms of microbiological safety. One must also be aware of the psychological barrier of using wastewater in food products. This is an impediment to zero discharge systems. Current improvements are more focused on partial water recovery. In order to achieve this, the remaining wastewater fraction needs to be regenerated up to a level similar to the freshwater currently used in the company.

### *10.6.2 Waste Minimization*

Waste minimization in the yeast industry faces certain unique challenges. First, the yeast-making process itself was developed from very old classical practical activities. This limits the application of modern technologies to minimize waste in many yeast factories. Second, as a general rule in the yeast industry, many poor practices are often not classified as “poor,” but as common practices. This mindset renders the implementation of waste management in the yeast industry a challenging task. Third, industrial operations are a mixture of both batch and semi-batch processes.

These uncontrollable variations adversely affect resources to the point where waste minimization is being compromised during actual production operations. These limitations offer an explanation as to why waste management is practiced as end-of-pipe technologies in numerous yeast plants [200]. However, owing to rapidly growing global demand on manufacturing processes and final products to exert minimal or no environmental footprints [201], the yeast industry has begun to experience legislative pressure to become more efficient [199, 200]. Thus, the increasing demand for the greening of industrial production processes and products, both from customers and legislative authorities, coupled with rising operational and waste treatment costs in the yeast industry, has started to move toward the adoption of integrated waste preventative approaches, as opposed to the traditional reparatory environmental engineering practices [203]. To effectively analyze waste generated in the yeast industry, a systematic methodology was followed comprising waste source identification, causative evaluation of waste, and qualitative derivation of feasible waste minimization alternatives [204].

It is highly suggested to concentrate on the entire production process and consider comprehensive waste minimization strategies for each waste stream, process, or unit operation.

Process flowsheets were evaluated from the inception of the raw materials up to the packaging of the products. The visual representation of the flowsheets facilitated the identification of critical points within the production processes, where major releases and discharges were likely to occur. This also helped prioritize the waste streams in need of further investigation. The standard production process consists of preparation, fermentation, clarification, dewatering, and drying. However, various companies use different process routes, which significantly impact waste management for both intrinsic and extrinsic wastes. In that sense, it is impossible to derive all feasible waste minimization strategies. Such a wide spectrum of process routes highlights the need for a systematic analysis to determine alternatives for eliminating or improving the handling of waste streams from the production processes [202, 203].

Changing process technology is an important technique for reducing waste volume and strength. Some examples are the following:

1. Alternation in cleaning procedures such as using counter-current washing, recycling of used water, and reducing the cleaning frequency. In the production process, it is possible to reuse the last yeast wash water as the first wash water. The $BOD_5$ values of this washing water range from 200 to 500 mg/L. This water can be reused again in the washing process after heat sterilization. It is suggested that filtration followed by sterilization is likely to produce treated wastewater suitable for recycling as the next batch fermentation first water.
2. Employing new methods in production line cleaning in baker's yeast industry. Several feasibility studies indicated that the installation of an automatic CIP control system not only reduced the extent of waste treatment but also permitted wastewater recycling [205].

3. Changing waste transport methods such as self-draining piping systems will help reduce the amount of wash water used within the plant.

Changing products, which can serve the purpose of those that they substitute, can bring waste minimization as in the cases of yeast cream as a final product, instead of block or dry yeast products.

The utilization of glucose syrup instead of molasses as a fermentation medium is a good example of changing input material. About 85% of the pollution load (COD) of the yeast industry is due to molasses, and it originates from the fermentation stages. Therefore, it is necessary to reduce the pollution load of these stages. Molasses can be partially substituted (up to 50%) for glucose syrup to improve waste minimization as well as a reduction in COD and color load in the effluent.

#### 10.6.2.1 Minimizing Extrinsic Waste

Yeast production and its complementary products are accompanied by the generation of large quantities of waste streams, namely organic waste and wastewater. Variations adversely affect resources to the point where waste minimization is being compromised during actual production operations. These constraints offer an explanation as to why waste management is practiced as "end-of-pipe" technologies in numerous yeast plants, notably wastewater treatment and landfilling of solid wastes. However, owing to the rapidly growing global demand for manufacturing processes and final products to exert minimal or no environmental footprints, the yeast industry has begun to experience legislative pressure to become more efficient. Thus, the increasing demand for the greening of industrial production processes and products, both from customers and legislative authorities, coupled with rising operational and waste treatment costs in the yeast industry, has started to move toward the adoption of integrated waste preventative approaches, as opposed to the traditional reparatory environmental engineering practices [206].

To effectively analyze waste generated in the yeast industry, a systematic methodology needs to be followed comprising waste source identification, causative evaluation of waste, and qualitative derivation of feasible waste minimization alternatives.

Several generic methods for developing waste minimization options at an industrial scale have been discussed, and a case study on industrial food processing has been reported by Van Berkel [204].

Waste minimization is addressed through the acquisition of process knowledge of fermentation operations. Knowledge acquisition can be achieved through regular observation of a variety of yeast production processes and unit operations.

Two approaches for evaluating process flowsheets to identify feasible strategies for achieving waste minimization in any industrial process have now been established [207]. The techniques are broadly classified as quantitative and qualitative approaches, and the latter approach was employed in this case, owing to the general

lack of reliable plant data in production processes. Second, a qualitative approach provided a feasible way of evaluating flowsheets of existing processes, unlike quantitative methods where large sets of data of high integrity are required to precisely determine feasible waste minimization alternatives. Third, most data and information in the yeast industry have been acquired through experience, and qualitative techniques were found most suited in synthesizing process flowsheets for the identification of waste minimization opportunities.

It is necessary to concentrate on the entire yeast production process and consider comprehensive waste minimization strategies for each waste stream, process, or unit operation [208].

#### 10.6.2.2 Recovery of Waste

Betaine recovery from the wastewater is a good example of *"Resource recovery"* in the yeast industry. High-strength waste generated at the end of the fermentation process contains both high- and low-molecular-weight organic loads. It was shown that betaine can be separated from the weak vinasse by using an ultrafiltration process with a membrane of the molecular cutoff size of 4000. This does not help reduce the load of the waste treatment but also by-product recovery of a commercially valuable chemical named "betaine."

In the yeast industry, strength of wastewater is influenced by the organic load content of the discharge. As much organic load as possible should be recovered to reduce the strength of wastewater by installing appropriate technology. Examples of resource recovery and reuse may be listed as follows:

1. Vinasse has been found to be quite suitable for animal feed when properly prepared and treated. The animal feed can substitute molasses.
2. The recovered betaine is used as a valuable animal feed additive.
3. The sludge would be suitable as a fertilizer due to its nutrient content and can be used as a source of nitrogen, potassium, and magnesium.
4. Methane can be produced as a by-product through anaerobic digestion. This can be utilized for domestic purposes or in the industry itself.

Despite being a highly polluting effluent, vinasse could be used in the production of single-cell proteins for feed supplementation due to its high carbon content. The generated microbial biomass by biological treatment of vinasse with yeast presented a low anti-nutritional value and an average protein content of 46.85% [178].

Through the application of the conceptual framework, several alternatives for reducing, eliminating, or reusing extrinsic waste should be identified. It is remarked that acceptable effluent quality and quantity can be achieved through the implementation of integrated waste and production management strategies.

The yeast production industry has been generating a large amount of yeast waste (the main sub-product of yeast production and the liquid fraction obtained by decanting) rich in organic matter that could be used as soil improvers or organic fertilizers. Yeast waste can be applied to an acidic sandy soil as organic fertilizer

since it provides not only nitrogen and potassium to plants but also contributes to increasing soil organic matter content. But the potential nitrate leaching and increase of $CO_2$, $N_2O$, and $CH_4$ have to be carefully monitored to avoid environmental problems [209].

#### 10.6.2.3 Methodological Approach

To help frame the problem of waste minimization in yeast production, two critical issues need to be clarified. On the one hand, it was important to establish an understanding of the product route from raw materials to the final product. The yeast production route was established through interviews, actual plant observations, and reviewing the literature. The final waste matrix was found to be a combination of interactive factors. Examples of such factors are the type of technology used, reuse and recovery of useful by-products, and the operating practices within a given factory. On the other hand, different production scenarios were examined as they had a critical influence on the consumption of raw materials and effluent quantity and quality.

#### 10.6.2.4 Product Route Determination

Process flowsheets were evaluated from the inception of the raw materials up to the packaging of the products. The visual representation of the flowsheets facilitated the identification of critical points within the production processes, where major releases and discharges were likely to occur. This also helped prioritize the waste streams in need of further investigation. The standard production process consists of preparation, fermentation, clarification, dewatering, and drying. However, various companies use different process routes, which significantly impact waste management for both intrinsic and extrinsic wastes. In that sense, it is impossible to derive all feasible waste minimization strategies. Such a wide spectrum of process routes highlights the need for a systematic analysis to determine alternatives for eliminating or improving the handling of waste streams from the production processes [202, 203].

#### 10.6.2.5 Inventory Tools

The identification and quantification of waste sources in the yeast industry were accomplished using inventory tools [204]. The tools are classified as either product-oriented or process-oriented. In the fermentation industry, process-oriented tools, i.e., material mass balance and process flow chart methods, are preferred. At any process or unit operation, the material balances are carried out to identify the components of the waste streams generated during the fermentation process.

The process flow chart method facilitated the identification of all possible sources of waste generated at any stage of the fermentation process. The process was divided into unit operations. Note that a unit operation in this context refers to an area of the process, or a piece of equipment where input materials are processed and output material streams are generated, which could either be a product, a by-product, or waste.

The qualitative mass balance method was used to establish the material flow at each level of the production process or unit operation. As a result, the method enhanced the understanding of the relative significance of different sources and causes of waste, as well as clarifying the composition of wastewater streams and thus the sources of pollutants. The sequence of defining the problem until all possible waste minimization alternatives has been identified.

#### 10.6.2.6 Waste Reduction: Methodological Evaluation of Waste Minimization Strategies

Data collected on different stages of the fermentation process were obtained and analyzed to identify waste minimization opportunities. Some of the data obtained comprised flow rates of materials, the composition of generated waste effluent, and volumes of yeast produced, as well as operating conditions and practices in different processes. To ensure systematic identification of waste minimization strategies, a structured methodology was followed. The methodology is composed of three-step sequential approaches, namely waste source identification, qualitative evaluation of waste causes, and finally the derivation of feasible alternatives for waste minimization.

#### 10.6.2.7 Waste Source Identification

Optimal formulation of waste minimization strategies requires unambiguous identification of all possible sources of waste, such as the inputs and consequent outputs from a given unit operation or process in order to trace material balances at each stage of the yeast production process. Using waste classification proposed by Berglund and Lawson [210] and Douglas [211], the fermentation wastes were classified as intrinsic (process) or extrinsic (utility). The intrinsic wastes are inherent in the fundamental process configuration, while on the other hand, the utility wastes are a function of auxiliary aspects of the operation [210]. Waste identification process was achieved through waste stream analysis and process analysis [212]. Note that, in the context of waste minimization and particularly in deriving the reduction strategies based on this classification, the two classes of waste types were found to be dependent on each other in the yeast industry. In that sense, care was taken in understanding the interactions and interconnections between the two waste types to ensure that root causes of various waste streams were adequately established. For the systematic identification of intrinsic waste sources, the production process was

broadly divided into several categories. After completion of each production stage, water and cleaning chemicals were used for a wide range of activities. These activities may include, but are not limited to, cleaning, cooling, and sanitizing of equipment. Using the above classification scheme for the process, different wastes, by-products, or product losses were identified from various unit operations and processes based on process flow path decomposition.

#### 10.6.2.8 Causative Analysis of Waste

In the first stage of the conceptual framework, waste inventories and characterization profiles provided valuable baseline data regarding the nature of pollutants generated in the yeast plants. However, before comprehensive strategies for waste reduction or minimization could be formulated, it was crucial to understanding when, how, and why different kinds of wastes were generated. Therefore, a causative analysis provided the understanding of the core influencing factors to the effluent quantity and quality, as well as the reasons for product and by-product losses. Thus, understanding of the causality formed a sound basis. In addition, causality facilitated the grasping of cause-and-effect relationships that govern the fermentation unit operations and processes that, in reality, are complex and multidimensional and are invariably influenced by diverse factors. It was not possible to provide definitive answers on causality and explicitly identify the differences that exist among various causes of waste.

#### 10.6.2.9 Causes of Waste Related to Input Material Characteristics

Generally, in the process industries, the feedstock of any process or unit operation has certain properties such as toxicity or nontoxicity. Based on these properties, the input materials may require special handling to reduce waste generation. In the case of the yeast industry, the key raw material is molasses, which are neither toxic nor hazardous. However, molasses have high organic content, and second, they contain dark colors, which are an unwanted but nevertheless unavoidable component of the input material. Hence, to minimize or eliminate cross-media pollution, molasses as raw materials require proper and effective handling. The same principle also holds in handling the inevitable by-products and the high volumetric fluid product. In facilities where improper handling of the raw materials, intermediate products, by-products, or finished products occurs, the resultant effluent composition is characterized by high organic content, high conductivity, and dark color. Such an effluent can be highly disruptive to the environment. On the other hand, certain cleaning and sanitizing chemicals are not environmentally benign, owing to their toxic and hazardous properties. For instance, while chlorine and ammonia solvents are effective cleaning and sanitizing materials, their toxicity and hazardousness have led to their substitution in certain fermentation plants by more benign agents, such as hydrogen

peroxide, ozone, or hot steam. This also reduced the need for higher quantities of rinsing water to remove the chemicals.

#### 10.6.2.10 Causes of Waste Related to Technology

This category accounts for the causes of waste related to "technological-based" factors, such as a type of material used for equipment design, equipment sizes, piping layout, and equipment efficiency, among others, which influence the quantity or other characteristics of waste streams as a result of some equipment or unit operations changes. For example, low equipment efficiency or poor design generally leads to increased waste generation; in addition, the technology used has a considerable impact on the effectiveness of managing and harnessing useful, but inevitable, by-products generated at various unit operations and processes. For example, during the pressing process where yeast and fermentation medium are separated, the use of modern separators reduces yeast losses significantly in comparison to old-style low-speed separators. On the other hand, it was observed that the efficiency of equipment used for cleaning and sanitizing showed a strong correlation with the quantities of potable water and chemical demand in plant operations. For instance, if open hosepipes were used, water and chemical consumption was found to be higher than in an operation where high-pressure low-volume cleaners or CIP systems were used.

#### 10.6.2.11 Causes of Waste Related to Process Execution and Management

In industrial chemical production processes, waste generation mechanisms can be regarded as functional dependent variables [213]. In the food and beverage industry, and particularly in the yeast industry, procedural, administrative, and institutional practices are the key causes of waste generation. These practices are simply good housekeeping and have a significant effect on waste profile in terms of volume, composition, and dispersion to other environmental media. One distinctive feature of these practices is their requirement for relatively simple in-plant changes regarding the operating procedures or methods of handling wastes. Such changes lead to the reduced waste or concentration of the contaminants in a waste stream.

#### 10.6.2.12 Causes of Waste Related to Recovery and Reuse

The degree of effectiveness in waste management depends on the nature of the industry. This is due to the uniqueness of the feedstock materials as well as the specific nature of the products and the intermediate by-products formed during the manufacturing process. The yeast industry is not an exception to this rule and was found to experience unique waste management constraints that can only be addressed adequately through effective reuse, recycling, or recovery. The best waste minimization strategy was to recycle the waste and by-products, reuse them in other

processes, recover them in order to be sold, or use as input materials in other industries. The recycling should be done based on the understanding that by-products and waste cannot be recycled in the process(es) generating them in an attempt to produce the same product or perform the same function.

High health standard requirements for food-based products, as stipulated by the industrial food production act, render by-products and recyclable wastes not easily reusable in the process(es) generating them owing to the risk and uncertainties associated with microbial contamination. From this perspective, products, by-products, and waste recovery and recycling in other associated industries were viewed as the most feasible waste management alternative.

The rinse water produced from cleaning and sanitizing of equipment, which is not heavily contaminated with yeast, can be reused on cleaning floor surfaces or pre-rinse water for heavily contaminated equipment.

Credible evidence also indicates that where reuse and recycling are implemented ineffectively, or not at all, high effluent volumes are generated and the solids from various processes may result in odors, high organic content in the wastewater stream, and other catastrophic environmental consequences, such as sodicity and salinity when the effluent is used for irrigation without proper handling [203].

#### 10.6.2.13 Formulation of Waste Minimization Strategies

Within the theoretical framework, the development of alternative strategies was aimed at eliminating, reducing, and controlling the causes of waste generation or segregating useful materials from the waste streams. This was guided by the identification and analysis of both waste streams and the processes giving rise to these streams [212] and consequently establishing their true causes through systematic causative analysis. Alternative strategies were derived from personnel expertise and information from the technical literature. The information and knowledge from this causative analysis are crucial in identifying viable waste minimization strategies.

The waste minimization techniques were classified into the following categories: technological modifications, input substitution, operational practices, and waste/product recovery and reuse. Note that the strategies derived for a waste stream, operation, or process do not imply that they are applicable to every yeast plant, and in certain cases, the information and knowledge presented here are inappropriate to some of the current practices in the yeast industry.

By using the type of waste generated as a unit of classification, three distinct waste minimization classes were identified:

1. Strategies for minimizing fundamental waste
2. Strategies for minimizing extrinsic waste
3. Strategies for improvement of effluent quality

#### 10.6.2.14 Strategies for Minimizing Intrinsic Waste

In any industry, including the yeast industry, intrinsic waste poses the greatest challenge to be eliminated or reduced [210]. This is because most waste minimization alternatives with the capability of achieving a reasonable reduction or elimination of intrinsic waste ought to be technology based. As a result, high capital investment is required for the acquisition, installation, and operating costs of the equipment. Nevertheless, the advantages of reducing waste generation are diverse such as reduction of waste treatment costs as well as increasing the yield per unit throughput. Note that most common alternatives are in the category of "operating practices." Technology-related alternatives have the greatest potential for reducing intrinsic waste or enhancing the effectiveness of handling unavoidable waste.

## Glossary

**Active dried yeast** It is the form of yeast that consists of coarse oblong granules, with live yeast cells encapsulated in a thick jacket of dry cells with some growth medium.

**Biomass** Biomass is biological material from living or recently living organisms, most often referring to plants or plant-derived materials.

**Budding yeast** Yeast with an asymmetric cell division process for the production.

**Compressed yeast** Compressed yeast contains about 70% water and 30% yeast solids. The water content of the yeast cream is reduced to form by passing the yeast cream through a rotary vacuum filtration unit.

**Emulsifier** An emulsion is a dispersion of droplets of one immiscible liquid within another. Emulsifiers allow for a stable and homogeneous mixture of two liquids, which do not normally mix.

**Melanoidins** Melanoidins are brown, high-molecular-weight heterogeneous polymers that are formed when sugars and amino acids combine at high temperatures and low water activity. They are also present in the wastewater of sugar refineries, necessitating treatment in order to avoid contamination around the outflow of these refineries.

**Mesophilic microorganism** An organism that grows best in moderate temperature, neither too hot nor too cold, typically between 20 and 45 °C. The term is mainly applied to microorganisms.

**Molasses** Molasses is a viscous by-product of the refining of sugarcane or sugar beet, and the syrup left from the final crystallization stage is called molasses. Beet molasses is about 50% sugar by dry weight, predominantly sucrose, but also contains significant amounts of glucose and fructose.

**Mycelium** Mycelium is the vegetative part of a fungus, consisting of a mass of branching, thread-like hyphae.

**Seed yeast** Small amount of pure laboratory-grown yeast culture, which will be used for the startup of the commercial fermentation vessels.

**Spentlees** The concentrated part of the aqueous distillery effluent stream.
**Spentwash** The aqueous distillery effluent stream.
**Stillage** The residue grain from the manufacture of alcohol from grain; used as a feed supplement.
**Strains** A *strain* is a subset of a yeast species differing from other yeast of the same species by some minor but identifiable difference.
**Sugar beets** Sugar beet is a plant whose tuber contains a high concentration of sucrose, and it is grown commercially for sugar production.
**Sugarcane** Sugarcane predominantly grows in the tropical and subtropical regions. It belongs to the grass family and is an economically important plant. The main product is sucrose.
**Trehalose** Trehalose is a disaccharide formed by a 1,1-glucoside bond between two α-glucose units.
**Vinasses** The residue in a still after distillation.
**Yeast cream** At the end of the fermentation, the yeast broth is concentrated using a series of combined centrifugation and washing steps into a yeast cream with a solids concentration of approximately 20%.

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