Raman Kumar · Anil Kumar Sharma
Sarabjeet Singh Ahluwalia *Editors*

Advances in Environmental Biotechnology

Springer

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Preface

Recent advancements in environmental biotechnology have been instrumental to deal with many environmental concerns and global challenges. This frontier branch of biotechnology has always been a fascinating field for the research and academic fraternity, which is consistently attracting the scientific community to delineate complex mechanisms in order to resolve global environmental issues. Today, we face many challenges with respect to our environment, which is predominantly being affected by the exhaustive human activities, increased urbanization, and industrial growth. Therefore, it becomes increasingly important to understand intricate details about this association of human beings with the environment. Majority of the natural resources have reached up to the level of extinction as a result of excessive exploitation and increased consumption rate. Environment-friendly sustainable tools and technologies are being employed to considerably reduce the pollution. Less environmentally sustainable chemical means as employed earlier have many disadvantages associated with them, making the scientific fraternity to look for modern biotechnological sustainable alternatives. Modern tools of environmental biotechnology could provide us a better platform at least to improve our quality standards and the overall environment and to explore the possibility of using renewable raw materials. Current trends demand for a cost-effective, eco-friendly sustainable technology for the production of products maintaining high-quality standards and recycling of waste products further, reducing the pollution.

This book comprises of various chapters started with the basic of Introduction to Environmental Biotechnology, followed by Measurement of Environmental Pollution: Types and Techniques. Many chapters were incorporated on various aspects of bioremediation such as removal of pollutants, technologies for decolorization of effluent, and tannery wastewater treatment in addition to advanced technologies for wastewater treatment and perspectives of bioreactors in wastewater treatment. The advance trend of application of biosensors, nanotechnology, genetically modified microorganisms in heavy metal bioremediation, phytoremediation technology for sustainable environmental biotechnology, and agricultural biotechnology was discussed.

The use of microbial community to degrade or remove environmental contaminants such as heavy metals, pesticides, dyes, etc. needs to be strongly encouraged. For the sustainable use of microbial community for the bioremediation

process, there is a need to understand the metal-microbial interaction so that in-depth mechanism of bioremediation and biodegradation could be delineated. Keeping in view of the above concerns, authors have brought forward to bring major advancements employing modern environmental technologies through this book under the umbrella of Springer Publishers. The book aims to provide a comprehensive view of advanced environmental approaches for wastewater treatment, heavy metal removal, pesticide degradation, dye removal, waste management, microbial transformation of environmental contaminants, etc.

Our readers have been well apprised of the modern trends and scope of employing various state-of-the-art approaches to clean up and save our environment. The continued success of the books published under the banner of Springer Publishers is the result of a joint effort of a dedicated editorial and publishing team, and we will continue to evolve progressively for the benefit of our contributors and readers. In preparation for this book, we have benefitted from the guidance and advice of a large number of biotechnology instructors across the country. We gratefully acknowledge our debt to the reviewers, who provided constructive criticism and valuable suggestions at various stages. While thanking all the contributors, we reiterate our commitment for the ethical and quality work published through this book *Advances in Environmental Biotechnology*. We anticipate that this book would be able to provide a comprehensive, accessible, up-to-date information about sustainable approaches in making an eco-friendly environment. Moreover, this book offers an instant access to a wealth of data for environmental biotechnologists and microbial and biochemical technologists along with student fraternity from diverse streams of environmental engineering and industrial biotechnology.

We would like to acknowledge our spouse and families who have provided the constant invaluable support throughout the writing process.

Finally, we have an enduring appreciation for our young researchers, whose comments and suggestions provide insight and remind us their need as this book is for them.

"If you don't worry about it now, Its too late, later on" …….. (**Karl Sagan**)

Ambala, Haryana, India — Raman Kumar
Ambala, Haryana, India — Anil Kumar Sharma
Patiala, Punjab, India — Sarabjeet Singh Ahluwalia

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About the Editors

Dr. Raman Kumar is working as associate professor in the Department of Biotechnology, Maharishi Markandeshwar University, Mullana (Ambala), Haryana, India, since 2011. He has also worked as senior research fellow of the Division of Soil and Crop Management, Central Soil Salinity Research Institute, Karnal, India, from 2007 to 2010. He has experience of about 10 years in the field of teaching and research in the area of microbial and environmental biotechnology. He has published more than 30 publications in various national and international peer-reviewed journals. He developed many bacterial and fungal strains for the removal of heavy metals from wastewater. His current research areas are bioremediation and biodegradation of toxic pollutants, pesticides, and azo dyes using microbial consortium from the industrial effluents, mechanism involved in the heavy metal bioremediation.

Dr. Anil Kumar Sharma is a full professor and head of the Department of Biotechnology with about 15 years of teaching and research experience after PhD. He did his doctorate from the PGIMER, Chandigarh (year 2000), in the field of medical biotechnology. He worked as a senior research scientist and a postdoctoral research fellow (molecular biology) in the Microbiology and Immunology Department at the UIC College of Medicine in Chicago, IL, USA, for about 7.5 years. He has more than 80 publications in peer-reviewed journals of national and international repute with a cumulative impact factor of his publications around 115. He has won many prestigious awards and accolades in his career. In 2000, he was felicitated with a MGIMS Young Scientist Award from the Association of Clinical Biochemists of India (ACBI), while in 2013, he won the Bharat Excellence Award from the FFI, New Delhi, India, for his research accomplishments in basic sciences. His research interests are diverse ranging from understanding the metal regulation in prokaryotes and eukaryotes, drug resistance, cancer biology, and nanomedicines to the development of microbial strains for remediation of heavy metals and pesticides.

Dr. Sarabjeet Singh Ahluwalia is presently working as an assistant professor at the Department of Biotechnology, at General Shivdev Singh Diwan Gurbachan Singh Khalsa College, Patiala, Punjab (India). He did his MSc in biotechnology

from Punjabi University, Patiala, Punjab (India), and PhD from Thapar Institute of Engineering and Technology, Patiala, Punjab (India). He has 22 years of research experience including 10 years of teaching microbiology, environmental biotechnology, and microbial technology for graduate students. Dr. Ahluwalia has published 25 research papers in peer-reviewed international and national journals; in addition, he has presented 33 research papers in conferences and symposia along with three patents in his credit. Dr. Ahluwalia has reviewed a number of research papers/manuscripts. Dr. Ahluwalia has received the grants from the UGC, Government of India, New Delhi, and CSIR, New Delhi, for research projects.

Further, he has developed the technology for the removal of Cr (VI) from chrome effluent. Besides that, he is a fellow member of *Research Journal of Chemistry and Environment* as well as a life member of the Biotech Research Society of India, Association of Microbiologists of India, and Punjab Academy of Sciences. His research interests are in the field of environmental biotechnology, bioremediation, and environmental monitoring.

Introduction to Environmental Biotechnology

1

Roshan Gul and Raman Kumar

Abstract

Environmental biotechnology is a coordination of scientific and engineering acquaintance associated with the use of microorganisms and their degraded products after treatment in the prevention of environmental pollution through biotreatment of wastes and biomonitoring of environment and treatment processes. Environmental biotechnology is the multidisciplinary combination of sciences and engineering in order to employ the enormous biochemical efficacy of microorganisms and plants, for the restoration and perpetuation of the environment and for the sustainable utilization of resources. The most significant considerations for purpose of biotechnology in waste treatment are technically and economically sound for biodegradability or detoxification of substances during biotechnological treatment, huge amount of treated wastes, and capability of natural microorganisms to degrade substances. The unique role of environmental biotechnology in the future is intensified taking into account the possibilities to append with innovative solutions and guidelines in remediation of environments contaminated with pollutants, minimizing future waste release and creating pollution prevention alternatives.

Keywords

Environmental pollution • Waste treatment • Bioremediation • Pollutants

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1.1 Introduction

Environmental biotechnology (EB) is the relevance of biotechnology to all aspects of the environment. Environmental biotechnology in the beginning was a science of wastewater treatment and was extended to soil remediation, off-gas purification, surface and groundwater cleaning, deposition techniques for solid waste in sanitary landfills, composting and bioorganic recycling, as environmental biotechnology plays tremendously imperative role in management of water pollution. Further it is the multidisciplinary integration of sciences and engineering in order to utilize the vast potential of microorganisms and plants for the restoration and conservation of the environment and for the sustainable utilization of resources. Environmental biotechnology is utilized to widen advanced technologies based on biological systems to develop efficient and reduce or utilize wastes to promote a wide range of industries and the environment and commercial efforts in three main areas, i.e., biofilm prevention and dispersal, rapid in-field microbial detection and control, and bioprocesses such as bioremediation and industrial wastewater treatment (Meadows et al. 1972). Environmental biotechnology brings jointly the multidisciplinary skills of researchers, engineers and industry participants to bring out innovative technologies in environmental, agricultural, industrial, mining, veterinary, and medical applications.

The International Society of Environmental Biotechnology promotes awareness in environmental biotechnology and offers the exchange of information pertaining to the development, usage and regulation of biological processes for remediation of contaminated environments and for eco-friendly processes (green manufacturing technologies and sustainable development).

1.2 Issues and Implications Associated to Environmental Biotechnology

In the detection of the premeditated significance of biotechnology, integrated plans are established and constructed in several countries in the different sectors (Gavrilescu and Chisti 2005). EB is apprehensive with the application of biotechnology as promising alternative in the perspective of environmental protection. Environmental biotechnology is not a new field, but there are some issues which are of great concern such as wastewater treatment and composting etc., which are common examples of old technologies. In early stages environmental biotechnology has evolved from chemical engineering, but in a while other disciplines such as environmental engineering, environmental microbiology etc., also add to environmental biotechnology (Hashim and Uijang 2004). The progress of industrialization and increase of various human activities have increased pollution of air, water, soil and the usage of nonbiodegradable materials and its improper disposal. Researchers demonstrated that pollutants can be degraded by means of microorganisms, plants, and animals. Advanced techniques are now possible to treat toxic pollutants by the use of living organisms to develop such efficient products and treatment processes

that generate less waste and protect the integrity of our ecosystem and as a result of which ensuring health of the environment through bioremediation, biomonitoring, and environmental protection.

1.3 Environmental Remediation by Biotreatment/ Bioremediation

Biological treatment and bioremediation have been a rapidly increasing area over the last decade. Environmental hazards and risks that occur as a result of accumulated toxic chemicals or other pollutants could be diminished by the use of biotechnology in the form of bioremediation/biotreatment (using microorganisms). US Environmental Protection Agency (USEPA) defined bioremediation as a treatment process in which microorganisms are employed to degrade or modify toxic pollutants to less harmful forms, thus diminishing environmental pollutants generated by various anthropogenic activities. Bioremediation methods are processes to degrade and remove pollutants from environment including water, soil, and air. Four processes such as removal, separation, degradation and immobilization can be considered acting on various contaminants present in the environment. Removal, separation and degradation are the processes to minimize the pollutants from the contaminated sites. On the contrary, immobilization regulates the transfer of a contaminant to sensitive receptors without reducing the contamination. Biodegradation and biotransformation are conceded by an individual organism or a consortium, are the center of environmental biotechnology and are the most important part of applied processes for environmental cleaning. Biodegradation is a very broad field and involves use of ample range of microbes to remove hazardous pollutants from the environment. Biotransformation processes use microbes that play a central role in the field of foodstuff and pharmaceutical industry. Biological treatments rely on the basis of microbial activities such as degradation and detoxification of harmful pollutants such as organic, inorganic, metal transformation, applied to gaseous, aqueous and solid waste (Fig. 1.1).

1.3.1 Environmental Remediation Using Microbes and Plants

The all forms of life can be considered as having prospective competence in environmental biotechnology. However, microbes and plants are of great apprehension even as frequently present in their natural environment (Evans and Furlong 2003). Microorganisms have the capability to degrade most noxious substances. Microorganisms may work either individually or in mixed cultures (consortia), which are of great significance in various applicable environmental technologies. The role of plants in environmental cleaning is exerted through the oxygenation of a microbe-rich environment, filtration and uptake of toxic contaminants.

The use of microorganisms for the removal of pollutants is based on the conception that all of microbes could transform and/or remove substances from

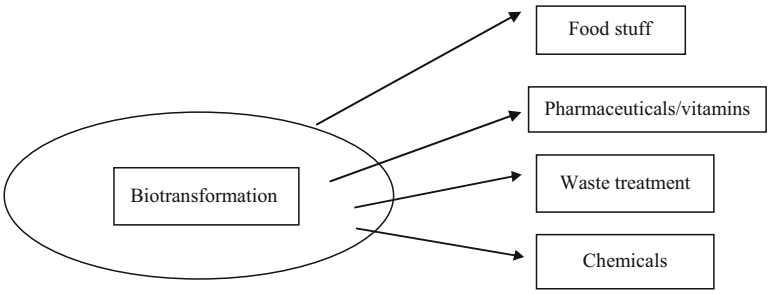

Fig. 1.1 Applications of biotransformation

the environment for the purpose of their own growth and metabolism (Wagner et al. 2002; Doble et al. 2004). Both bacteria as well as fungi are considered significant for the remediation of toxic substances to less toxic forms. Besides, plants also have been proven appropriate to take up nitrogen, phosphorus, sulfur and minerals and metals from the environment. Microorganisms employed in biological processes include aerobic and anaerobic bacteria (Timmis et al. 1994; Wagner et al. 2002; Moharikar et al. 2005) (Table 1.1).

1.4 Applications and Scope of Environmental Biotechnology

Highly developed technologies are now available to remove and degrade toxic pollutants by microorganisms or to build up such treatment methods that produce less waste and preserve the natural resources (Olguin 1999; Gavrilescu and Chisti 2005; Chisti 2007):

- *Bio-composting* involves combining of organic materials in definite controlled conditions that decomposes them at more quickly pace than they would decompose under natural conditions in free environment.
- *Bioenergy* involves fuels like biogas, biomass and hydrogen being used for industrial and domestic purposes. These fuels belong to the group of bioenergy. The need of the bioenergy has become alternate resources of energy that are clean and equally competent. Energy production from biomass is the best example of green energy. These are all eco-friendly solutions to pollution problems. Biomass energy supply-demand balances have become a part of energy sector study and planning and assumed greater importance in countries.
- *Bioremediation* is a pollution control technology that uses natural biological species to catalyze the degradation of various lethal chemicals to less harmful forms (Vidali 2009). Microorganisms like bacteria, fungi and algae have been shown to degrade and biotransform azo dyes from wastewater (Banat et al. 1996). Biotreatment offers a cheaper, eco-friendly and the most desirable approach for cleaning up the toxic environmental pollutants.

Table 1.1 Microorganisms used in environmental remediation

Microorganisms	Abilities	References
Streptococcus	Degrades hydrocarbon and dairy industry wastes	Atlas (1981)
		Ince (1998)
Vibrio cholera	Heavy metals	Bitton (2005)
Pseudomonas sp.	The bacterium was completely able to decolorize different azo dyes	Shah et al. (2013a)
Pseudomonas aeruginosa	Was found to be capable of maximum degradation of all the dye samples collected from various textile industry effluent samples	Prasad (2014)
Bacillus cereus and *Bacillus megaterium*	Degrading of azo dyes	Shah et al. (2013b)
Ganoderma sp.	Decolorize and detoxify Reactive Orange 16	Ma et al. (2014)
Aspergillus niger, *A. flavus*, *Fusarium* sp., *Penicillium* sp., *Curvularia verruciformis*, and *Mucor racemosus*	Successfully decolorize red HE7B (C.I. Reactive Red 141) and Yellow FN2R (C.I. Reactive Yellow 206)	Balaji et al. (2012)
Aspergillus terreus	Degradation of Congo red and Methylene blue dyes from solutions	Ramamurthy and Umamaheswari (2013)

- *Biotransformation* is a process of biological alterations of complex compound to simpler toxic to nontoxic. It is employed in manufacturing industries where toxic substances are released as by-products.
- *Biomarker* is a biological reaction to a chemical that gives a degree of exposure and of toxic effect. Biological markers can provide molecular verification of the correlation among oils and their sources.

  Unfortunately, several environmental pollutants are of concern because they are toxic and are accumulated in the environment rapidly. Moreover, various conventional methods can be used for the treatment purpose but at the same time generate other contaminants in the environment which are supposed to be toxic and increasing the attention for the development of an alternative, eco-friendly and cost-effective biological treatments (Krieg 1998; Chen et al. 2005).

At least main four points are taken into consideration for environmental biotechnology interventions to detect by means of using biosensors and biomonitoring, to prevent the manufacturing processes by replacement of conventional processes and products with the use of modern biotechnology in various industries like as food, pharmaceutical and textiles, and to control and remediate the discharge of pollutants into the environment by degradation of toxic substances through wastewater treatment, soil refinement and management of solid waste (Olguin 1999; Chen et al. 2005; Das 2005).

1.5 Biotechnology for a Safer Environment

Till date, several industrial processes and agricultural practices have damaged the environment greatly. It is now mandatory to modify the existing technologies for reducing the degree of degradation of the environment by these processes. This has encouraged researchers to widen technologies that have scope for the utilization of microorganisms to generate bioresources and to reduce the impact on the existing natural resources. Environmental biotechnology aims at the protection of the environment and purification of the environment from toxic contaminants and helps in the prevention of the degradation of precious natural resources such as soil, air, water and mineral resources.

(a) *Treatment of Wastewater*

Wastewater of domestic and industrial origin contains oxidizable organic matters. Extensive progress has been made in the development of treatment systems for efficient and fast removal of organic matters and nutrients from wastewater. Biological degradation of organic substances and other organic chemicals such as hydrocarbons and xenobiotic substances using microbes is an eco-friendly approach. There is also considerable progress in accelerating the rate of degradation, to treat wastewaters efficiently. Extensive research is needed to optimize the process to increase the water treatment efficiency and to produce readily usable biomass.

(b) *Transformation and Removal of Heavy Metals*

Chemical and biological transformation of heavy metal ions are prevailing processes in the environment. Heavy metals come in wastewater treatment plants from industrial discharges, storm water etc. The release of these toxic metals may damage the biological treatment process, being frequently inhibitory to both aerobic and anaerobic processes. Biological transformation involves both microbes and higher plants. However, the efficiency of microorganisms and higher plants to transform toxic metal forms to nontoxic or less toxic forms varies.

Microbial transformation of heavy metals includes extracellular chelation, biosorption, and intracellular complexation (Bitton 2005). Many species of bacteria, cyanobacteria and green algae release organic acids to the soil and aquatic environment and these charged organic compounds form complexes with metals, thereby minimizing their toxicity. The mechanisms involved in metal removal from wastewater include adsorption to cell surface, complexation and solubilization of metals, precipitation, volatilization and redox transformation of metals (Kulbat et al. 2003; Bitton 2005). For example, Cd^{2+} can be accumulated by bacteria such as *E. coli*, *B. cereus* and fungi *(Aspergillus niger)*. Biosorption is the process of adsorption of charged metal species to the surface of the cell wall. During treatment, this has been proven as a potential technology for removing metals from the environment as well as from wastewater.

(c) *Degradation of Organic Pollutants and Pesticides*

The organic wastes present in wastewater are carbohydrates, proteins, lipids, hydrocarbons and organic acids. A great variety of microbes use these organic materials as substrates for their growth and maintenance. There are many organic molecules in the wastes, which are not only complicated to degrade but also need special environmental conditions.

In the past decades, there are many examples of bioaccumulation of different insecticides and pesticides, due to their abandoned use. The environmental effects and the potential of these chemicals in causing human health hazards have been fully realized. Many bacteria species have been isolated, cultured and studied broadly so that they can be used for retrieval of pesticide-affected soil and also for the management of insecticide-containing solid wastes and sludge.

1.6 Development of Environment-Friendly Processes

Scientific developments have modified the industrial processes of the present time to make them more competent and less environmentally damaging. New technologies are replacing old ones, as for as possible, predominantly in developed countries, while in developing countries investment on production of environment-friendly technologies is gradually increasing. Though, none of these technologies have become totally safe from the environmental degradation point of view, as each of them generates pollutants and discharges them into the environment in one or the other way. Thus, new methods of environmental purification as well as resource production are needed.

(a) *Development and Designing of Bioreactors*

The prerequisite for improving effluent quality from wastewater treatment has led to an increasing difficulty in the design and operation of plants. In most cases inappropriate design of the treatment systems renders the treatment system operation, time and energy consumption. To reduce and control the operation, it is necessary to have a proper design, which can be achieved by the use of dynamic models, by considering each treatment system as a bioreactor. Different bioreactors have been designed to treat wastes. Among them the most important ones are solid-state fixed bed bioreactors, solid-state bioreactors with natural ventilation and slurry bioreactors. In the field of bioreactor development and designing, future research may generate more efficient, time saving and economically viable bioreactors.

(b) *Integrated Waste Management*

Integrated waste management is a new approach for managing urban solid wastes and the liquid wastes from point sources. It involves the integration of different waste treatment methods for treating wastes like aerobic and anaerobic solid waste treatment and sanitary landfilling. The practical relevance of this system has not yet been fully achieved, but it has succeeded in making the

waste treatment system self-sustained, economically and in respect of energy requirement. New research is needed to make this system adequate and more efficient.

(c) *Integrated Pest Management*

Integrated pest management termed as the environment-friendly pest control method has emerged as a combined pest control tactic, involving natural, biological and chemical control processes. It is more positively considered as the integrated population management of insect pests or any other pest types that are often under the influence of the total ecosystem. The goal is to make pest management sustainable in long run.

1.7 Biotechnology for Conservation of Biodiversity

Biotechnology is more and more being used for the genetic improvement of crops. The organisms produced are genetically modified organisms (GMOs) – plants, animals and microbes, threatening the survival and use of local biodiversity. Biodiversity provides the foundation for ecosystems and their services, upon which all people are basically dependent. Since the beginning of human civilization, biodiversity is considered as the base of agriculture, source of all recent crops and domestic livestock species. Biotechnology has been used to get better and increase crop productivity, as well as to preserve, appraise and make use of the various aspects of biodiversity. The focus of the conservation action has been shifted from protecting individual species to conserving the habitat and ecosystems. Loss and degradation of habitats are the most imperative factor causing loss of species. Soil erosion, introduction, invasion, fire and flood are the main forces for destroying and threatening the forest diversity. The loss of agro-biodiversity is predominantly due to the introduction of high yielding hybrid varieties and GMOs, followed by soil contamination, enrichment, and loss of habitat of the wild relatives of crops. The Convention on Biological Diversity (CBD) recognizes the significance of invasive species as a global problem and calls upon contracting parties to prevent the introduction of and control or obliteration of, those alien species that intimidate the ecosystem, habitats and species diversity. The prime emphasis is on habitat protection through identification and propagation of species that is (or are) endemic to the region and has a competitive advantage over the alien and invasive species. The future approach should be to develop elevated yielding and adaptive plant and forest species for their introduction to degraded forest land, which can not only satisfy the energy demand but also prevent topsoil erosion and soil moisture loss. There is an urgent need for the conservation of biodiversity. Biotechnology offers innovative ways of conserving biodiversity. Conservation may be in situ or ex situ, either in the natural or seminatural habitat, or in some purpose-built environments. Biotechnologies including tissue culture, micro-propagation, conventional breeding, marker-assisted breeding, transgenic crops and genomics are all relatively helpful for conserving and propagating biodiversity in many distinctive ways.

1.8 Environmental Biotechnology for Pollution Prevention and Cleaner Production

Biotechnology is considered as the motor for integrated environmental protection. Pollution prevention works to diminish pollution at its source by using a number of practices including competent raw materials, addition of less toxic substances and eliminating them from production process. The great concern to end the pollution globally resulted in increased pressure for economical branches (agriculture, industry, and market transport) to focus on pollution prevention rather than end-of-pipe cleanup, since biotechnology could eliminate the hazardous pollutants at their source prior to their release into the environment.

The relevance of biotechnology as an eco-friendly alternative proved to be very constructive for prevention of pollution during source reduction, minimization of waste, recycling and rescue. Therefore, this result in reducing cost making, less pollution and resource consumption may be taken as immense strength of biotechnology for sustainable development. Once the biotechnological systems are established and are proved economical than conventional methods, these changes will not only add to protection of environmental but also save the companies from money loss (Olguin 1999; Evans and Furlong 2003). The techniques of modern molecular biology are useful in the industries and in the environment to improve effectiveness and also to lessen the environmental impact. The improvement of innovative biological processes followed by the modification of earlier existing processes by the introduction of biological processes, hence based on microbial or enzyme activity, is increasingly being used as an imperative prospective area of primary pollution prevention (Olguin 1999).

1.9 Conclusion

Environmental biotechnology plays an important role in wastewater treatment and hence pollution control. The technology has proven its potential in a number of areas and future developments to widen its scope. These techniques make genetically modified organisms that efficiently deal with specific tasks. The potential of environmental biotechnology can be exploited to make major contribution for protection and remediation of the environmental pollutants. Environmental biotechnology has payed attention on those alternatives which are intended to prevent environmental pollution or to remove the contaminants from the environment in a cost-effective way. The aim is to develop the production standards of soil, water bodies, and forest resources, keeping the quality of the environment unchanged. Pollution has caused loss of resources as wastes, decline of resource quality and high rate of consumption of fossil energy resources. Environmental biotechnology aims to improve the resource quality as well as to introduce renewable raw materials as a replacement of fossil fuels. Development of environmentally enhanced production technologies with maximum utilization of the substrate and

production of minimum pollutants is the most important theme of environmental biotechnology.

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Measurement of Environmental Pollution: Types and Techniques

2

Rajeev Kumar, Arsum Pathak, Moondeep Chauhan, and Sushma Negi

Abstract

Since the onset of industrial revolution is up till the recent surge in technological processes, environmental pollution has grown at an alarming rate causing distress to living beings and irreplaceable damage to the earth. With the recognition of the severity of this environmental damage and increase in interest of using technological advancement, a number of successful pollution control strategies have emerged over the years. However, the measurement and quantification of environmental pollution is the most pragmatic first step for identifying various management and mitigation strategies to control environmental pollution. This chapter aims to study a range of proven measurement techniques for quantitatively determining the concentration of various environmental pollutants in the atmosphere. This is particularly important in the formulation of cost-effective control measures and strategies for environmental pollution. Furthermore, to elucidate the concept of pollution measurement, certain parameters which are considered of high importance for environmental monitoring and reflect the quality of a healthy (or unhealthy) environment, especially with respect to soil, water and air, are also discussed in the initial parts of the chapter.

Keywords

Environmental • Pollution • Monitoring • Instruments • Soil

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2.1 Introduction

Environmental pollution is defined as the undesirable change in physical, chemical and biological characteristics of our air, land and water.

Different constituents of environment, such as plants, animals and human beings, interact with each other and maintain a balance in nature. However, anthropogenic activities like deforestation, industrialization, agriculture *etc*. give rise to an imbalanced environment which is the root cause of several environmental problems, pollution being the most severe of them.

Based on the source of origin, pollution can be either point source pollution or non-point source pollution. Point source pollution is the type of pollution where the source of pollution can be easily identified as it has been originated from a stationary location. Smoke coming out of an industrial unit, oil spillage from ships and chemical leakage from pipes are some common examples of point source pollution. In contrast, the type of pollution where the source cannot be traced back is known as non-point source pollution. Such type of pollution does not have a specific outlet or a single point of origin which can be identified. Common pollution activities happening in day-to-day community life, for example, heaps of trash, defecation of living beings and the use of pesticides and fertilizers in agriculture can be categorized as non-point sources of pollution.

In order to spread either kind of environmental pollution, certain agents, called pollutants, play a vital role. The Environment (Protection) Act, 1986, defines environmental pollutant as any solid, liquid, or gaseous substance present in such concentrations as may be, or tend to be, injurious to environment. The acuteness of a pollutant can be determined by its ability to persist in the environment, the amount in which it is present (concentration), and its chemical nature.

Environmental pollution is further divided into different types based on the different components of the environment. Major of these are air pollution, soil pollution, water pollution, noise pollution, thermal pollution, radiation pollution *etc*. Let us discuss some significant types of pollution individually.

Air Pollution Air pollution is caused by the chemical and particulates (solid, liquid, or gaseous) in the atmosphere higher than the acceptable concentrations causing harm to the living beings and interfering with the normal environmental processes. Carbon monoxide, lead, nitrogen dioxide, ground-level ozone, particulate matter and sulfur dioxide are the six criteria pollutants responsible for causing smog, acid rain, and other health hazards in humans.

Water Pollution Water (Prevention and Control of Pollution) Act, 1974, defines water pollution as such contamination of water; such alteration of the physical, chemical, or biological properties of water; or such discharge of any sewage or trade effluent, of any other liquid, gaseous, or solid substance into water (whether directly or indirectly) as may, or is likely to, create a nuisance or render such water harmful or injurious to public health or safety, or to domestic, commercial,

industrial, agricultural, or other legitimate uses, or to the life and health of animals or plants, or of aquatic organizers. Toxic chemicals, waste, nutrients, biodegradable organics and bacterial and viral pathogens are the predominant water contaminants.

Soil Pollution Addition of undesirable substances (pesticides, herbicides, fertilizers etc.) to soil which adversely affects its quality, making it infertile for vegetation, is known as soil pollution. Polluted water streams, low-density polyethylene (LDPE) plastic bags and industrial sources like fly ash and chemical residues are the common sources of soil pollution. In addition, the increased use of pesticides and fertilizers for enhancing agricultural productivity also contribute to soil pollution.

2.2 Environmental Pollution Analysis

The most important aspect, which the chapter dwells upon, is the need of measuring and monitoring environmental pollution. The basic answer would be simply to analyze the extent of pollution. The appropriate analytical techniques help to study environmental pollution by providing the exact estimation of the quantitative and qualitative composition of pollutants. On-site measuring of environmental pollution helps in studying the interaction range of specific pollutants and their accumulation by living beings can be estimated. It also helps in figuring the composition variability of pollutants in time and space along with estimating their source of emission and intensity.

Pollution control measures are dependent on reliable data and figures depicting the exact level of pollution. Any pollution control measurement will be rendered ineffective due to the absence of authentic data about the different types of pollution (air, water, soil, sewage management, noise pollution, effluents) (McDonald 2003). Thus, analysis of environmental pollution is the most effective way to decide which pollution control strategy to adopt, to know whether the strategy would be viable based on the extent of the pollution in that particular area and to ensure the overall economic feasibility of the particular control measure (Mitchell 2002; Stevens 1994).

2.2.1 Parameters to Study Environmental Pollution

In order to determine the quality of environment and its different components, a number of wide categories of physical, chemical and biological parameters are to be analyzed. These parameters help in determining whether the particular component of environment under study (soil, water, or air) can be characterized as polluted or not. The parameters are also known as quality indicators and play an important role in the process of environmental monitoring. According to a report published by USDA Natural Resource Conservation Service, attributes of a good

Table 2.1 Indicators used in the measurement of soil quality

Chemical indicators	Physical indicators	Biological indicators
Electrical conductivity	Aggregate stability	Earthworms
Soil nitrate	Available water capacity	Particulate organic matter
Soil reaction (pH)	Bulk density	Potentially mineralizable nitrogen
Soil electrical conductivity	Infiltration	Respiration
Reactive carbon	Slaking	Soil enzymes
Phosphorus	Soil crusts	Total organic carbon

indicator are sensitivity to change, ease of measurement and interpretation, and repeatable methodology and reversibility so that both development and decay can be monitored. Environmental monitoring is defined as the systematic sampling of air, water, soil, and biota in order to observe and study the environment, as well as to derive knowledge from this process (Artiola et al. 2004; Wiersma 2004).

2.2.1.1 Indicators of Soil Quality

Doran and Parkin (1994) defined soil quality as the capacity of the soil to function within ecosystem and land use boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health (Table 2.1).

2.2.1.2 Indicators of Water Quality

The major factors that determine the quality of water are listed as follows:

- Temperature and dissolved oxygen
- Conventional variables (pH, total dissolved solids, conductivity and suspended sediment)
- Nutrients
- Metals
- Hydrocarbons
- Industrial chemicals
- Fecal coliform

As oxygen is required by the microbial community in the water, concentration of molecular oxygen dissolved in water, also known as dissolved oxygen (DO), is an important parameter to analyze the health of a water ecosystem. Discharges of wastes rich in organic matter (e.g., from sewage treatment plants, paper manufacturing, food processing, and other industries) can reduce the concentration of DO which can be harmful to fish and other aquatic organisms. Turbidity provides an approximation of the dirtiness or cloudiness of water caused by floating matter, such as clay, silt, finely divided organic and inorganic matter, soluble colored organic compounds and plankton and other microscopic organisms. A high turbidity can also be a result of the presence of *E. coli* (the most commonly occurring fecal coliform bacteria) in water which indicates the occurrence of disease-causing bacteria. Probable diseases and illnesses carried by such waters are gastroenteritis,

typhoid fever, hepatitis, dysentery, swimmer's itch and ear infections. (Chapman 1996)

2.3 Environmental Pollution Analysis: Instrumentation and Measurement Techniques

2.3.1 Colorimetry/Spectrophotometer

Colorimetry is a sensitive analysis technique which involves the measurement of the amount of light absorbed by the color developed in a sample. Reacting chemicals, also known as reagents, are used to develop a characteristic color to the material used for analysis. The amount of light absorbed is dependent upon certain factors: the length at which light travels, the concentration of absorbing material and the overall chemistry involved.

Colorimeter is the device used for making accurate and precise measurements using colorimetry. It is an instrument which compares the intensity of light between an unknown solution and a pure sample solvent by determining the difference between the intensity of initial and transmitted light. The essential parts of a colorimeter are:

1. A light source (can be a tungsten filament light bulb)
2. An adjustable aperture
3. A detector used for measuring the light that has passed through the solution
4. Glass or plastic cuvettes for placing the solution
5. Set of filters which passes light of the color which is absorbed by the treating sample.

However, for a solution to be analyzed by a colorimeter, it has to be colored and free from any type of bacterial or other contaminations (Figs. 2.1 and 2.2).

Transmittance and absorbance are both inversely proportional to each other, *i.e.*, as the transmittance increases, absorbance decreases exponentially. For making calorimetric measurements, the intensity of light passing through the pure solvent, intensity of the light, i.e., passing through the unknown sample, and the two quantities of transmittance and absorbance are calculated (Table 2.2).

2.3.2 Spectrophotometers

Spectrophotometers, more delicate and costly than colorimeters, are used for more precise and interference-free measurements. The underlying principle of spectrophotometry lies in breaking a light of color using a prism or diffraction grating. The prism (or grating) is rotated periodically to select the color of light absorbed by the sample. There are two types of spectrophotometers:

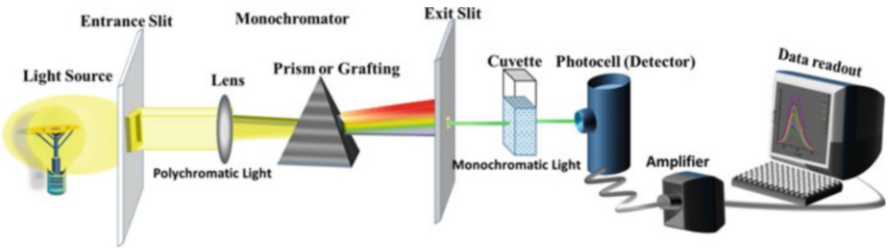

Fig. 2.1 A systematic diagrammatic representation of colorimeter

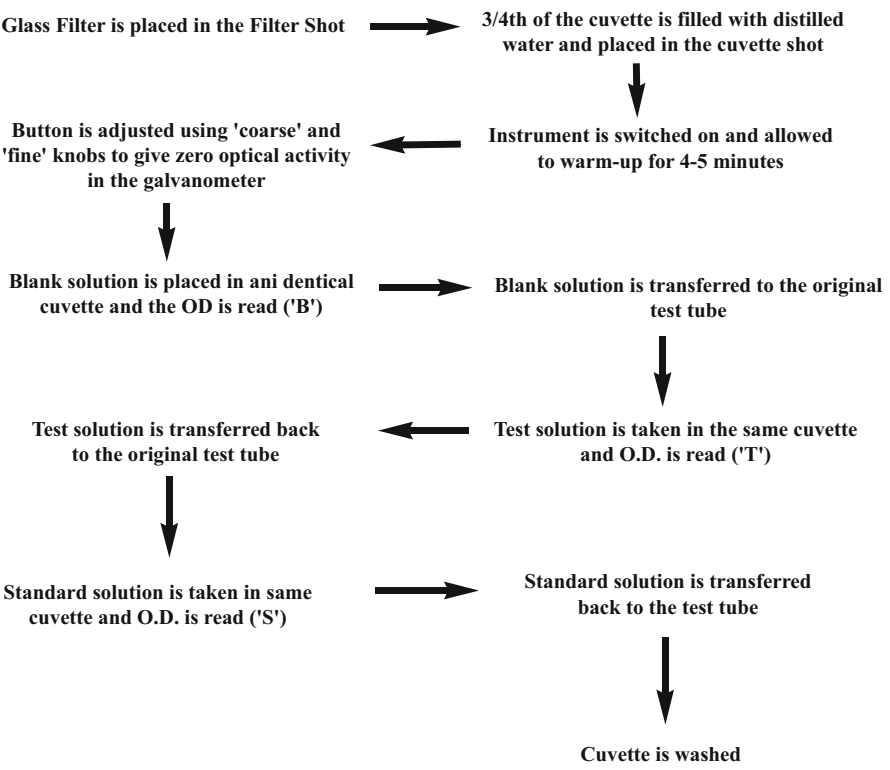

Fig. 2.2 Working of colorimeter/spectrophotometer

Table 2.2 Calculation of transmittance and absorbance

Transmittance	Absorbance
The amount of light that passes through a solution is known as transmittance	Negative logarithm of transmittance is called absorbance
$T = I_t/I_0$ (Where I_t = intensity of transmitted light and I_0 = intensity of the initial light beam)	$A = \log (1/T)$

2.3.2.1 Single Beam Spectrophotometer

Single beam spectrophotometer – the source of energy provides a stable source of light radiation, and wavelength selectors permit the partition of wavelength of the preferred one from other radiations. Light radiation passes through a glass container or cell with sample and the purpose of the detector is to measure the energy as it passes through the sample. The read-out device calculates the quantity of light absorbed by the sample and displays the signal from the detector as absorbance or transmittance.

2.3.2.2 Double Beam Spectrophotometer

Double beam spectrophotometer – is an instrument in which two beams of light are created by a V-shaped mirror called a beam splitter. Out of the two beams, one beam passes through the reference solution to a photodetector, while the other one passes through the sample to a second photodetector simultaneously. The obtained two outputs are amplified, and their ratio or the log of their ratio is obtained electronically/computed and displayed on the output device. Double beam spectrophotometer can be further categorized into double beam in-space spectrophotometer and double beam in-time spectrophotometer based on the axis of operation (Figs. 2.3 and 2.4).

2.3.3 Components of Spectrophotometer

A spectrophotometer consists of:

1. A stable light source with proper intensity. Depending on the electromagnetic region of the light, hydrogen or deuterium discharge lamp (having wavelength of 10–200 nm), tungsten lamp (200–1000 nm), or Nernst glower (1000–1,000,000 nm) can be used.
2. Filters and monochromators which allow the radiation of specific wavelength to pass, while absorbing others at the same time. Filters, made up of glass and gelatin, have high absorbing capacities as only a small section of frequencies can pass through them. Monochromators (slit or other dispersive element, i.e., prism and grating) are optical devices used for selecting particular frequencies from a range of wavelengths.
3. Sample vessel, generally called cuvette, is made up of the material which does not absorb any light or interfere with the process of absorption of light by the solution. Quartz and glass cuvettes are used for UV and visible range, respectively. In case of infrared region, cuvettes made up of sodium chloride or potassium bromide can be used.
4. Detector photovoltaic cell (barrier layer cell), phototubes (photoemissive tube), and photomultiplier tubes are the three most common types of detectors used for measuring the absorption or transmission of the light.
5. Recorder is used for digital recording of the different results (absorption, transmission, graph of wavelength versus absorption, etc.).

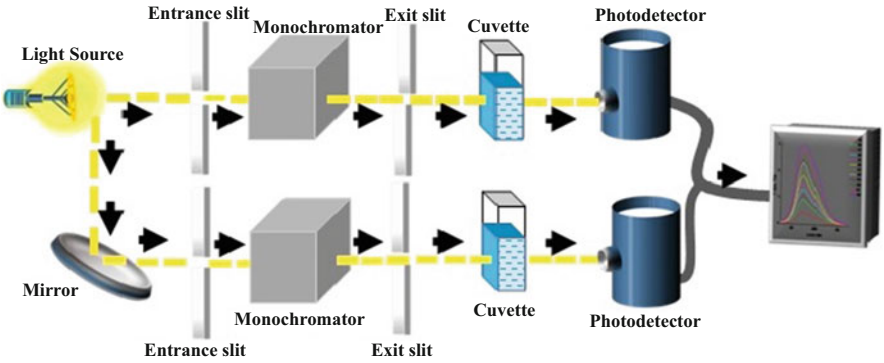

Fig. 2.3 Double beam in-space spectrophotometer

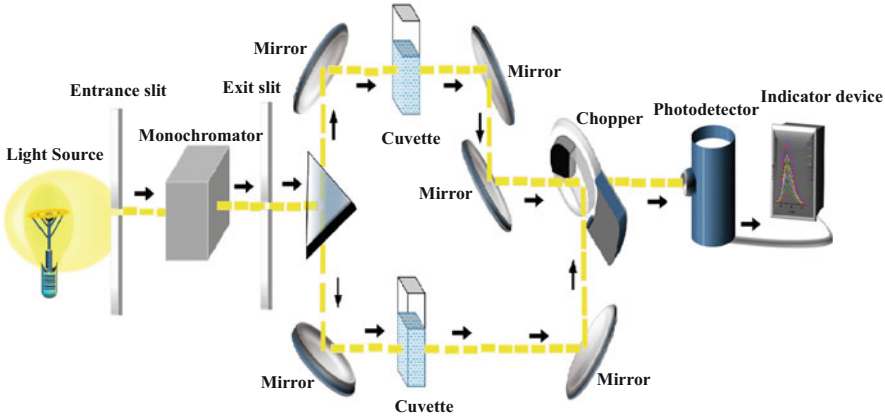

Fig. 2.4 Double beam in-time spectrophotometer

6. Photomultiplier tube consists of a photocathode coated with light reflecting material. It is used to measure the intensity of light by generating electrons from the light source (Table 2.3).

2.4 Atomic Absorption Spectroscopy

Atomic absorption spectroscopy, first developed in 1954, is a very simple and reliable technique for detecting the concentration of metals (and metalloids) in a sample. In an environmental analysis, it is very helpful in finding the levels of heavy metal pollution in water and air pollution measurement. It involves quantifying the absorption of free atoms in an atomizer from ground state to the vapor state. While

Table 2.3 Components of a spectrophotometer

	Prism	Grating
Material	Quartz, calcite, or glass	Aluminum or any bright surfaced material
Dispersion of light	Not sharp	Very sharp
Purity of spectrum	Less pure than grating	Much more pure
Working wavelength	Between 400 and 1000 nm regions	Between 200 and 800 nm regions
Range of ray band acquired	10–25 nm	5 nm
Ghost spectrum	Not acquired	Acquired in case of improperly placed lines

transitioning from ground level to higher energy state, the atoms absorb UV or visible light. This radiation absorbed by the atom in the sample is used to determine the concentration of an analyte. Depending on the advancement of technology, atomic absorption spectroscopy is further categorized into three types: flame photometry, atomic absorption spectrophotometry (AAS) and inductively coupled plasma-atomic emission (ICP-AES).

2.4.1 Flame Photometry

Flame photometry, also known as atomic emission spectrometry, is an environmental analysis technique used to determine the concentration of alkali metal salts, mainly sodium, calcium, potassium, lithium and cesium, with the help of a photoelectric flame photometer. A solution, containing the metal, is subjected to excitement with the help of light or flame which evaporates the solvent. However, in addition to evaporation, the flame atomizes the metal present in the solution as well as exciting the valence atom to higher energy state simultaneously. As the atoms return to the ground state, they emit light. The intensity of this light is measured, and characteristic wavelength of the color helps in determining the particular element under investigation. Similarly, the quantity of the element present can be determined by the intensity of the flame. The technique, although simple and relatively inexpensive, can be somewhat less accurate in case of ions with higher concentration (Fig. 2.5).

A typical photoelectric flame photometer consists of:

1. Source of flame for spraying the sample solution. A premixed burner in which sample, fuel and oxidant are well mixed is used as it gives a uniform flame at a constant temperature for the process.
2. A nebulizer is used for breaking the liquid into fine droplets into a flame. For removing large droplets from the stream, an atomic modifier can also be used.

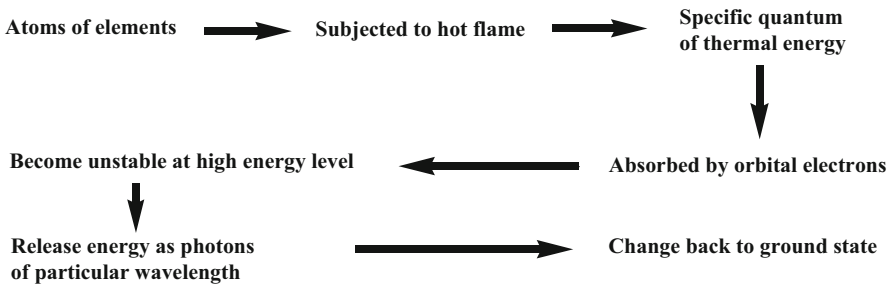

Fig. 2.5 Working of flame photometer

3. An optical system made up of a convex mirror, lens, and filter are used for transmitting the light emitted from the atom, focusing it on a slit and, thus, isolating the wavelength, respectively.
4. A photodetector in the form of photomultiplier tubes, photoemission cell, or photovoltaic cell is also used. It reads the intensity of emitted light in the form of an electric signal and measures the intensity of radiation (Fig. 2.6).

2.4.2 Atomic Absorption Spectrophotometry

Although expensive than flame photometry, atomic absorption spectrophotometry or AAS is a very versatile and widely used technique as it helps in determining a large number of metals in soil, water, air, and blood, present at fairly low concentration (parts per billion or parts per million). A hollow cathode lamp which produces monochromatic light is the main component of an atomic absorption spectrophotometer, and the instrument functions at a very high temperature (2500–3000 °C). The metal that has to be studied or investigated is used for making the hollow cathode. The cathode is by and large cylindrical in shape which plays an important role in giving direction to the emerging beam and redepositing sputtered atoms back as cathode. The anode is generally made up of tungsten. The electrodes of anode are surrounded by noble gases which are ionized by the electrons produced by cathode when given high voltage. These ionized atoms, in turn, bombard the cathode causing the sputtering of atoms from the surface of cathode. These free atoms are excited by high-speed electrons and, therefore, emit the line spectrum characteristics of the particular elements of which the cathode is made up (Figs. 2.5 and 2.7).

2.4.3 Inductively Coupled Plasma Mass Spectroscopy (ICP-MS)

ICP-MS is a combination of inductively coupled plasma which is considered as an ideal source for the generation of ions, and mass spectrometry is for the

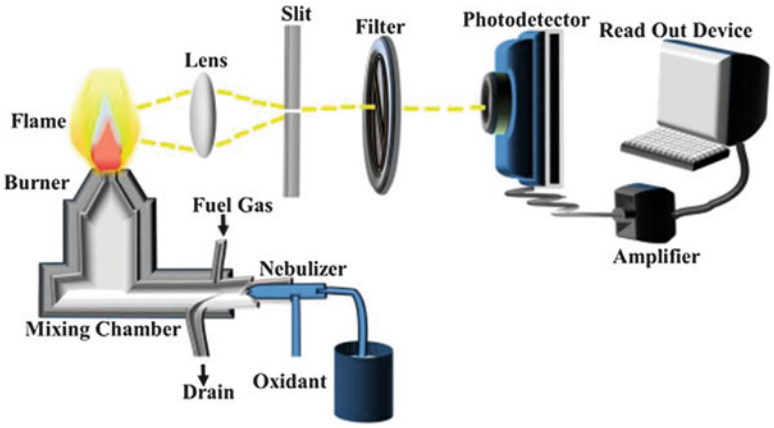

Fig. 2.6 Diagrammatic representation of flame photometer

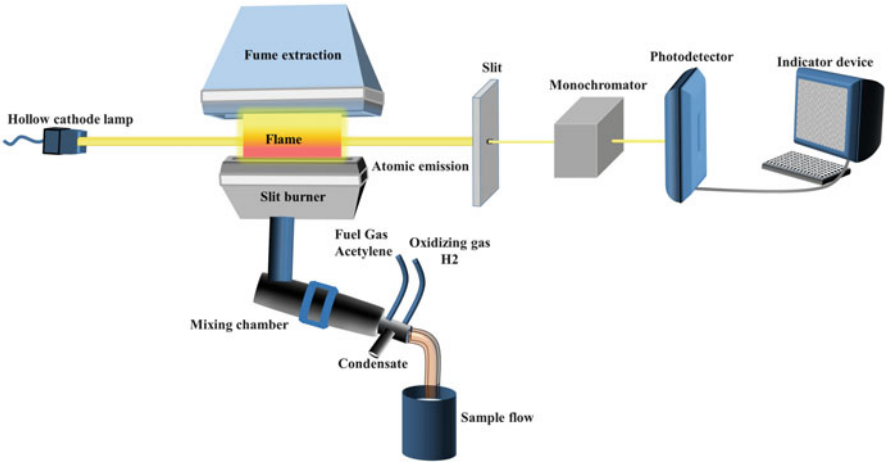

Fig. 2.7 Diagrammatic representation of atomic absorption spectrophotometer

detection of ions, a technique based on the measurement of the mass of an element. It is a highly efficient technique with a wide elemental coverage ranging from the levels of ppt to ppb in a single analysis. When the samples are introduced into ICP, a complete decomposition of samples into analyte atoms takes place owing to its high temperature and the atoms are simultaneously ionized. These ions are, then, extracted from the plasma into mass spectrometer region (Fig. 2.8).

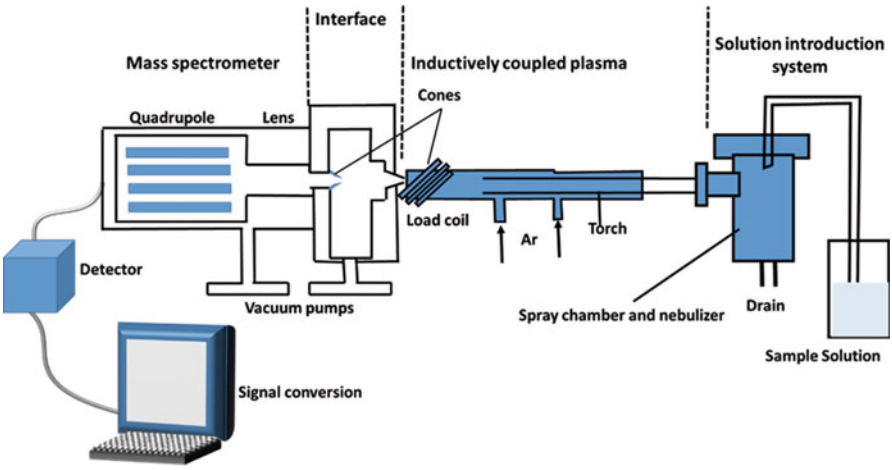

Fig. 2.8 Inductively coupled plasma mass spectrometry (ICP-MS)

A typical ICP-MS system can be divided into three parts which includes a solution introduction system, inductively coupled plasma and mass spectrometer along with an interface region between the latter two parts and an ion detector.

1. Solution introduction system consists of autosamplers, tubes, peristaltic pumps, spray chamber and nebulizer. While solid samples are introduced by laser ablation, liquid samples are mostly introduced via peristaltic pumps which are further converted into fine aerosol mist with the help of the argon gas present in nebulizer. Nebulizers are generally made up of quartz glass and can be either concentric, cross flow, or micro flow depending upon the efficiency and size of the matrix. The function of a spray chamber is to separate small fine droplets from large ones for further analysis and suppress sample flow variation caused by peristaltic pumps.
2. Inductively coupled plasma is a highly ionized gas which is generated with the help of carrier gas, argon and a plasma source known as torch which causes the vaporization, decomposition, atomization, and ionization of the sample. The torch consists of three quartz concentric circles assembled in a single piece for the flow of carrier gas at different rates (main plasma gas, Ar flow ~ 15 L/min; auxiliary gas, Ar flow ~ 0.75 L/min; Nebulizer Gas, Ar flow ~ 1 L/min). The interface region, coupling atmospheric ICP source and high vacuum mass spectrometer, is situated between two nickel or platinum (having high thermal conductivity and resistance to corrosion) cones, called sampler cone and skimmer cone, through which the sample ion passes. Sampling cone has a small orifice of about 1 mm and is used to create a difference in pressure to draw the ions in the plasma and plasma gas at low pressure region. The cone with a 2.5 times larger orifice called skimmer cone helps in the passage of ions and expanding stream of plasma gas when placed behind the sampler cone.

3. Mass spectrometer consists of ion lens optics, collision-reaction cell and quadrupole mass analyzer attached to a detector for signal conversion. Ion lens optics consists of one or more positively charged lenses causing the separation of positively charged ions by applying differential voltage. These ions pass through the collision-reaction cell to remove major spectral interferences generated by ions derived from plasma gas. Quadrupole, a type of mass analyzer, is a very important component of the system used for separating ions based on their mass-to-charge ratio. It consists of four parallel conductive rods of the same length (typically 15–20 cm with 1 cm diameter). The cylindrical or hyperbolic rods are made up of gold-coated ceramic or molybdenum for corrosion resistance. Electrostatic field is generated between the rods when a direct current (DC) potential is applied to one pair of opposite rods, and an alternating current of radio frequency (RF) is applied to the other opposites of the rod. By adjusting and accurately optimizing the suitable DC/RF ratios, analytes of specific mass-to-charge ratios can be selected while the other unstable ones are ejected from the quadrupole. After passing through the quadrupole, ions reach the ion detector which is typically an electron multiplier. It is used for the quantification of the total ions from the quadrupole. The ions are converted into electrical pulses, and their magnitude shows the number of analytes present in the sample.

ICM-PS is used in environmental analysis for metal toxins as well as heavy metals in surface and groundwaters (fresh to saline), sediment extracts and waste in fish bone and tissue. Though the technique has a wide range of applications and analysis is possible for almost all elements in the periodic table, it is an expensive technique with a high level of training, maintenance and service required.

2.5 Chromatography

Chromatography, meaning color writing or color picture, can be broadly defined as the technique of separating color pigments from one spot on a paper. It is used to separate a combination of organic and inorganic compounds. A solvent containing different components gives a series of spots when it is allowed to move through a paper. The two important components that explain the concept of chromatography are (1) an immovable static part known as stationary phase and (2) a movable mobile phase. The mobile phase (i.e., the solvent) moves along the stationary phase, picking up and absorbing the compound under investigation. The distance a particular compound travels in a solvent is known as *retention factor* (R_f). It is a quantitative indicator to identify a compound and to understand the similarity between an unknown and a known compound. The closer the R_f value of two compounds, the more will be the similarity between those two compounds under investigation and vice versa (Ettre 1993) (Fig. 2.9).

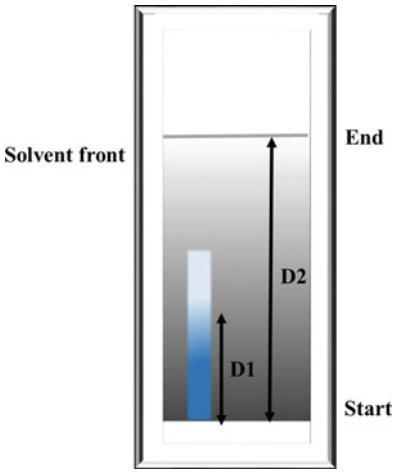

Fig. 2.9 TLC plate

Mathematically, R_f can be given as:

$$R_f = D_1/D_2$$

where D_1 is the distance traveled by the compound and D_2 is the total distance traveled by the solvent.

2.5.1 Types of Chromatography

Based on the difference in mobile phase and stationary phase, chromatography can be further categorized as (Cramers et al. 1999; Unger et al. 2008):

2.5.1.1 Gas Chromatography

It consists of a narrow coiled inert material (made up of glass, silica, or stainless steel) for injecting a mixture of substances for their separation and purification. The technique is ideally suited for volatile compound having a molecular weight up to 500 g. Gas chromatograph (GC), the instrument used to carry out the separation, consists of narrow capillary column which are made up of oil-coated powdered mineral. This is also known as the stationary phase and is thermally stable in nature. The mobile phase is composed of a gas, known as a carrier gas, usually neon or helium, which is continuously passed through a column during the whole operation. The column is mounted on an oven which can be set at a temperature up to 300 °C. At the end of the column is attached an inlet for carrier gas and an injection head which contains a rubber septum through which the sample can be injected by a syringe. A small volume of mixture to be separated is injected into the column where it is vaporized sometimes with the help of a flash heater. Based on the speed of different components on the stationary phase, the mixture is separated into discrete bands which are eluted one after the other through the detector. This device senses the presence of an organic compound in the gas stream and sends an electrical signal to the chart recorder or a visual display unit. Each compound in a

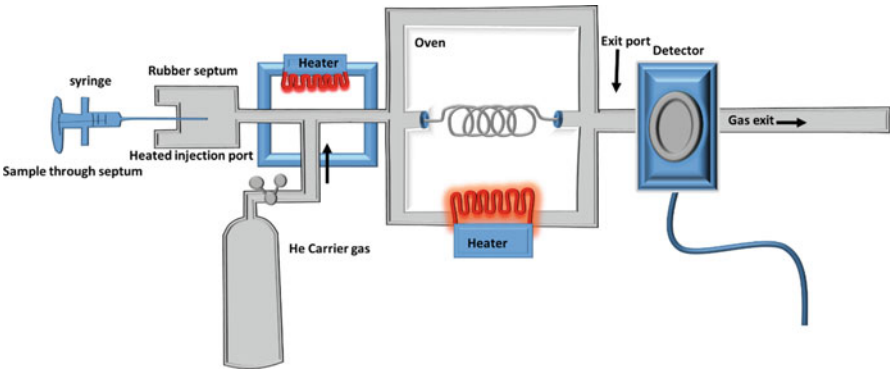

Fig. 2.10 A systematic diagrammatic representation of gas chromatography

mixture, therefore, produces a peak on the recorder and the more volatile a compound, the faster it will pass through the column.

The most important part of the GC is the packed column which is made up of copper or stainless steel tubing. Various diameters of tubing are used, but 3 mm–6 mm sizes are the most common. The material chosen for stationary phase is generally a liquid, a wax, or a low-melting solid which is relatively nonvolatile in nature. Some commonly used materials are silicon oils, waxes, polymeric esters, and amides (Fig. 2.10).

2.5.1.2 Liquid Chromatography

This type of chromatography is different than GC as it utilizes a liquid as a mobile phase instead of a gas. Silica gel or alumina is generally used as a stationary phase. It is best suited for analyzing organic compounds in a solution.

2.5.1.2.1 High-Pressure or High-Performance Liquid Chromatography (HPLC)

Some substances have a tendency to show instability at higher temperatures and have very high boiling points. For such substances, HPLC is the preferred method which utilizes organic solvents for mobile phase. A finely divided stationary phase, a solvent reservoir and mixing system, a high-pressure pump, a sample inlet and a column and together with a detector and recording unit constitute the apparatus of an ideal HPLC unit. The appropriate solvent from the reservoir is allowed to enter the mixing chamber when a homogenous mixture is obtained. A pump capable of maintaining high pressure draws the solvent from the mixing chamber and pushes it through the column. The sample is injected into a port with the high-pressure liquid carrier stream between the pump and the column. The separation takes place on the column which may vary from 8 cm to 20 cm in length and 2 mm to 3 mm in diameter. The typical flow rate is 1–2 mm per minute with a pressure of up to 1000 s of psi. The column effluent passes through a nondestructive detector where the properties such as ultraviolet absorbance, refractive index and molecular fluorescence are monitored, amplified, and recorded as a typical detector response versus

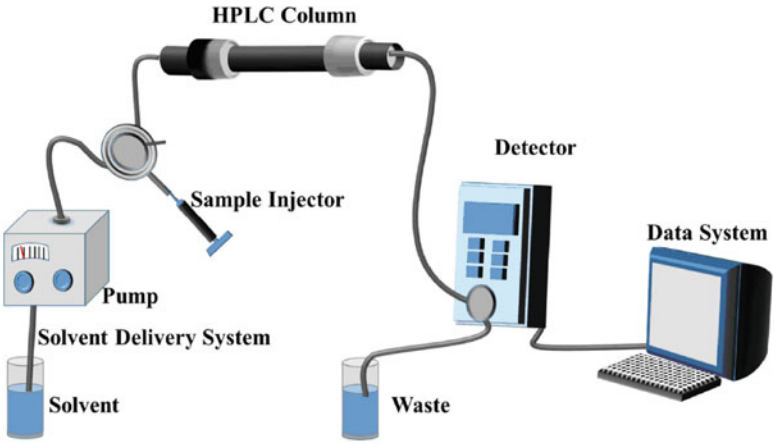

Fig. 2.11 A systematic diagrammatic representation of high-pressure or high-performance liquid chromatography

retention time chromatograph. The effluent can be discarded, reused, or further saved for studies in a fraction collector which is synchronized with the detector (Fig. 2.11).

2.5.1.3 Reverse Phase Liquid Chromatography

In reversed-phase high-performance liquid chromatography (RP-HPLC), the separation of molecules is based on their hydrophobicity, i.e., attraction toward water. The separation totally depends on the hydrophobic binding property of the solute molecule from the mobile phase to the immobilized hydrophobic ligands attached to the stationary phase, i.e., the sorbent (Garcia-Alvarez-Coque et al. 2015).

2.5.1.4 Paper Chromatography

It is the most common type of chromatography which involves placing a drop of solution of compounds to be separated on a piece of paper and allowing them to dry. The mixture is separated with the help of capillary action which pulls the solvents up and separates the solute. A strip of paper or paper fiber acts as a stationary phase which causes the partition of the different solutes. Mobile phase ranges from solvents as common as propanone, pentanol, or ethanol to aqueous and solvent systems with different polarities.

2.5.1.5 Thin-Layer Chromatography

Thin-layer chromatography, also known as TLC, is one of the fastest methods to determine the purity of an organic solution. The mixture, to be separated, is placed at the bottom of the TLC plate, which after getting dry is placed in a closed vessel containing the solvent (mobile phase) to ensure complete saturation and minimize any kind of evaporation. The small particle size of stationary phase makes it an extremely efficient process with easy to reproduce results as well (Fig. 2.9).

Table 2.4 Different types of chromatography and its applications

Type of chromatography	Mobile phase	Stationary phase	Applications
Gas chromatography	Gases (neon, helium)	Capillary or packed columns	Volatile organic compounds, permanent gases
Thin-layer chromatography	Vapor of organic solvents	Impregnated plates	Organic compounds
Paper chromatography	Mixture of dyes or inks	Paper or the cellulose fibers in the paper	Inks from fiber-tipped pens, food colorings, and dyes
HPLC	Water and organic solvents	Porous silica particles	Pesticides and herbicides, antioxidants, additives, antibiotics, sedatives
RP-HPLC	Organic solvents	Porous silica particles	Proteins and peptides

Table 2.4 summarizes different types of chromatography and its application in different areas.

2.6 Infrared Spectroscopy

IR spectroscopy is a nondestructive technique used for the quantification of samples and structural elucidation to determine their functional group based on the measurement of IR radiations, absorbed or emitted by them. IR has a wide range of applications as different inorganic and organic compounds acknowledge the infrared light. IR spectroscopy is also known as vibrational-rotational spectroscopy as the applied infrared frequency is directly proportional to the natural frequency of vibration of a molecule. Molecular vibrations are characterized into two types – stretching and bending vibrations.

1. Stretching vibrations occur along the line of bond at high energy and causes a change in bond length which can be symmetrical or asymmetrical. (a) Symmetrical stretching where both bonds increase and decrease in length simultaneously with respect to central metal and (b) in asymmetrical stretching, one molecule is moving toward the central atom, while another one is moving away from central atom.
2. Bending vibrations occur at low energy and alters the bond angle. Bending can be either in plane or out plane bending. When two atoms approach each other causing a decrease in bond angle, it is a type of scissoring "in plane" vibration; however, if the movement takes place in the same direction, it is called rocking "in plane" vibration. Out plane bending can be further divided into wagging and twisting where both the atoms either move up and down in the same direction or in the opposite direction, respectively (Pavia et al. 2012) (Fig. 2.12).

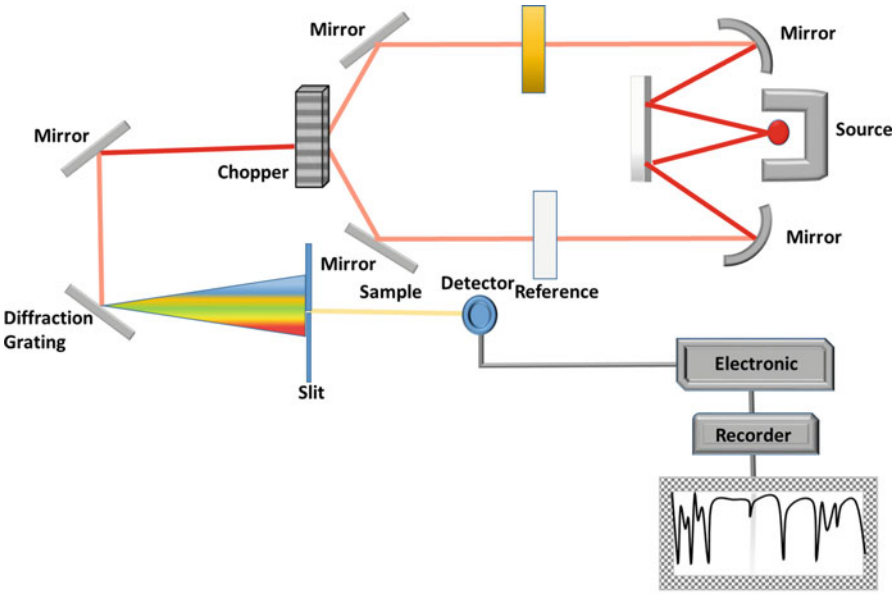

Fig. 2.12 A systematic diagrammatic representation of IR spectrometer

An IR spectrometer consists of the following parts:

1. *Source*, namely, tungsten incandescent lamp (black body source), Nichrome (or rhodium) wire (coiled and heated by resistance to incandescence), Nernst Glower (rare earth oxides) (more intense emitted radiation), Globar (a rod of silicon carbide 6–8 mm in diameter) and carbon dioxide laser (useful for narrow radiation bands).
2. *Fore optics* consists of mirrors for alternately allowing the reference and sample beam to pass through.
3. A *monochromator* is used for splitting the polychromatic radiation to component wavelengths by applying the use of prisms or grating or both. Rock salt prism or diffraction grating is usually used as a monochromator in IR.
4. *Chopper* is a tune amplifier used for modulations.
5. *Detector* measures the radiant energy by its heating effect, whereas detectors used are thermopiles bolometer and Golay cells. The conductivity of the material is measured continuously by a bridge network when radiation is allowed to fall on photoconducting material. Once the sample absorbs radiation, there will be difference between the two radiations, and signal will be produced.
6. *Recorder* – the signal which is amplified is used to move an attenuator which cuts down the radiation coming out of the reference beam until energy balance is restored. This is attached by a motor which drives the comb into the reference

beam when an absorbing band is encountered and out of the beam when the band is passed over (Colthrup et al. 1990).

Nowadays, three different types of IR instruments are available, namely, (a) Fourier transform infrared (FTIR) spectrometers, (b) nondispersive infrared (NDIR) instruments, and (c) dispersive infrared (DIR) instruments.

2.7 X-Ray Diffraction

X-ray diffraction or XRD is a nondestructive analytical technique pioneered in 1912 for the identification of crystalline materials. There are primarily two types of XRD known as X-ray powder diffraction and single-crystal XRD. The most important role of XRD is to identify and characterize the crystalline compound under investigation and analyze unit cell dimensions. When radiation enters a crystalline substance (minerals, ores, aggregates, limestone, silica sands, and clay), diffraction of X-ray from the unique arrangement of atoms in a crystal structure takes place. These X-rays are produced by a cathode-ray tube which is filtered to generate monochromatic radiation, collimated to concentrate and directed toward the sample. The interaction of incident rays with the sample produces constructive interference when conditions satisfy Bragg's law ($n\lambda=2d\sin\theta$) (Cullity 1978).

The basic components of XRD machine includes a (1) monochromatic X-ray source; (2) sample, finely powdered or polished surface, may be rotated against the center; and (3) data collector – like a film, chart strip, or magnetic medium/storage. The X-ray radiation commonly used is emitted by copper whose characteristic wavelength is 1.5418°A. When the incident beam strikes a powdered sample, diffraction occurs in every possible orientation of 2θ. The diffracted beam may be detected using a movable detector such as "Geiger Counter" which is connected to a chart recorder. The scanning speed of the counter is usually 2θ of 2° per minute, and, therefore, about 30 min are needed to obtain a trace of sample (Skoog et al. 2007) (Fig. 2.13).

2.8 Application of Environment Biotechnology in the Environment

Environmental biotechnology in particular is the application of processes for the protection and restoration of the quality of the environment. Environmental biotechnology can be used to sense, prevent and remove the emission of pollutants into the environment in a number of ways. All the wastes, i.e., solid, liquid, and gaseous can be transformed, either by recycling to make new products or by purifying so that the end product is less damaging to the environment. Replacing chemical supplies and processes with biological technologies can diminish environmental damage. In this way environmental biotechnology can make a significant

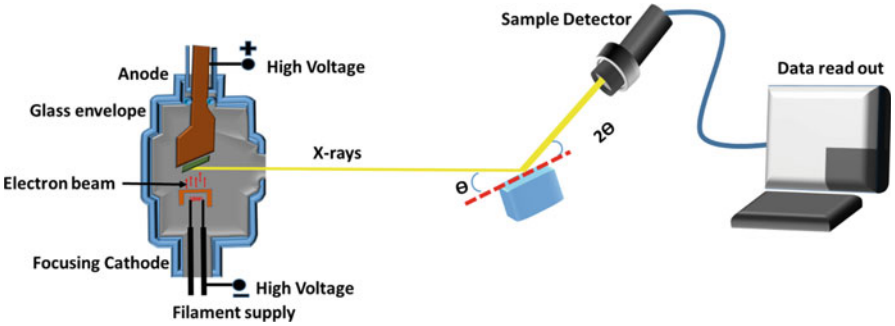

Fig. 2.13 A systematic diagrammatic representation of X-ray diffraction

contribution to sustainable development. Environmental biotechnology is one of today's fastest growing and most practically useful scientific fields. Research into the genetics, biochemistry, and physiology of exploitable microorganisms is rapidly being translated into commercially available technologies for reversing and preventing further deterioration of the earth's environment.

There are various major different types of applications of environmental biotechnology. These are as follows *(Source: www.biotechonweb.com)*:

2.8.1 Environmental Detection and Monitoring

A wide range of biological methods are in use to detect pollution and for the continuous monitoring of pollutants. The techniques of biotechnology have novel methods for diagnosing environmental problems and assessing normal environmental conditions so that human beings can be better informed of the surroundings. Applications of these methods are cheaper, faster and also portable. Rather than gathering soil samples and sending them to a laboratory for analysis, scientists can measure the level of contamination on site and know the results immediately. Biological detection methods using biosensors and immunoassays have been developed and are now in the market (Mataveli et al. 2010). Microbes are used in biosensor contamination of metals or pollutants. *Saccharomyces cerevisiae* (yeast) is used to detect cyanide in river water, while *Selenastrum capricornutum* (green alga) is used for heavy metal detection (Park et al. 2010). Immunoassays use labeled antibodies (complex proteins produced in biological response to specific agents) and enzymes to measure pollutant levels. If a pollutant is present, the antibody attaches itself to it making it detectable either through color change, fluorescence, or radioactivity.

2.8.2 Bioremediation

The process of cleaning up the hazardous substances into nontoxic compounds is called the bioremediation process. This process is majorly used for any kind of technology cleanup that uses the natural microorganisms. The use of different types of contaminants and fungi are used to clean the environment, and it plays a very vital role to keep the pollutants away from the environment. The bacteria are considered as one of the vital microbes since they break the dead organisms or the materials into useful organic matter and nutrients. As per the research, not all the contaminants affecting the environment can be destroyed using the process of bioremediation, e.g., lead and cadmium are not the contaminants that can be decomposed by the microorganisms (Vilar et al. 2007; Kouba et al. 2010).

The process of bioremediation takes place in two conditions – aerobic and anaerobic conditions. When the microbes need oxygen to perform, its process is in the case of aerobic condition; if they can have ample amount of oxygen, they can be able to give maximum amount of water and carbon through the conversion of contaminants and toxins. In the case of anaerobic conditions, the microbes perform their work without the presence of oxygen; the chemical compounds present in the soil help the anaerobic to perform its duties efficiently.

2.8.3 Wastewater and Industrial Effluents

Water pollution is a serious problem in many countries of the world. Rapid industrialization and urbanization have generated large quantities of wastewater that resulted in the deterioration of surface water resources and groundwater reserves. Biological, organic and inorganic pollutants contaminate the water bodies. In many cases, these sources have been rendered unsafe for human consumption as well as for other activities such as irrigation and industrial needs (Nova and Manaie 2010). This illustrates that degraded water quality can, in effect, contribute to water scarcity as it limits its availability for both human use and the ecosystem. Treatment of the wastewater before disposal is of urgent concern worldwide. In sewage treatment plants, microorganisms are used to remove the more common pollutants from wastewater before it is discharged into rivers or the sea (Gavrilescu 2002). Increasing industrial and agricultural pollution has led to a greater need for processes that remove specific pollutants such as nitrogen and phosphorus compounds, heavy metals, and chlorinated compounds. Methods include aerobic, anaerobic and physicochemical processes in fixed-bed filters and in bioreactors in which the materials and microbes are held in suspension (Cohen 2001). Sewage and other wastewaters would, if left untreated, undergo self-purification, but the process requires long exposure periods. To speed up this process, bioremediation measures are used.

2.8.4 Enzyme Application

Enzymes are widely employed in industries for many years. Enzymes, nontoxic and biodegradable, are biological catalysts that are highly competent and have numerous advantages over nonbiological catalysts. The use of enzyme by man, both directly and indirectly, have been for thousands of years. In the recent years, enzymes have played important roles in the production of drugs, fine chemicals, amino acids, antibiotics and steroids (Johnston 2003; Das 2005). Industrial processes can be made eco-friendly by the use of enzymes. Enzyme application in the textile, leather, food, pulp and paper industries help in the significant reduction or complete elimination of severe chemicals and are also more economic in energy and resource consumption (Chang et al. 2000; IBEP 2006). Biotechnological methods can produce food materials with improved nutritional value, functional characteristics and shelf stability. Plant cells grown in fermenters can produce flavors such as vanilla, reducing the need for extracting the compounds from vanilla beans. Food processing has benefited from biotechnologically produced chymosin which is used in cheese manufacturing; alpha-amylase, which is used in production of high-fructose corn syrup and dry beer; and lactase, which is added to milk to reduce the lactose content for persons with lactose intolerance (Cantor 2000). Genetically engineered enzymes are easier to produce than enzymes isolated from original sources and are favored over chemically synthesized substances because they do not create by-products or off-flavors in foods.

2.8.5 Soil and Land Treatment

As the human population grows, its demand for food from crops increases, making soil conservation crucial. Deforestation, overdevelopment, and pollution from man-made chemicals are just a few of the consequences of human activity and carelessness. The increasing amounts of fertilizers and other agricultural chemicals applied to soils and industrial and domestic waste disposal practices led to the increasing concern of soil pollution. Pollution in soil is caused by persistent toxic compounds, chemicals, salts, radioactive materials, or disease-causing agents, which have adverse effects on plant growth and animal health. Many species of fungi can be used for soil bioremediation (Gadd 2007). *Lipomyces* sp. can degrade herbicide paraquat. *Rhodotorula* sp. can convert benzaldehyde to benzyl alcohol (Schatzmeyr et al. 2003). *Candida* sp. degrades formaldehyde in the soil. *Aspergillus niger* and *Chaetomium cupreum* are used to degrade tannins (found in tannery effluents) in the soil thereby helping in plant growth (Wainwright 1999). *Phanerochaete chrysosporium* has been used in the bioremediation of soils polluted with different chemical compounds, usually recalcitrant and regarded as environmental pollutants. A decrease of PCP (pentachlorophenol) between 88–91% within 6 weeks was observed in the presence of *Phanerochaete chrysosporium* (Bogan et al. 1996). Bioremediation of contaminated soil has been used as a safe, reliable, cost-effective and environment-friendly method for degradation of various

pollutants. This can be affected in a number of ways, either in situ or by mechanically removing the soil for treatment elsewhere. Research in the field of environmental biotechnology has made it possible to treat soil contaminated with mineral oils. Solid-phase technologies are used for petroleum-contaminated soils that are excavated and placed in a containment system through which water and nutrients percolate (Ahn et al. 2005). Biological degradation of oils has proved commercially viable both on large and small scales, in situ and ex situ.

2.8.6 Air and Waste Gases

With the onset of human civilization, the air is one of the first and most polluted components of the atmosphere. Most air pollution comes from one human activity: burning fossil fuels – natural gas, coal and oil – to power industrial processes and motor vehicles. When fuels are incompletely burned, various chemicals called volatile organic chemicals (VOCs) also enter the air. Pollutants also come from other sources. For instance, decomposing garbage in landfills and solid waste disposal sites emits methane gas and many household products give off VOCs. Expanding industrial activities have added more contaminants in the air. The concept of biological air treatment at first seemed impossible. With the development of biological waste gas purification technology using bioreactors – which includes biofilters, bio trickling filters, bio scrubbers and membrane bioreactors – this problem is taken care of. The mode of operation of all these reactors is similar. In the biofilters, the air is passed through a bed packed with organic materials that supplies the necessary nutrients for the growth of the microorganisms (Andres et al. 2006). This medium is kept damp by maintaining the humidity of the incoming air. Biological off-gas treatment is generally based on the absorption of the VOC in the waste gases into the aqueous phase followed by direct oxidation by a wide range of voracious bacteria, which include *Nocardia* sp. and *Xanthomonas* sp. (Arnold et al. 1997).

2.8.7 Benefits

The major benefit of environmental biotechnology is it keeps our environment safe and clean for the use of the future generations. It helps the organisms and the engineers to unearth useful ways of getting adapted to the changes in the environment and keep the environment green, safe and clean. The benefit of environmental biotechnology helps us to stay away from the use of hazardous pollutants and wastes that affect the natural resources as well as the environment. The upliftment of the society should be done in such a way that it helps to protect our environment and also helps us to develop it. The environmental biotechnology has a role to play in the removal of the pollutants. It is becoming an advantage for the scientists and the environmentalists to find ways to convert the waste to reusable products. The applications of environmental biotechnology are becoming a benefiting factor for

the environment; the applications which include genomics, proteomics, bioinformatics and sequencing and imaging processes are providing large amounts of information and new ways to improvise the environment and protect the environment.

2.9 Conclusion

This chapter of "Measurement of Environmental Pollution: Types and Techniques" is intended for students of any branch of science (chemistry, environment, biotechnology, etc.). This chapter is also a useful tool for the students engaged in research. Our aim is to give insight of various instruments, their basic principle and working. We also tried to focus on the important aspects of each spectroscopic techniques without dwelling excessively on theory or complex mathematical analysis. Brief studies of various biotechnological applications in relation to environment have also been incorporated.

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Need for the Advanced Technologies for Wastewater Treatment

3

Jagjit Kaur, Sandeep Punia, and Kuldeep Kumar

Abstract

Water is one of the basic needs of a living organism to sustain life on earth. But due to the rapidly increasing population, urbanization, and industrialization, the quality of portable water is depleting. If the wastewater is not treated efficiently, then it generates a number of problems such as malodor and health problems, gives birth to disease-causing agents, etc. Therefore, it is the need of the day to develop some new techniques which are more efficient in treating the wastewater. In this chapter, the use of new techniques such as membrane bioreactor, advanced oxidation techniques and nanotechnology for the treatment of wastewater have been discussed. The nanocomponents such as nanosorbents, nanocatalysts, molecularly imprinted polymers (MIPs), and nanostructured catalytic membranes (NCMs) are the recent techniques which treat wastewater very efficiently. The water recovered after these treatments meet the human consumption criteria.

Keywords

Wastewater • Nanoparticles • Advanced oxidation technologies • Membrane bioreactors

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3.1 Introduction

Waste is generated by mankind in all forms: water emulsions and solid wastes. Wastewater is defined as the residual water containing the waste of the institutes, residential, industrial, commercial and agricultural community after it has been used for a number of purposes. If the wastewater is allowed to accumulate, it will lead to various problems such as the production of malodorous gases, human gut infection etc. This is so because the wastewater contains a number of pathogenic microorganisms and increased growth of aquatic plants (eutrophication). It may also contain certain mutagenic or carcinogenic compounds. Therefore, it is very necessary to treat wastewater, remove the pollutants, reuse and safely discharge the residual water in the rivers. Population, urbanization and industrialization are causing stress on the freshwater resources which have created an alarm to search for new technologies which could treat the wastewater and make it available for human consumption. Rio Earth Summit (1992) introduced the concept of recycle, reuse and recovery for the sustainable development. The conventional wastewater treatment technologies that have been used do not treat the water to that extent which can be used for human use. A lot of water for domestic use is wasted in gardening, flushing and cleaning. For the purpose of gardening and flushing, the recycled water can be used (Ngo et al. 2007).

3.1.1 Conventional Treatment Technologies

To improve the quality of wastewater by removing nutrients, solids, and organic matter, water is given preliminary, primary, and secondary treatments. These processes are explained below:

3.1.1.1 Preliminary Treatment

In the preliminary treatment, the wastewater is screened for any debris and their separation. Sticks, toys, rags, leaves, sand, food particles and gravels are removed from the water using grit chambers, bar screens and comminutors. The separated out debris are then disposed of in a landfill.

3.1.1.2 Primary Treatment

Primary treatment involves sedimentation and skimming for the removal of inorganic and organic materials. The floatable materials are skimmed off the wastewater. The heavy metals associated with solids, organic nitrogen and phosphorous are removed, but the dissolved solids remain unaffected. During the primary treatment, around 65% of oil and grease, 50–70% total suspended solids and 25–50% BOD are removed.

3.1.1.3 Secondary Treatment

The effluent from the primary treatment is subjected to aerobic processes for the removal of remaining organic matter and suspended solids. The aerobic biological

treatments involve the decomposition of organic matter into inorganic compounds such as CO_2, NH_3 etc. in the presence of aerobic microorganisms.

3.2 Need for Advanced Wastewater Treatment Technologies

Since the past two decades, the efficiency of conventional wastewater treatment process has been reduced due to the three major factors (Langlais et al. 1991; Mallevialle et al. 1996): (1) population, (2) awareness, and (3) industrialization.

3.2.1 Population

With the rapid increase in the population, the water resources are depleting at alarming rates. The municipal and industrial wastewater need to be treated to remove the pollutants so that it may be reused. This treatment becomes more important in the semiarid and arid regions where a lot of money is spent on the import of the irrigation and portable water. The conditions are made worse by the release of toxic compounds in the water. These problems can be solved using the advanced wastewater treatment technologies which help in the removal of harmful compounds much more efficiently than the conventional wastewater treatment technologies.

3.2.2 Awareness

The public has become more aware about the water pollution and its consequences and thus desires strict laws to be made for MCLs (Maximum Contaminant Levels) of different pollutants and their removal. The US Environmental Protection Agency (USEPA) had formulated the Interim Enhanced Surface Water Treatment Rule to prevent the ill-effects of outbreaks of *Cryptosporidium* oocysts and *Giardia* cysts under which it is mandatory to destroy these microbes before release to the environment. For setting the new MCLs for haloacetic acids (HAA) and lowering the MCLs for trihalomethanes (THM), the synthetic organic compounds (SOCs) and nutrients like phosphorous and nitrogen cause a number of harmful impacts on environment and public health, and thus strict regulations need to be made for their discharge.

3.2.3 Industrialization

With the advancements in industrialization, the processes used have become more versatile and costly. Wiesner et al. (1994) used life cycle analysis and found that as compared to the cost of new pressure-driven membrane filtration plants for 20,000 m^3/day, the cost of conventional treatment processes was more.

These problems could be overcome using various advanced technologies that have been proposed, tested and applied for the wastewater treatment. The advanced wastewater treatment technologies have been discussed in detail as follows:

3.3 Advanced Wastewater Treatment Technologies

Some of the advanced wastewater treatment technologies are biological, physicochemical and hybrid technologies. Biological treatment technologies include biologically enhanced phosphorous removal (BEPR) systems and intermittently decanted extended aeration lagoons (IDEAL) systems for the removal of nitrogen. These do not produce the water to be reused but lays the platform for next treatment processes. Physiochemical processes include membrane filtration and deep bed filtration. Both these methods produce the reusable water and serve the advantages of minimum sludge production and simplicity, respectively. Membrane reactors, the combination of both biological and physiochemical processes, fall under hybrid treatment technologies providing the above said benefits in one single step.

3.3.1 Biological Treatment Technologies

3.3.1.1 Biologically Enhanced Phosphorous Removal (BEPR) Systems

Microbes need phosphorus for their metabolism and an activated sludge contains nearly 1.5–2% phosphorus. The microbes, if grown anaerobically, utilize the polyphosphates from the sludge to consume phosphorus and also generate phosphate store houses for other microbes. In this phosphate can be removed from the sludge if anaerobic conditions are provided in the initial phase and then aerating the sludge. The advantage of this process over chemical processes used for phosphorous removal is that the sludge generated is composed of biological matter which could be disposed of safely. However, this process suffers from the major drawback that it can only be used as a supplementary process to chemical processes and cannot be used alone.

3.3.1.2 Intermittently Decanted Extended Aeration Lagoon (IDEAL) Systems

Domestic waste consists of nitrogen which can be removed by the two-step process. The first step involves the nitrification catalyzed by *Nitrobacter* and *Nitrosomonas* bacteria, in which the ammonia nitrogen is converted to nitrate nitrogen. While in the second step, denitrification takes place, i.e., nitrate nitrogen is converted to nitrate gas. Both the nitrification (aerobic process) and denitrification (anaerobic process) take place in the same tank periodically. The clarified effluent is decanted by lowering the weir of the lagoon. Sewage water is treated using IDEAL systems in Australia for the removal of nitrogen, for example, Quakers Hill STP (Sewage Treatment Plant) in New South Wales.

3.3.1.3 Membrane Bioreactor Technology (MBR)

The separation process in which a semipermeable membrane is used to separate the feed stream into permeate (material passing through the membrane) and retentate (material left behind) is known as membrane filtration (Mallevialle et al. 1996). The combination of activated sludge process and membrane separation process to treat the wastewater is known as membrane bioreactor technology (MBR). In MBR the process is operated in the similar way as activated sludge treatment process without the need of secondary and tertiary treatment steps (sand filtration and clarification). Instead, the effluent is separated from the activated sludge using low-pressure membrane filters such as ultrafiltration (UF) or microfiltration (MF), reverse osmosis (RO) and nanofiltration (NF). Two types of models of MBR are available: sidestream configuration and submerged configuration (Fig. 3.1), but for the treatment of municipal wastewater, submerged configuration is used. Diclofenac, estrone (E1), 17α-ethinylestradiol (EE2), and ibuprofen could be removed using membrane reactors (Kruglova et al. 2016) (Fig. 3.1).

3.3.1.3.1 Submerged Membrane Bioreactor (MBR)

The membranes used in submerged MBRs may be plate membrane design or hollow fiber membrane. The wastewater should be prefiltered with the membrane filter of 3 mm grid distance to prevent the clogging of submerged MBRs. The membrane fouling should be controlled as it would otherwise reduce the flux. The shear forces produced by the turbulence of uprising air and liquid produce a stable flux as it controls the cake layer formation. In submerged MBRs nitrification, denitrification and phosphorous removal can be carried out simultaneously under low sludge loading conditions. Operational parameters could be controlled flexibly with submerged MBRs as it provides sludge retention time (SRT) and hydraulic retention time (HRT). The slow-growing specialized microorganisms are produced when the activated sludge is allowed to remain in contact with the critical class of substrates for a longer period of time. This helps in the removal of low biodegradable pollutants from the wastewater. The trace organic materials discharged from the treated sewage cause a number of pollution and health problems. These include personal care products, pharmaceuticals, and endocrine-disrupting compounds. These compounds can be removed with the help of MBR (Melvin and Leusch 2016).

3.3.1.3.2 Design of MBR

The membrane material used in the reactor greatly influences the success of the MBR. The membrane to be used should be cheap, durable and resistant to chemicals and contaminants and should provide greater permeate flux. The rate with which the permeate passes through the unit area of the membrane is defined as the permeate flux. New membranes have been developed which prove to be efficient for the MBR (Wiesner and Chellam 1999). The inorganic membranes provide resistance to high temperature and chemicals but are expensive and brittle due to which they are not used commercially. Therefore, commercially organic membranes are preferred as they provide higher chemical resistance and water

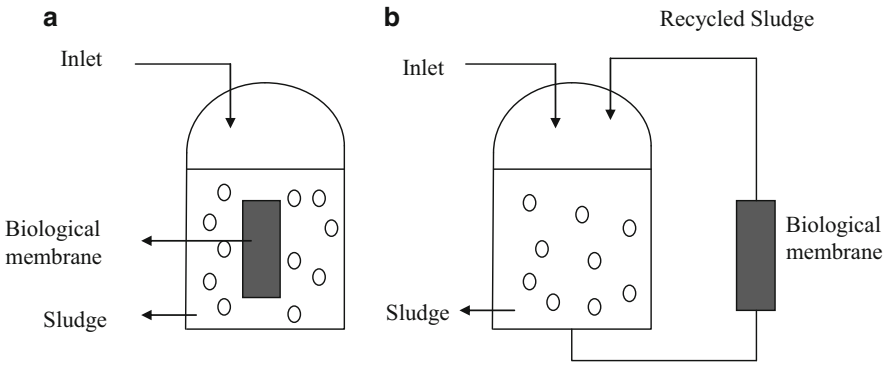

Fig. 3.1 Types of MBR: (**a**) submerged membrane bioreactor; (**b**) sidestream membrane bioreactor

permeability. They are prepared by coating the microporous support with thin layer of active polymer such as polysulfones, polyamides, cellulose acetates and polypropylene. While selecting the membrane, the most important factor that needs to be considered is the molecular weight cutoff (MWC) or the pore size which determines the amount of the solute to be rejected. The membranes are used to separate the microorganisms and particles of about 0.5 μm size under small pressure difference and high flux. RO is used for the desalting of seawater and brackish water as it has the smallest pore size and operates under low permeate flux and high pressure difference. UF and NF are used for the removal of SOCs and natural organic matter (NOM). The applicability of membranes for wastewater treatment has gained much importance as they are available with wide range of pore size and used for the removal of large number of contaminants. The UF and MF consist of hollow fibers which provide high surface area to volume ratio and backwash, but they consume a lot of energy to facilitate high cross-flow velocity. On the other hand, RO and NF have spiral wound configuration which provides reduced concentration polarization/fouling, higher turbulence, and lower deposition of particle cake.

For the reduction of membrane fouling and cost, new designs of MBRs have been proposed and used. A rotating disk membrane filter (Fig. 3.2) was developed by Reed et al. (1997) which consisted of a pressurized membrane with hollow rotating shaft and hollow membrane-covered disks stacked along it. This generates high shear at the membrane surface. The fluid dynamics were studied by Mallubhotla and Belfort (1997), and they developed a special curved model of MBR which reduces the membrane fouling by increasing the vortex at the surface. Another remarkable development of MBR model which helped in the reduction of energy consumption, tolerance to solid loading and high turbidity was the use of hollow submerged fiber bundles. Slight vacuum is applied to the hollow submerged fiber bundles directly mounted in the reactor and permeate is withdrawn.

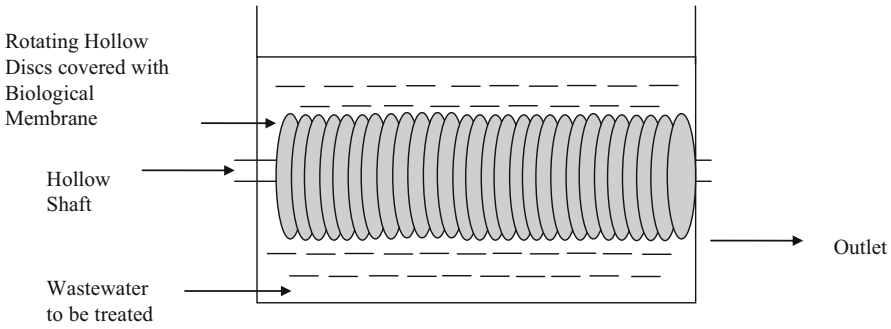

Fig. 3.2 Rotating disk membrane filter

Membrane fouling is reduced by introduction of air from the base which cleans and scours the outer surface of the membrane by creating turbulence.

3.3.1.3.3 Advantages of MBR

MBR serves a number of advantages over the conventional activated sludge treatment process. Some of them are listed below:

1. Higher sludge age due to decreased sludge production.
2. Lower sensitivity to contamination peaks.
3. Due to the use of membrane filters, the effluent is of high quality and more consistent.

3.3.1.3.4 Disadvantages of MBR

Despite the advantages and efficient operation, the MBR has some loopholes such as:

1. Parameters such as temperature, pH and pressure need to be maintained to meet the membrane tolerance.
2. Expensive to install and needs skilled operator.
3. The membrane needs to be frequently monitored and maintained.
4. Some chemicals may damage the membrane.

3.3.1.3.5 Applications of MBR

MBRs serve a number of advantages due to the wide range of pore size available. Out of these the main applications of MBRs are inorganic removal, organic removal and solid-liquid separation.

I. *Inorganic Removal*

The largest application of wastewater treatment is the removal of inorganic compounds by RO and NF. Heavy metals, hardness and nitrates can also be

removed by using NF and RO (Waypa et al. 1997). For the small surface water plants with no facilities of groundwater treatment plants, RO has been considered the best technology by USEPA (US Environmental Protection Agency).

II. *Organic Removal*

Dissolved organic compounds such as pesticides from groundwater, dyestuff from textile effluent etc. can be removed from industrial and municipal wastewater by membrane filtration (Brindle and Stephenson 1996; Mallevialle et al. 1996). Other applications of membrane filtration can be in the petroleum industry where the oil is concentrated from oil field brines, treatment of leachate from landfills, product recovery from food processing plants and decolorization of pulp and paper mill effluents. Pretreatment of wastewater with coagulation at pH 5–7 prior to membrane filtration increases the efficiency to remove organic compounds. Pesticides, NOM and DBPs from water with total organic carbon (TOC) concentration greater than 8 mg/L can be removed using RO and NF.

III. *Solid-Liquid Separation*

The solid-liquid separation is usually successful with MF and UF as they can be operated at low-pressure differentials. Chlorine-resistant pathogens (*Cryptosporidium* and *Giardia* species) and turbidity of a wastewater is removed using different types of membrane processes (Yoo et al. 1995; Ventresque et al. 1997). In a comparative study by Jacangelo et al. (1995), it was found that 0.3–0.9 log units of MS2 virus were removed using MF, whereas 6.8 log units of MS2 virus were removed with UF. The efficiency of the MBRs to remove microbial particles and suspended solids can be enhanced by pretreating the samples with coagulation (Wiesner et al. 1989), but excessive coagulation should be prevented as it would cause membrane fouling. This can be overcome if most of the newly formed floc is removed prior to membrane filtration. In addition a disinfectant needs to be added to prevent the regrowth of microbes in the water samples. For the efficient microbial removal of microbial particles, it is necessary to maintain the integrity of the membrane by bubble point testing, sonic sensor, seeded microbial monitoring and air pressure test. One way to maintain the integrity is to use the artificially defective membrane which had a needle hole in it (Adham et al. 1995). It helped in increasing the microbial count in the membrane, but there was no significant effect on the turbidity of the sample.

3.4 Nanotechnology in Wastewater Treatment

Nanotechnology is one of the most finest and advanced ways for the treatment of wastewater. Various reasons behind the success of nanotechnology are that nanoparticles have very high interacting, absorbing and reacting capacities due to their small size with large surface area. They can even be mixed with aqueous

suspensions to form colloidal solutions. Energy conservation is achieved by nanoparticles because of their small size. Since water treatment by using nanoparticles has high technology demand, hence its usage cost should be managed according to the existing competition in the market (Crane and Scott 2012). There are various recent advances on different nanomaterials (nanostructured catalytic membranes, nanocatalysts, bioactive nanoparticles, nanosorbents, biomimetic membrane and molecularly imprinted polymers (MIPs)) which have been used for the removal of disease-causing microbes, removal of toxic metal ions and also removal of inorganic and organic solutes from water. Different types of nanomaterials used are listed below:

(a) *Nanocatalysts*

Nanocatalysts have high surface area due to which their catalytic activity is high. Due to this nanocatalysts are used for the treatment of wastewater as it increases the reactivity of contaminants and rate of degradation. Environmental contaminants such as azo dyes, halogenated aliphatics, polychlorinated biphenyls (PCBs), organ chlorine pesticides, halogenated herbicides and nitro aromatics can be degraded by nanocatalysts like semiconductor materials, zero-valence metals and bimetallic nanoparticles (Xin et al. 2011). ZrO_2 nanoparticles, silver (Ag) nanocatalysts and N-doped TiO_2 catalysts are very efficient in degrading microbial contaminants. They have an additional advantage that these nanocatalysts can be reused (Shalini et al. 2012). The TiO_2-AGs are used for the remediation of Cr (IV) in wastewater. The TiO_2 nanoparticles are modified, and due to this their absorption band shifts from UV region to visible region. Thus, TiO_2-AGs are very efficient in removing Cr (IV) from the wastewater. The contaminants like halogenated organic compounds (HOCs) cannot be degraded easily and require advanced nanocatalytic activities. Therefore, the HOCs are first treated with Pd nanocatalysts and then biodegraded in the treatment plant. Depending on the level of contamination, hydrogen or formic acid can be used as reducing agent in the reaction. The nanocatalyst being used possesses ferromagnetism due to which it can be easily separated from the reaction mixture and then reused (Hildebrand et al. 2008). *E. coli* cells could be removed using WO_3 nanocatalysts (Khalil et al. 2009) and palladium-incorporated ZnO nanoparticles (Khalil et al. 2011). Marcells et al. (2009) studied the reduction of Cr (IV) to Cr (II) using palladium nanoparticles (PdNPs). For the combined sorption and degradation of the contaminants, the nanocatalysts could be combined with nanosorbents. Remediation of organic dyes can be achieved by activating the silver and amidoxime fiber nanocatalysts using tetrahydrofuran treatment (Zhi et al. 2010). A mono azo dye, Acid Blue 92 (AB92), could be removed efficiently with Sm (samarium)-doped ZnO nanoparticles (Khataee et al. 2016).

(b) *Nanosorbents*

The nanosorbents for the water treatment processes are mainly being used in Asia and the United States. They have specific and high sorption capacity toward

different contaminants. The nanosorbents serve an advantage that they can be removed from the treatment site which reduces the toxicity. Moreover, regenerated nanosorbents are cost-effective and preferred commercially. Ion exchangers, magnetic forces, cleaning agents and many more are used for the removal of nanosorbents from the treatment sites. The specific ligands with specific affinity are coated on the magnetic nanoparticles for the development of magnetic nanoparticles (Apblett et al. 2001). Silver ions can be removed as silver nanocrystals using nanosorbents, poly (aniline-co-5-sulfo-2-anisidine) (Li et al. 2010). Hydrocarbon dyes and phosphorus are removed using nanoclays. The organic contaminants can be removed from the wastewater using magnetic nanosorbents (Campos et al. 2012). Carbon-based nanosorbents have good adsorption capacity, high specific surface area, excellent mechanical strength and chemical resistance. They are used for the treatment of nickel-containing water (Lee et al. 2012). Due to their unique chemical and physical properties, mesoporous silica, chitosan and dendrimers are used as nanosorbents for the removal of heavy metal ions from the contaminated water (Vunain et al. 2016).

(c) *Bioactive Nanoparticles*

Bioactive nanoparticles are chlorine-free biocides which are emerging as a new tool in the treatment of wastewater. MgO nanoparticles and cellulose acetate (CA) fibers with embedded Ag nanoparticles are very effective biocides against gram-positive bacteria, gram-negative bacteria and bacterial spores (Nora and Mamadou 2005). Mesoporous silica nanoparticles could also be used for wastewater treatment processes as they are nontoxic, biologically compatible and easily modified with functional groups (Gunduz et al. 2015). Current and emerging nanotechnology approaches for the detection of microbial pathogens will aid microbial and pathogen detection as well as diagnostics.

(d) *Molecularly Imprinted Polymers (MIPs)*

Molecular imprinting is the process of free radical polymerization to a cross-linker. Molecularly imprinted polymers (MIPs) are one of the finest emerging techniques used in biological, environmental and pharmaceutical applications. They are cheap, simple, robust, selective and nonbiodegradable (Hande et al. 2015; Mattiasson 2015). The specific binding sites are provided to MIPs by the semi-covalent, covalent and non-covalent binding of the functional groups of suitable monomer to the template. Due to this modification, the MIPs are highly selective in nature and also are good absorbents. It is used for the treatment of wastewater and detection of the pollutants even in very low concentration (Caro et al. 2006). The selective nature of the MIPs is a great advantage over other techniques used. Mini-emulsion polymerization technique is used to develop nano-MIPs for the adsorption of micropollutants from hospital wastewater. The particle size of the nano-MIPs is 50–500 nm. For the removal of nano-MIPs from the wastewater after treatment, they could be coated with magnetic core (Tino et al.

2009). The pollution caused during wastewater treatment is treated with MIPs encapsulated in nanofibers using electro-spinning method. A sensor was developed using MIPs for the detection of phosphate levels in wastewater. The developed sensor had a detection limit of 0.16 mg P/L and was simply handheld and did not require filtration of the sample to be monitored like conventional methods such as colorimetry (Warwick et al. 2014). MIPs could be used for the removal of Cd (II), Pb (II), As (V), Hg (II), Ag, Au, Pt, Pd, acitindes, and lanthanides (Hande et al. 2015).

(e) *Nanostructured Catalytic Membranes (NCMs)*

Nanostructured catalytic membranes (NCMs) are preferred due to their optimization capability, limited contact time of catalyst, uniform catalytic sites, easy industrial scale-up and allowed sequential reactions. Membranes under UV-visible light and nanostructured TiO_2 films help in the inactivation of microorganisms, physical separation of water contaminants, anti-biofouling action and decomposition of organic pollutants (Hyeok et al. 2009). The metallic nanoparticles could be immobilized into various membranes such as chitosan, polyvinylidene fluoride (PVDF), cellulose acetate, polysulfone and many more. The immobilized metallic nanoparticles serve a number of advantages such as lack of agglomeration, high reactivity, reduction of surface passivation and organic portioning (Jian et al. 2009). Nanocomposite films have been prepared from polyetherimide and palladium acetate and specific interactions between hydrogen and the Pd-based nanoparticles have been studied proving the efficiency in water treatment. The metal nanoparticles were generated within the matrix by annealing the precursor film under different conditions using both in situ and ex situ method. This provides opportunities to design materials having tunable properties (Clémenson et al. 2010). The N-doped "nutlike" ZnO nanostructured materials showed antibacterial activity, produced clean water with constant high flux and removed water contaminants efficiently by increasing the photodegradation activity (Hongwei et al. 2012).

3.5 Conclusion

Water is an essential requirement for mankind to survive on earth, but with the globalization, the water consumption as well as contamination has increased. Water has been treated using various techniques such as filtration, sedimentation etc., but these techniques cannot generate the water that could be reused by the humans. Therefore, advanced techniques need to be applied to generate water of good quality. Nanosorbents, nanocatalysts, MBRs, NCMs, MIPs and bioactive nanoparticles have been used to treat wastewater. Using these techniques dyes, heavy metals, lanthanides and organic contaminants can be removed from the water and make it fit for human consumption.

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Perspectives of Bioreactors in Wastewater Treatment

4

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Abstract

Wastewater treatment has become compulsory by government regulations in most parts of the world owing to the importance of maintaining the sanitation of freshwater and preserving the environment. Bioreactors are the core of any biotechnology-based process for enzymatic or microbial biotransformation, bioremediation, and biodegradation. This present chapter summarizes the perspective of the most concerning widespread reactors, such as rotating biological contactor, biological fluidized bed reactor, packed bed reactor, membrane bioreactor, continuous stirred tank bioreactor, upflow anaerobic sludge blanket reactor and photobioreactor, etc., that are most commonly used for treatment of different industrial wastewater. The performance studies of bioreactors carried out by different researchers have also been reviewed.

Keywords

Aerobic and anaerobic treatment • Bioreactors • Bioremediation • Municipal wastewater

4.1 Introduction

With the escalation in globalization and industrialization, the requirement of clean water in the developing countries has become grievous to attain because of a progressive trend on population, urbanization, and water usage per capita. Enormous quantity of wastewater is generated by residential, commercial, industrial, and institutional establishments. The availability of fresh clean water will become

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severely limited in many areas of the world in the coming years. Water scarcity and water quality are problems facing both developed and undeveloped countries. This encourages many governments on establishing stricter regulations on water resources and water pollution (Copeland and Taylor 2004). For example, excessive usage of water can be reduced by the water-saving campaign and restriction on the use of groundwater. Wastewater standards are also tightened up to protect water resources, such as river, lake, and sea from pollution. These water management regulations made industries do their best efforts on finding a suitable and/or advanced wastewater treatment technology as treated effluent is considered to be environmentally safe and can be used for landscaping purposes or for flushing toilets. Hence, wastewater treatment has become compulsory by government regulations in most parts of the world due to the importance of maintaining the sanitation of freshwater and preserving the environment.

Conventional water and wastewater treatment processes have since long been established in eliminating many physical, chemical, and microbial contaminants of concern to public health and the environment. However, the effectiveness of these processes has become limited over the last two decades because of the new challenges. Firstly, increased knowledge about the consequences from water pollution and, secondly, the public desire for better quality water have promoted the implementation of much stricter regulations by expanding the scope of regulated contaminants and lowering their maximum contaminant levels (Mallevialle et al. 1996). Wastewater is treated through physical, chemical, and biological processes in order to remove contaminants from it so as to generate treated effluent. Some emerging treatment technologies, including membranous filtration, advanced oxidation processes, and the electrochemical method, hold great promises to provide alternatives for better protection of public health and environment. The application of biotechnological processes involving microorganisms, with the objective of solving environmental pollution problems, is gradually growing. Bioremediation processes, which take advantage of microbial degradation of organic compounds, can be defined as the use of microorganisms (especially bacteria) to detoxify and remove environmental pollutants from soils, waters, and sediments.

The bioremediation process, presenting countless advantages in relation to other processes employed, is an evolving method for removal and transformation of many environmental pollutants including those produced by various industries, being one of the most efficient methods to treat polluted environments (Gargouri et al. 2011). Biological treatment using activated sludge in aerobic condition is one of the regularly used treatment methods for industrial effluent. Stabilization ponds, aerated lagoons, or percolating filters are widely applied in aerobic treatment. In aerobic treatment, dissolved oxygen is utilized by microorganism, and, finally, wastes are converted into more biomass and carbon dioxide. Organic matter is partially oxidized, and some of the energy produced is used for generating new living cells under the formation of flocs. Once the flocs are settled down, they are removed as sludge. Bioreactors are the core of any biotechnology-based production processes for vaccine, proteins, enzymatic or microbial biotransformation, bioremediation, and biodegradation (Chishthi and Young 1994). Examples describing

above processes, based on immobilized bacteria and fungi, are available at laboratory and industrial scale in fixed-bed reactors (Zhang et al. 1999), trickling filter reactors (where the biofilm is slightly humidified by water or another liquid) (Messner et al. 1990), and rotating biological contactors (where the biofilm develops on the surface of vertical disks that rotate within the liquid) (Kapdan and Kargi 2002). Hollow fiber or membrane biofilm reactors (microbial layer is attached to a porous gas permeable membrane) can provide an efficient gas supply to the base of the biofilm and are considered to be the promising technologies (Lema et al., 2001). Further, the integration of aerobic and anaerobic degradation pathways in a single bioreactor is capable of enhancing the overall degradation efficiency. The integrated bioreactors are classified into four types, which are the following:

- Integrated bioreactors with physical separation of anaerobic–aerobic zone
- Integrated bioreactors without physical separation of anaerobic–aerobic zone
- Anaerobic–aerobic sequencing batch reactors (SBR)
- Combined anaerobic–aerobic culture system

4.2 Various Bioreactors Used in Wastewater Treatment

Different types of bioreactors are utilized for the treatment of wastewater that are reliable, cost-efficient, and effective in eliminating a wide range of pollutants. The following sections summarize the potential of different bioreactors such as rotating biological contactor, biological fluidized bed reactor, packed bed reactor, membrane bioreactor, continuous stirred tank bioreactor, upflow anaerobic sludge blanket reactor and photobioreactor, etc., that are most commonly used for treatment of different industrial and domestic wastewater.

4.2.1 Rotating Biological Contactor (*RBC*)

Rotating biological contactor is an efficient sewage treatment plant developed on the basis of the original biological filter. It is constituted by a series of closed disks (diameter 1–3 m) made of lightweight materials, such as hard plastic plate, glass plate, etc., that are fixed on a horizontal axis (Fig. 4.1). Nearly half of the disk area is under effluent in the sewage of the oxidation tank, but the upper half is exposed to the air. The rotating horizontal axis is driven by the rotating device that makes the disk rotating slowly. Due to the disk's rotating, the sewage in the oxidation tank is completely mixed. There is a layer of biofilm on the disk surface; when the rotating disk immersed in the sewage inside of the oxidation tank, the organic matter in the sewage would be adsorbed by the biofilm on the disk. When the rotating disk rotates to the air, the water film which is brought up by the disk will drip down along the biofilm surface, and at the same time, the oxygen in the air will dissolve into the water film constantly. Under the catalysis of enzyme, through absorbing the

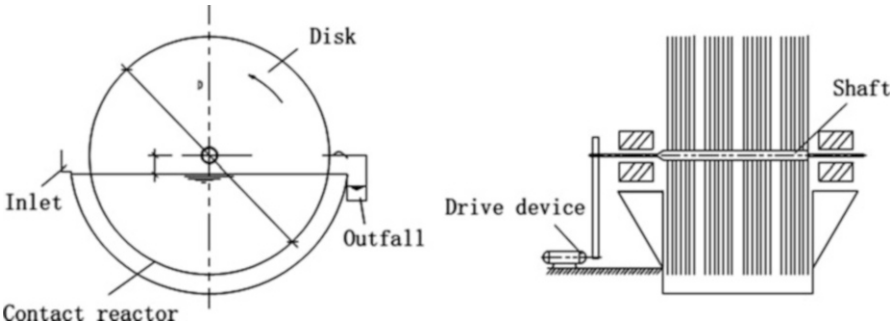

Fig. 4.1 Diagrammatic scheme of biological rotating contractor

dissolved oxygen in the water film, microorganism can oxidate and decompose the organic matter in the sewage and excrete the metabolite. During disk rotation, the biofilm on the disk gets in touch with the sewage and the air constantly alternating, completing the process of adsorption–oxidation–oxidative decomposition continuously to purify the sewage. The advantages of the biological rotating contactor are power saving, large shock load capability, no sludge return, little sludge generated, little noise, easy maintenance and management, and so on.

The main parameters of affecting the process performance are rotation speed, sewage residence time, reactive tank stage, disk submergence, and temperature. The efficiency of sewage treatment depends upon the consistency sewage, i.e., BOD <300 mg/L; the rotation speed is under 18 m/min. Alternately, at high-BOD consistency sewage, increasing the rotate speed is equivalent to increase the contact, organic loading, hydraulic retention time (HRT), dissolved oxygen, temperature, and submergence (Waskar et al. 2012). Tawfik et al. (2006) investigated the performance of RBC for treatment of domestic wastewater at a temperature of 12–24 °C. The overall nitrification efficiency was 49% at total organic loading rate (OLR) of 11 gm COD/m^3/d, and the overall removal efficiencies for chemical oxygen demand significantly decreased when decreasing the total HRT from 10 to 2.5 h and increasing the OLR from 11 to 47 gm COD/m^3/d. Moreover, to achieve an effluent quality of BOD (<25 mg/L) and COD (<60 mg/L), the system must have to be operated at organic loadings of about 22 gm BOD/m^3/d and 65 gm COD/m^3/d, respectively (Ghawi and Kriš 2009).

4.2.2 Fluidized Bed Reactor (*FBR*)

A tubular reactor is a vessel through which fluid flow is continuous, generally at steady state, and configured so that conversions of the chemicals and other dependent variables are functions of position within the reactor rather than of time. In the ideal tubular reactor, the fluids flow as if they were solid plugs or pistons, and reaction time is the same for all flowing material at any given tube cross section. Fluidized bed process is also called suspended carrier biofilm process, which is a

new efficient sewage treatment process. Figure 4.2 shows the diagrammatic scheme of fluidized bed reactor. The method which adopts the solid particles fluidization technology can keep the whole system at a fluidized state to enhance the contact of solid particles with fluid and achieve the purpose of sewage purification. The growth of microorganism in the fluidized bed reactor was followed by a count of viable cells in both liquid phase and the biofilms attached to the support. An increased number of viable cells were observed inside the reactor when it was used to degrade higher organic loads, with most of the cells on the support. The higher concentration of active biomass was responsible for achieving a relatively high absolute degradation of the wastewater containing the high organic load (Souza et al. 2004). The treatment efficiency is about 10–20 times higher than conventional activated sludge process. It can remove higher organic matter in short time, but the land acreage is only about 5% of the common activated sludge process. Gonzalez et al. (2001) investigated the 90% degradation of phenolic wastewater by the pure culture of immobilized cell of *Pseudomonas putida* ATCC 17484 in fluidized bed bioreactor at a loading rate of 0.5 gm phenol/L/d. Sokół and Woldeyes (2011) evaluated the performance of inverse fluidized bed biological reactor for treating high-strength refinery wastewaters and achieved 96% COD reduction (from 54,840 to 2190 mg/L), when the reactor was operated under optimized operating conditions, i.e., at the ratio (Vb/VR) = 0.55, air velocity u = 0.046 m/s, and time t = 65 h. Haribabu and Sivasubramanian (2016) achieved 97.5% COD reduction at an initial concentration of 2 g/L and for a superficial gas velocity of 0.00212 m/s at HRT of 40 h using fluidized bed reactor containing biocarrier made up of low-density (870 kg/m^3) polypropylene of surface area 524 mm^2 per particle. Further, anaerobic treatment of textile wastewater was possible with the supplementation of substrate additives as external carbon sources such as 0.6 gm/L of glucose (Haroun and Idris, 2008) and 2.0 gm/L of glucose (Sen and Demirer 2003), and a further increase in the external carbon source added to textile wastewater did not improve the color removal efficiency of the anaerobic FBR reactor. The study implied that 98% soluble COD, 95% BOD_5, and 65% color reduction were possible by an anaerobic FBR for HRT of around 24 h and OLR of 3 kg COD/m^3/d. Aerobic digestion of starch industry wastewater was carried out in an inverse fluidized bed bioreactor using low-density (870 kg/m^3) polypropylene particles (Rajasimman and Karthikeyan 2007). Constant biomass loading was achieved over the entire period of operation, and maximum COD removal of 95.6% occurs at an OLR of 1.35 kg COD/m^3/d and a minimum of 51.8% at an OLR of 26.73 kg COD/m^3/d.

4.2.3 Packed Bed Reactor (*PBR*)

These reactors are tubular and filled with microbial biomass/pellets, and the biochemical reaction takes place on the surface of the microbial biomass. A fixed-bed reactor usually consists of a cylindrical vessel packed with microbial pellets that are easy to design and operate. The metal support grid and screen is placed near the

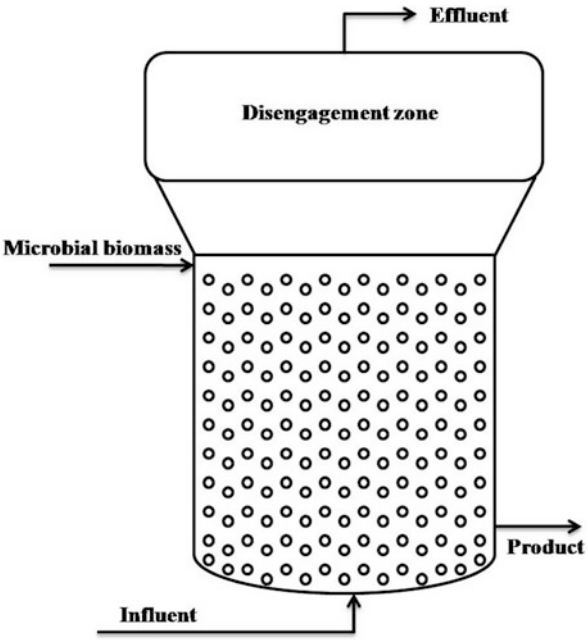

Fig. 4.2 Diagrammatic schematic of fluidized bed reactor

bottom to support the microbial pellets. Inert ceramic balls are placed above the microbial biomass bed to distribute the feed evenly (Fig. 4.3).

An anaerobic packed bed reactor was employed to treat highly polluted pharmaceutical wastewater having high COD (80,000 mg/L) (Chelliapan et al. 2011). Seventy-three percent COD reduction was attained at an average reactor OLR of 1.58 kg COD/m^3/d (HRT 5.6 d). Further, with the increase in OLR from 2.21 to 4.66 kg COD/m^3/d, the COD removal efficiency decreased gradually up to 60–70%. The average COD and SS removal efficiencies for domestic wastewater were 75.92% and 94.25%, respectively, in an upflow anaerobic packed bed reactor (Bhuyar 2013). On changing pH from 7.2 to 4.2, biogas was produced 0.50–0.59 L/d on same HRT. The performance of an anaerobic fixed bed reactor installed at a chemical industry producing organic peroxides was investigated for sulfate removal from sulfate-rich wastewater (sulfate concentrations ranging from 12,000 to 35,000 mg SO_4^{2-}/L) (Silva et al. 2002). A maximum sulfate removal efficiency of 97% was reached during discontinuous and semicontinuous operations. Further, Abdullah et al. (2016) achieved the removal of 98% COD and 93% TOC during the treatment of high-strength organic brewery wastewater with added acetaminophen (AAP) with an anaerobic packed bed reactor (APBR) operated with an organic loading rate of 1.5gm COD/L and 3 days HRT. The average CH_4 production decreased from 81 to 72% is counterbalanced by the increased CO_2 production from 11 to 20% before and after the injection of AAP, respectively. Similarly, the hydrogen sulfide (H_2S) removal by sulfur-oxidizing bacteria isolated from the sludge of the wastewater of a biogas plant, attached as a

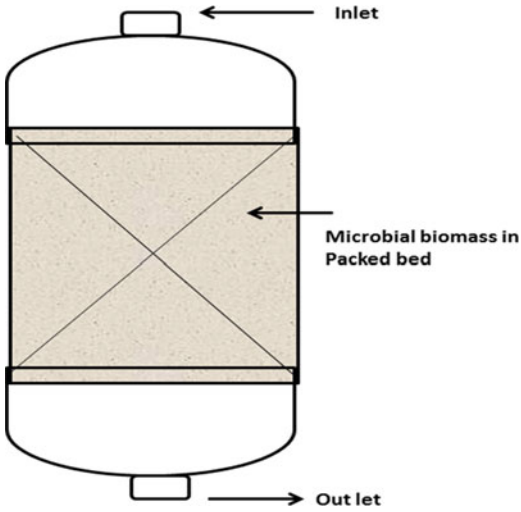

Fig. 4.3 Diagrammatic scheme of packed bed reactor

biofilm on salak fruit seeds, was studied with packed bed reactor by Lestari et al. (2016), and they observed the decrease in H_2S in biogas from 142.48 mg/L to 4.06 mg/L (97.15% removal efficiency) for a biogas flow rate of 8550 gm/m^3/h corresponding to a residence time of 4 h.

4.2.4 Membrane Bioreactor (*MBR*)

These are widely used nowadays for municipal and industrial wastewater treatment. These are suspended growth bioreactors which are integrated with a membrane process like microfiltration or ultrafiltration and are involved in treatment processes, which make use of a semipermeable membrane with a biological process. A membrane is simply a two-dimensional material used to separate components in the fluids on the basis of their relative size or electrical charge. The semipermeable membrane allows only specific components to pass through them without changing their properties. The filtrate part is known as permeate, while residual retained on membrane is called as concentrate or retentate. The typical diagrammatic sketch of membrane bioreactor has been in Fig. 4.4. Different types of membrane configuration that are currently in operation are hollow fiber, spiral wound, plate and frame, pleated filter cartridge, tubular type, etc. The advantages of MR technology are (a) secondary clarifiers and tertiary filtration processes are eliminated, thereby reducing plant footprint; (b) can be designed to prolong sludge age, hence lower sludge production; (c) high effluent quality; and (d) high loading rate capability.

The municipal wastewater was treated with submerged membrane bioreactor technology (Zhidong et al. 2009) and observed the average removal rate of COD and BOD over 90% in addition to the 99% removal rate of NH_3–N. Moreover, Sima et al. (2011) revealed the effect of virus removal from the wastewater samples using

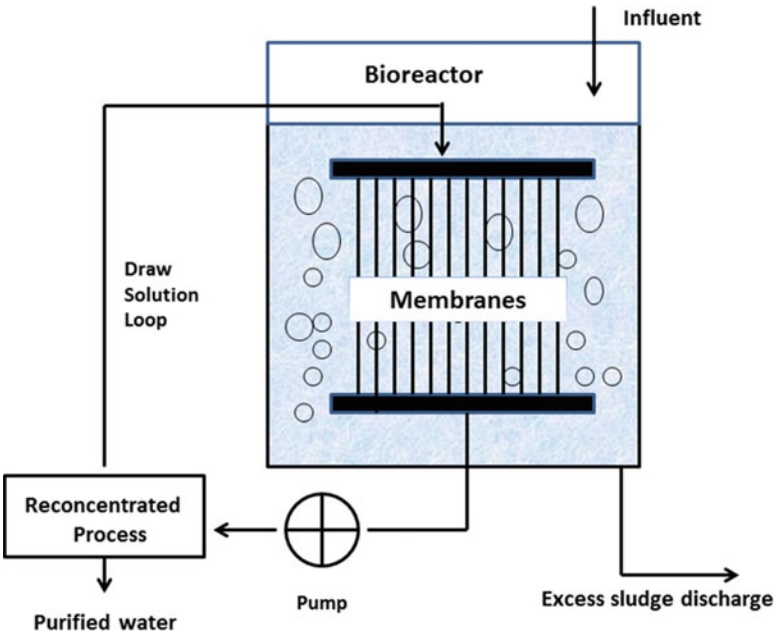

Fig. 4.4 Diagrammatic sketch of membrane bioreactor

membrane bioreactor wastewater treatment in southwest France and analyzed the calicivirus (*Norovirus* and *Sapovirus*), adenovirus, and *Escherichia coli*. The results demonstrated that the viruses were blocked by the membrane in the treatment plant and were removed from the plant as solid sludge. *E. coli* was found to be below the limit of detection in the effluent. Overall, the removal of calicivirus varied from 3.3 to greater than 6.8 log units, with no difference between the two main genogroups. Further, low-strength wastewater (Martinez-Sosa et al., 2011) and high-strength wastewater (Jager et al. 2013) were treated with an anaerobic submerged membrane bioreactor. The membrane area is determined by the hydraulic throughput and not the biological load; no sludge is wasted, and all bacteria are retained within the reactor, including specific bacteria capable of degrading the toxic, nonbiodegradable constituents present in textile wastewater. Besides biogas sparging, additional shear was created by circulating sludge to control membrane fouling. 75–97% reduction of COD was achieved over the 220-day test period, which was well within permissible limits of wastewater discharge standard. An average 91% conductivity rejection was achieved with conductivity being reduced from an average of 7700 to 693 μS/cm and the TDS reduced from an average of 5700 to 473 mg/L, which facilitated an average TDS rejection of 92% (Jager et al., 2013). Boonyungyuen and Wichitsathian (2014) studied the removal efficiencies of HMBR which were higher than MBR system, and the TKN removal of hybrid membrane bioreactor (HMBR) system is higher than MBR at 14.2% operated at HRT of 24 h under anaerobic digestion, which is due to the biofilm on activated

carbon surface that allows anoxic condition inside porous biofilm and enhances nitrite/nitrate removal efficiency. Membrane reactor is a promising technology for wastewater treatment and water reclamation, but even then it has certain disadvantages such as high operation and capital costs of membranes, membrane complexity and fouling, energy costs, etc.

4.2.5 Continuous Stirred Tank Bioreactor (*CSTR*)

The continuous stirred tank bioreactor (CSTR) is the idealized opposite of the well-stirred batch and tubular plug flow reactors. In the CSTR, the reactants and products are continuously added and withdrawn. In practice, mechanical or hydraulic agitation is required to achieve uniform composition and temperature, a choice strongly influenced by process considerations. Compared to other configurations, the CSTR provides greater uniformity of system parameters, such as temperature, mixing, chemical concentration, and substrate concentration. The CSTR is frequently used in research due to its simplicity in design and operation, but also for its advantages in experimentation. The primary design and operational target of the ASP for BOD removal is obtaining good solids settling properties in secondary clarifier. For instance, food to microorganisms (F/M) ratio is limited at 0.2–0.4 g BOD/gm MLSS/d in a typical ASP to obtain biosolids with a good sludge settling properties, although microorganisms can accommodate much higher F/M ratio. Usack et al. (2012) studied the use of continuously stirred anaerobic digester to convert organic wastes into biogas (Fig. 4.5).

The continuously stirred tank bioreactor was used to optimize feasible and reliable bioprocess system for the removal of dye (sulfur black) from textile effluent (Andleeb et al. 2010) as well as to treat hydrocarbon-rich industrial wastewater (Gargouri et al. 2011). In former case, the *Aspergillus terreus* SA3 isolated from the textile contaminated sites was used, and overall color, BOD, and COD in the continuous stirred tank bioreactor (CSTR) system were removed by 84.53, 66.50, and 75.24%, respectively, with 50 mg/L dye concentration and HRT of 24 h. The removal efficiency of the reactor decreased as the concentration of the dye was increased. This CSTR system was found very effective for efficient treatment of textile wastewater (up to 200 mg/L sulfur black dye) by the fungal strain *A. terreus* SA3, whereas in the latter case, an efficient acclimatized microbial consortium was used for decontaminating the hydrocarbon-rich wastewater. The performance of the bioaugmented reactor was demonstrated by the reduction of COD rates up to 95%. The residual total petroleum hydrocarbon (TPH) decreased from 320 to 8 mg TPH/L. It was further observed that, during the treatment process, the degradation of hydrocarbons was enhanced, implying that the aerobic treatment is an effective bioremediation technology. These encouraging results are mainly due to the development of an efficient microbial consortium and to the optimization of specific hydrodynamic conditions of the bioreactor.

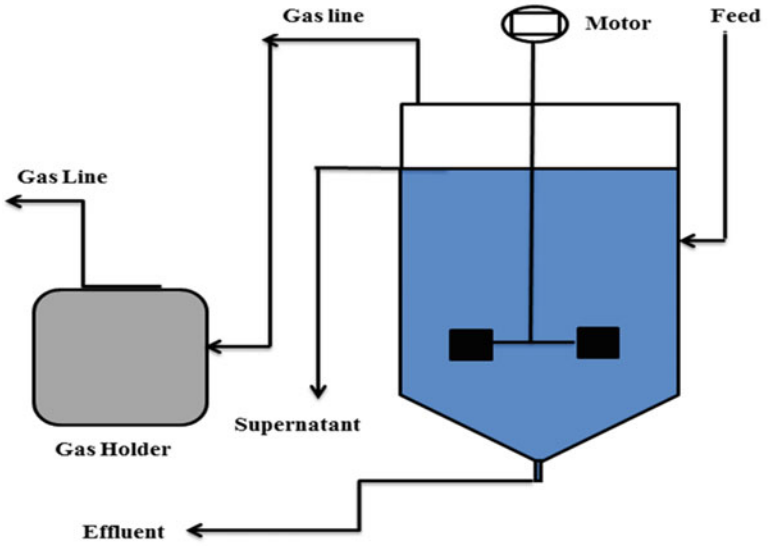

Fig. 4.5 Diagrammatic scheme of continuous stirred tank bioreactor (CSTR)

4.2.6 Upflow Anaerobic Sludge Blanket (*UASB*) Reactor

Upflow anaerobic sludge blanket technology, normally referred to as UASB reactor, is a form of anaerobic digester that is used for treatment of industrial wastewater. The UASB reactor is a methanogenic (methane-producing) digester that evolves from the anaerobic clarigester. The UASB reactor uses an anaerobic process while forming a blanket of granular sludge which suspends in the tank. The UASB is widely applicable for treating various types of wastewater and has advantages over aerobic treatment. Wastewater flows upward through the blanket and is processed (degraded) by the anaerobic microorganisms. The upward flow, combined with the settling action of gravity, suspends the blanket with the aid of flocculants (Fig. 4.6). Tiny sludge granules begin to form whose surface area is covered by aggregations of bacteria. In the absence of any support matrix, the flow conditions create a selective environment in which only those microorganisms capable of attaching to each other survive and proliferate. Eventually, the aggregates formed into dense compact biofilms are referred to as "granules." Bacteria living in the sludge break down organic matter by anaerobic digestion and transform into biogas. Solids are also retained by a filtration effect of the blanket. Baffles at the top of the reactor allow gases to escape and prevent an outflow of the sludge blanket.

Like aerobic treatments, the UASB requires a posttreatment to remove pathogens, but due to low removal of nutrients, the wastewater, as well as the stabilized sludge, can be used in agriculture. Three different types of upflow anaerobic reactor such as upflow anaerobic sludge blanket (UASB), upflow

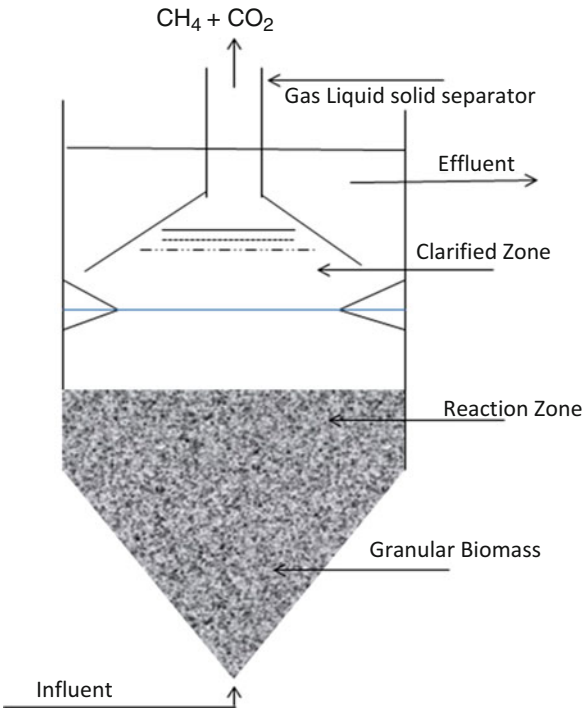

Fig. 4.6 Diagrammatic sketch of upflow anaerobic sludge blanket (UASB) reactor

anaerobic sludge-fixed film (UASFF), and upflow fixed film (UFF) reactors are used in the anaerobic process. In this anaerobic treatment, complex organic matter is converted into methane gas through the stages like hydrolysis, acidogenesis, and methanogenesis. Anaerobic hybrid reactor is a combination of upflow anaerobic sludge blanket (UASB) and upflow fixed film (UFF) reactors. The lower part of the UASFF reactor is the UASB portion where flocculants and granular sludges are developed. The upper part of the UASFF reactor serves as a fixed film bioreactor. The UASFF reactor has been used successfully for the treatment of various industrial wastewaters.

Researchers have used an upflow anaerobic sludge-fixed film (UASFF) reactor also termed as granular sludge bioreactor for the biological conversion of organic matter to biogas with the aids of aggregated microbial consortium in order to shorten the start-up period up to 4–5 days at 36 °C and HRT of 36 h. The organic loading rate was gradually increased from 7.9 to 45.42 gm COD/L/d. Further, a hybrid upflow anaerobic sludge blanket (HUASB) reactor was used for the treatment of domestic wastewater (Banu et al. 2007). The COD and BOD removal varied in the range of 75–86% and 70–91%, respectively. Methane content in the biogas was 62 ± 3%. VFA levels fluctuating between 100 and 186 mg/L and nutrient levels exhibited an increasing trend. The HUASB system could be designed with very short HRT of 3.3 h, which will reduce the treatment cost significantly. It appears to be a promising alternative for the treatment of domestic

wastewater in developing countries, like India. The anaerobic treatment of presettled cosmetic wastewater was studied in batch and continuous UASB reactor by Puyol et al. (2011). High COD and TSS removal efficiencies (up to 95% and 85%, respectively) were achieved over a wide range of organic load rate (from 1.8 to 9.2 gm total COD/L/day) in continuous treatment in an UASB reactor. Ferraz et al. (2011) evaluated the treatment of effluent from a jean factory using an upflow anaerobic sludge bed (UASB)-submerged aerated biofilter (SAB) system in different three phases, each with a different hydraulic retention time (HRT in hr) and organic loading rate (OLR in kg COD/m^3/d) up to 210-day operational period. In the first phase, best performance was achieved using the UASB (HRT 24 h, OLR 1.3) with COD and color removal efficiencies of 59 and 64%, respectively; the corresponding values were 77 and 86% for the final effluent. The use of a sequential anaerobic–aerobic system is promising for treatment of textile industrial wastewater.

4.3 Photobioreactor

A photobioreactor is a bioreactor that utilizes a light source to cultivate phototrophic microorganisms. These organisms use photosynthesis to generate biomass from light and carbon dioxide and include plants, mosses, macroalgae, microalgae, cyanobacteria, and purple bacteria. Algae have attracted much interest for production of foods, for bioactive compounds, and also for their usefulness in cleaning the environment (Fig. 4.7).

Kwangyong and Lee (2002) studied the microalgal nitrogen treatment with *Chlorella kessleri* in artificial wastewater with a low carbon/nitrogen (C/N) ratio with nitrate and glucose as a nitrogen and carbon source, respectively, along with abiotic control. The growth rates of the two cultures were almost identical when the aeration rate was over 1 rpm, and further, microalgae could successfully remove nitrogen from wastewater. Nitrate was successfully reduced to below 2 mg NO_3-N/ml from the initial nitrate concentration of 140 mg NO_3-N/ml in 10 days, even in the wastewater with no organic carbon source. However, the treatment of domestic wastewater with treatment (aerated and nonaerated) with *Chlorella vulgaris* under semi-controlled conditions in semi-closed photobioreactors in a greenhouse was performed (Marchello et al. 2015). Insignificant variations in pH and coliforms were observed between treatments. Nutrient concentrations were decreased supporting microalgae growth up to 107 cells/mL independent of aeration. Effluent is viable for the microalgae growth of *Chlorella vulgaris*, and at the same time the eutrophication potential decreased, contributing for better quality of the final wastewater. *Chlorella sorokiniana* isolated from White Sea, a suitable feedstock for biodiesel production was cultivated in semi-batch mode in a high-density photobioreactor for the bioremediation of alcohol distillery wastewater (Solovchenko et al., 2014). A decrease in COD from 20,000 to 1500 mg/L was achieved over 4 days with a decline in 95% nitrate, 77% phosphate, and 35% sulfate at pH 6.0–7.0. Another hollow fiber membrane photobioreactor (HFMPB)

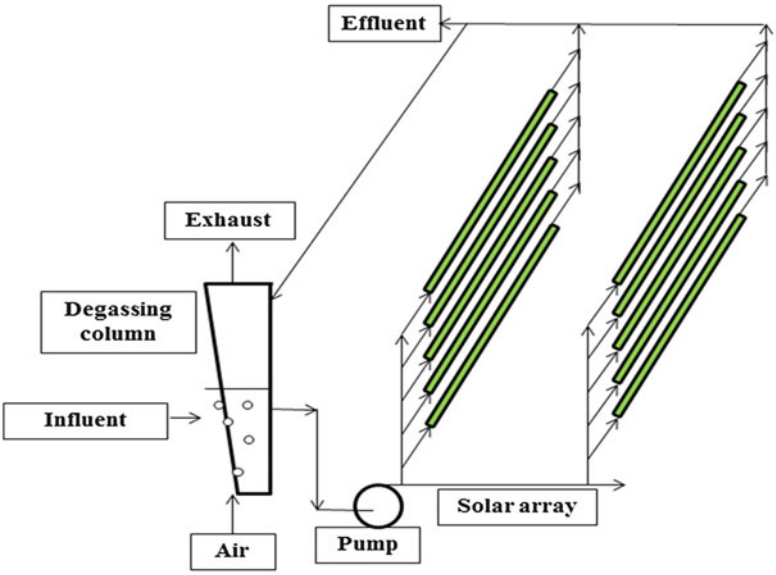

Fig. 4.7 Diagrammatic sketch of photobioreactor

containing *Spirulina platensis* used for biofuel production was operated with a 2–15% CO_2 supply (Kumar et al., 2010). The algal biomass concentrations and NO_3 removal efficiencies were 2131 mg/L and 68%, respectively. The combination of CO_2 sequestration, wastewater treatment, and biofuel production using *Spirulina platensis* in an HFMPB was found to be a promising alternative for greenhouse gas mitigation. Christenson and Sims (2011) reported the integration of microalgae-based biofuel and bioproducts production with wastewater treatment has major advantages for both industries. However, major challenges to the implementation of an integrated system include the large-scale production of algae and the harvesting of microalgae in a way that allows for downstream processing to produce biofuels and other valuable bioproducts. Although the majority of algal production systems use suspended cultures in either open ponds or closed reactors, the use of attached cultures may offer several advantages. With regard to harvesting methods, better understanding and control of autoflocculation and bioflocculation could improve performance and reduce chemical addition requirements for conventional mechanical methods that include centrifugation, tangential filtration, gravity sedimentation, and dissolved air flotation. There are many approaches currently used by companies and industries using clean water at laboratory, bench, and/or pilot scale; however, large-scale systems for controlled algae production and/or harvesting for wastewater treatment and subsequent processing for bioproducts are lacking. Further investigation and development of large-scale production and harvesting methods for biofuels and bioproducts are necessary, particularly with less studied one, but the promising approaches such as those involving attached algal biofilm cultures.

4.4 Conclusion

A number of significant trends are used in wastewater treatment, and they influence the near- and long-term alteration at wastewater treatment facilities. Undoubtedly bioreactor technology is advancing rapidly around the globe for municipal and/or industrial wastewater treatment both in research and commercial applications. Literary survey established that bioreactors are the core of biotechnological processes such as microbial transformation, bioremediation, and biodegradation which may hold the utmost promising alternative for efficient treatment of wastewater of a variety of strengths and compositions producing a pathogen-free treated water of excellent quality in addition to production of good quality fuel (biogas), a renewable energy. In spite of this, the adoption and commercialization of this technology at industrial scale is still in low pace.

The usage of bioreactors either independently or in combination as hybrids hold great promises in future, as they provide most effective and economical approach to deal with challenging environmental problems. Moreover, advance research is desirable for better understanding both synergistic and adverse effects, and further experiments are still needed to develop and evaluate the performance of hybrid bioreactors to clean the industrial effluent at low cost so that resultant could be recycled in the process to meet both current and anticipated treatment requirements. Nowadays, MBRs are widely used for aerobic wastewater treatment, as they are capable of producing a high-quality effluent with low-suspended solid concentration and small footprint relative to traditional aerobic treatment systems, but use higher energy to reduce membrane fouling. Researchers are focusing on new MBR design, the anaerobic fluidized membrane bioreactor, which combines a membrane system with an anaerobic fluidized bed reactor which will be more energy efficient and cost-effective.

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Bioremediation Technologies for the Removal of Pollutants

5

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Abstract

Bioremediation technology involves the use of living organisms like microbes and plants to reduce/degrade, eliminate and transform contaminants present in soils, sediments and water. The technology has gained wider acceptance in the recent years because of its potential to remove various organic and inorganic contaminants from various components of the environment. The technology provides an effective treatment of inorganic and organic contaminants under in situ and ex situ conditions by natural means. Potential of microbes and plants both have been exploited to achieve maximum removal/remediation of inorganic and organic contaminants. The biotechnological approaches and genetic engineering strategies have been employed by researchers to improve the efficacy of this technique for achieving complete degradation of contaminants. Enhancement in potential of both plants and microbes for achieving complete remediation of one or more than one pollutant can prove an asset for remediating contaminated sites. The present chapter highlights the role of microbial and phytoremediation in removal of pollutants from the environment.

Keywords

Bioremediation • Contaminants • Microbes • Plants

5.1 Introduction

Environmental contamination with inorganic and organic toxicants has increased over the years due to rapid industrialization, urbanization and anthropogenic activities. The organic contaminants such as petroleum hydrocarbons, pesticides,

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agrochemicals, pharmaceutical product and inorganic pollutants such as heavy metals are constantly added in the environment (Agarwal 1998; Zeyaullah et al. 2009). Most of the xenobiotic compounds resist degradation. The remediation or treatment of contaminants done by conventional methods (both physical and chemical) is a costly, time-consuming, invasive approach and causes environmental deterioration (EPA 1999, 2003). According to an estimate, the cleaning/restoring of the contaminated sites in the USA requires a capital investment of approximately US $1.7 trillion. Bioremediation has emerged as a safe, reliable, effective, low-cost and environmentally friendly alternative technology to achieve sustainable remediation of hazardous and recalcitrant pollutants. In this technique, treatment of contaminants can be done at site in a cost-effective, less disruptive, eco-friendly (no by-products, no requirement of complex setups and operations) manner.

The bioremediation technology uses biological processes and naturally occurring catabolic activity of microbes and plants to eliminate, attenuate, or transform inorganic and organic contaminants to less hazardous products such as carbon dioxide and water (Abruscia et al. 2007; Pandey and Fulekar 2012). Biological agents such as yeast, fungi, bacteria and plants remove contaminants by biotransformation and biodegradation mechanisms. The physiological and metabolic capabilities of organisms assist in degrading the pollutants converting them to nontoxic and environmentally safe products. In this technology, target compound is used as a carbon source. The complete mineralization of contaminants results in the formation of H_2O and CO_2 (Strong and Burgess 2008; Sharma and Fulekar 2009).

5.2 Bioremediation

Bioremediation processes have been broadly categorized into two groups.

5.2.1 Ex Situ Bioremediation

In this type of remediation, removal of the contaminant from soil and groundwater is done away from the site (Maheshwari et al. 2014). The treatment of contaminants has been done away from site. This includes bioreactors, biofilters, land farming, bioventing, biosparging, biostimulation and composting methods (Olaniran et al. 2006).

Ex situ bioremediation is of two types.

5.2.1.1 Solid Phase Treatment

It is a treatment process for land and soil contaminated with organic, industrial wastes, municipal wastes and sewage sludge. It includes:

- *Land Farming:* In this technique, contaminated soil is excavated and spread over a prepared bed and periodically tilled to achieve degradation of pollutants. Microorganisms facilitate aerobic degradation of contaminants.
- *Composting:* In this technique, contaminated soil is mixed with nonhazardous organic amendments such as manure or agricultural wastes. The presence of organic materials supports the growth of microbial population.
- *Biopiles:* Biopiles are a hybrid of land farming and composting. Engineered cells are constructed as aerated composted piles. Contaminated material is mixed with a bulking agent and aerobic, thermophilic bacteria are used in the treatment process.
- *Bioreactors:* In this technique, biodegradation is carried out by microbes in a container. It is used to treat organic contaminants from liquids or slurries.
- *Bioventing:* It involves supplying air and nutrients through wells to contaminated soil to stimulate the indigenous bacteria. The low airflow rates provide the amount of oxygen necessary for the biodegradation while minimizing volatilization and release of contaminants to the atmosphere. It is used to treat hydrocarbons.
- *Bioaugmentation:* It involves introduction of exogenic microorganisms (sourced from outside the soil environment) capable of detoxifying a particular contaminant. The addition of contaminant-degrading organisms accelerates the transformation rates (El Fantroussi and Agathos 2005; Thierry et al. 2008). Enhanced chlorpyrifos biodegradation has been reported via this process.
- *Biosparging:* This involves the injection of air under pressure to increase groundwater oxygen concentrations and enhance the rate of biological degradation of contaminants by naturally occurring bacteria.
- *Biostimulation:* This involves the addition of soil nutrients, trace minerals, electron acceptors, or electron donors to enhance the biotransformation of a wide range of soil contaminants (Li et al. 2010). Trichloroethene and perchloroethylene are reported to be completely converted to ethane by microorganisms in a short span of time with the addition of lactate as biostimulation (Shan et al. 2010). Electron shuttles such as humic substances (HS) stimulate anaerobic biotransformation of organic pollutants through enhancing the electron transfer speed.

5.2.1.2 Slurry Phase

In this type of bioremediation, contaminated soil is combined with water, other additives and microbes in a bioreactor. Nutrients and oxygen are added, and conditions are controlled to create the optimum environment for the microorganisms to degrade the contaminants. Slurry reactors are used for treatment of contaminated soil and water.

5.2.2 In Situ Bioremediation

In situ technique is applied to treat contaminated soil and groundwater. This involves addition of indigenous or naturally occurring microbial populations by feeding nutrients and oxygen to increase their metabolic activity. Oxygen, electron acceptors, and nutrients (nitrogen and phosphorus) promote microbial growth. The treatment is done on the site without any need to excavate or remove soils or water in order to accomplish remediation (Vidali 2001).

5.3 Microbial Remediation

5.3.1 Contaminants Removed by Microbes

Naturally occurring bacteria and fungi degrade/detoxify hazardous substances. Aerobic and anaerobic bacteria degrade various inorganic and organic contaminants (Kumar et al. 2011). Aerobic bacteria such as *Pseudomonas*, *Alcaligenes*, *Sphingomonas*, *Rhodococcus* and *Mycobacterium* degrade pesticides, hydrocarbons, alkanes and polyaromatic compounds and use the contaminant as the sole source of carbon and energy. Anaerobic bacteria degrade polychlorinated biphenyls (PCBs) and organic solvents such as trichloroethylene (TCE) and chloroform. Dioxygenases and monooxygenases are two of the primary enzymes employed by aerobic organisms during transformation and mineralization of xenobiotics, while anaerobic microbes use range of electron acceptors such as NO_3-, Fe, Mn, SO_4^{2-} and CO_2 depending on their availability and the prevailing redox conditions. Methane monooxygenase degrade various substrates such as chlorinated aliphatic trichloroethylene and 1,2-dichloroethane.

Microbes form an important part of consortium that assist in degrading contaminants. These include *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Beijerinckia*, *Flavobacterium*, *Methylosinus*, *Mycobacterium*, *Myxococcus*, *Nitrosomonas*, *Nocardia*, *Penicillium*, *Phanerochaete*, *Pseudomonas*, *Rhizoctonia*, *Serratia*, *Trametes* and *Xanthobacter* (Table 5.1). The complete mineralization involves synergism and cometabolism. Cometabolism of xenobiotics is required when the compound cannot serve as a source of carbon and energy. Hydrocarbons and persistent organic pollutants (POPs) such as dichlorodiphenyltrichloroethane (DDT), polychlorinated biphenyls (PCBs), dioxins etc. are degraded in the soil by bacteria present in the rhizosphere (Olson et al. 2003). Acidophilic bacteria like *Acidithiobacillus ferrooxidans* (Takeuchi et al. 2005) and sulfur-oxidizing bacteria remove high concentrations of As, Cd, Cu, Co, and Zn from contaminated soils. Pesticides have also been successfully removed by bacteria. *Providencia stuartii* strain depicts potential for degradation of chlorpyrifos (Surekha Rani et al. 2008). Isolates of *Bacillus*, *Staphylococcus*, and *Stenotrophomonas* from cultivated and uncultivated soil are able to degrade dichlorodiphenyltrichloroethane (DDT) (Kanade et al. 2012). Bacterial strains are able to degrade azo dyes under aerobic and anaerobic conditions (Dos Santos et al. 2007).

Table 5.1 Contaminants removed by bacterial species

Contaminants	Bacterial species
PCB	*Rhodococcus*, *Luteibacter*, *Williamsia*
Malathion	*Azospirillum lipoferum*
PAH	*Lysinibacillus*
Hydrocarbon	*Bacillus*, *Corynebacterium*, *Staphylococcus*, *Streptococcus*, *Shigella*, *Alcaligenes*, *Acinetobacter*, *Escherichia, Klebsiella*, *Enterobacter*
Aromatic hydrocarbon	*Mycobacterium*, *Corynebacterium*, *Aeromonas*, *Rhodococcus*, *Bacillus*
PCB	*Pseudomonas*, *Burkholderia*, *Ralstonia*, *Achromobacter*, *Sphingomonas*, *Rhodococcus*, *Janibacter*, *Bacillus*, *Paenibacillus*, *Microbacterium*
Pesticides (chlorpyrifos, DDT)	*Bacillus*, *Staphylococcus*, *Stenotrophomonas*
Dyes	*Proteus* sp., *Pseudomonas* sp., *Enterococcus* sp., *Shewanella decolorationis*
Metals (*Hg*)	*Alcaligenes faecalis*, *Bacillus pumilus*, *P. aeruginosa*, *Brevibacterium iodinum*

Table 5.2 Fungal species with the potential for removing various contaminants

Contaminants	Microbial species	References
Fungi		
Oil hydrocarbons	*Aspergillus*, *Cephalosporium*, *Penicillium*	Singh (2006)
Aliphatic hydrocarbons	*Cladosporium*, *Aspergillus*	Singh (2006)
Uranium (U), thorium (Th)	*Rhizopus arrhizus*	Treen-Sears et al. (1998)
Yeasts		
Alkane	*Candida lipolytica*, *C. tropicalis*, *Rhodotorula rubra*, *Aureobasidium (Trichosporon) pullulans*	De Cássia Miranda et al. (2007)
Diesel oil	*Rhodotorula aurantiaca*, *C. ernobii*	De Cássia Miranda et al. (2007)
Aniline azo dye	*C. methanosorbosa* BP-6	Mucha et al. (2010)

Mycoremediation is a form of bioremediation in which fungi especially white rot fungus such as *Phanerochaete chrysosporium* degrade diverse range of persistent or toxic environmental contaminants (Singh 2006) (Table 5.2). The fungal mycelium secretes extracellular enzymes and acids that break down lignin and cellulose (Eaton 1985). Microfungi transform aromatic organopollutants cometabolically including polycyclic aromatic hydrocarbons (PAHs) and biphenyls, dibenzofurans,

nitroaromatics, and various pesticides (Fritsche and Hofrichter 2008). Plant growth-promoting rhizobacteria (PGPR), endophytic bacteria and other rhizospheric bacteria have been shown to potentially degrade toxic organic compounds in contaminated soil (Sylvestre et al. 2009). *Pseudomonas* sp. specifically has shown potential for hydrocarbon-degrading capacity. Yeast species such as *Trichosporon cutaneum* also utilize aromatic compounds as growth substrates.

5.4 Mechanisms of Removal of Contaminants by Microbes

The inorganic contaminants removed by bacteria mainly include heavy metals and radionuclides. Heavy metals are removed via biosorption (metal sorption to cell surface by physicochemical mechanisms), bioleaching (heavy metal mobilization through the excretion of organic acids or methylation reactions), biomineralization (heavy metal immobilization through the formation of insoluble sulfides or polymeric complexes), intracellular accumulation and enzyme-catalyzed transformation (redox reactions) mechanisms (Lloyd and Lovley 2001). The resistance to heavy metal toxicity occurs by adsorption, uptake, methylation, oxidation, and reduction mechanism. Metals are also precipitated as insoluble sulfides via metabolic activity of sulfate-reducing bacteria. Heavy metal ions can be entrapped in the cellular structure and subsequently biosorbed onto the binding sites present in the cellular structure. They pass into the cell across the cell membrane through the cell metabolic cycle. Toxic radionuclides such as U and Th from nuclear waste streams are removed by similar mechanisms (PinakiSar et al. 2004).

Both anaerobic and aerobic bacteria are capable of metabolizing organic pollutants. The initial intracellular attack of organic pollutants is an oxidative process, and the enzymatic key reaction is catalyzed by oxygenases and peroxidases. Peripheral degradation pathways convert organic pollutants step by step into intermediates of the central intermediary metabolism. Cytochrome P450 alkane hydroxylases play an important role in the microbial degradation of oil, chlorinated hydrocarbons, fuel additives, and many other compounds. The degradation of hydrocarbons is carried out under aerobic condition and is mediated by specific enzyme system. Enzymes involved in degradation of xenobiotics mainly include oxygenases. Higher chlorinated PCBs are reduced by anaerobic microorganisms, while lower chlorinated biphenyls are oxidized by aerobic bacteria (Seeger et al. 2001). Aerobic catabolic pathway for PCB degradation involves steps catalyzed by enzymes, biphenyl dioxygenase (BphA), dihydrodiol dehydrogenase (BphB), 2,3-dihydroxybiphenyl dioxygenase (DHBD) (BphC) and hydrolase (BphD) (Taguchi et al. 2001).

Fungi are an important part of degrading microbiota because, like bacteria, they metabolize dissolved organic matter. Extracellular multienzyme complexes of fungi are efficient in breaking down the natural polymeric compounds. By means of their hyphal systems, they are also able to colonize and penetrate substrates rapidly and transport and redistribute nutrients within their mycelium (Fritsche and Hofrichter 2005). Hyphal penetration provides a mechanical adjunct to the

chemical breakdown affected by the secreted enzymes. The high surface-to-cell ratio characteristic of filaments maximizes both mechanical and enzymatic contact with the environment. Second, the extracellular nature of the degradative enzymes enables fungi to tolerate higher concentrations of toxic chemicals. Among the filamentous fungi, the ligninolytic ones have been specifically investigated because of their extracellular, specific oxidoreductive enzymes that have been already successfully exploited in the degradation of many aromatic pollutants. Studies with *Aspergillus niger* AB10 Cd and *Rhizopus arrhizus* M1 have indicated Pb binding occurs via the functional groups on the cell surface. The functional groups act as ligands for metal sequestration (Pal et al. 2010). The proteins in the cell walls of AMF appear to have similar ability to sorb potentially toxic elements by sequestering them. Filamentous fungi may degrade pesticides using two types of enzymatic systems: intracellular (cytochromes P450) and exocellular (lignin-degrading system mainly consisting in peroxidases and lactases) (Chaplain et al. 2011). Yeast species use n-alkanes and other aliphatic hydrocarbons as a sole source of carbon, and energy is mediated by the existence of multiple microsomal cytochrome P450 forms.

5.5 Phytoremediation

The capacity of plants for removing and degrading various inorganic and organic contaminants from different components of the environment is referred as phytoremediation (Salt et al. 1998; Meagher 2000; Pilon-Smits 2005). It is a cost-effective, nonintrusive, aesthetically pleasing technology that removes contaminants via processes such as degradation, sequestration, or transformation mechanisms (Raskin and Ensley 2000; Garbisu et al. 2002; McCutcheon and Schnoor 2003). The major advantage of using this technology is that treatment can be done under in situ. The plants have been successfully used in removing contaminants such as explosives (trinitrotoluene), herbicides, pesticides and metals from different areas such as military areas, agricultural fields, industrial areas, mine tailings, sewage, municipal wastewater, drainage water and landfill leachate. The plants species with an effective remediation potential include mustard, alpine pennycress, hemp, and pigweed. The major concern about phytoremediation technology is that it is a time-consuming process and depends on the plant's ability to grow and thrive in contaminated environment.

Potential of both terrestrial and aquatic plant species has been exploited for removing contaminants from the environment. Efficacy of phytoremediation varies according to varieties, cultivars, genotypes and type of pollutant (Dipu et al. 2011). The selection of the plant species is very crucial for the success of this technology. Plants with less maintenance and acclimatization in native climate conditions are favored. Each plant species depicts a variation in its ability to remove contaminants from the environment. The selection of plant species depends upon factors such as:

- Tolerance to the environment
- Uptake, translocation and accumulation ability of the plant
- High growth rates and biomass production
- Tolerance to environmental conditions such as drought, salinity, etc.
- Availability of the species (annual/perennial)

Among terrestrial plants, trees and grass species with the characteristics such as deep roots, high biomass production, and fast growth are commonly preferred for remediation (EPA 1998; Schnoor 2000). Trees stabilize a pollutant and minimize spread of contaminant. Strong and dense root system (around 3 meters deep) in grasses assists in higher uptake of contaminants. The tolerance to extreme climatic variations such as drought, flood, submergence, fire, and heat and wide range of soil acidity, alkalinity, salinity and sodicity establish plants as ideal candidates for phytoremediation. *Populus deltoides* (hybrid poplar), *Brassica juncea* (Indian mustard), *Helianthus annuus* (sunflower), *Thlaspi* sp. including *T. caerulescens* and *T. rotundifolium*, *Vetiveria zizanioides*, and *Paspalum conjugatum* are some of the plant species with high capacity for removal of contaminants.

Among aquatic plant species, free-floating, submerged, and emergent forms exhibit exorbitant capacity for removal of various contaminants including heavy metals, radioactive wastes, nutrients, explosives, organic xenobiotics, and herbicides/pesticides from municipal and industrial wastewater. Features such as easy cultivation, high biomass production, faster growth rate, surplus availability and high tolerance to survive adverse environmental conditions assist in removal of contaminants and make them an ideal and most suitable candidate for use in phytoremediation technology. Aquatic plant species with high contaminant removal ability include *Eichhornia crassipes* (water hyacinth), *Salvinia herzogii*, *Salvinia minima* (water ferns), *Pistia stratiotes* (water lettuce), *Nasturtium officinale* (watercress), *Spirodela intermedia*, *Lemna minor* (duckweeds), *Azolla pinnata* (water velvet), *Potamogeton pectinatus* (American pondweed), *Ceratophyllum demersum* (coontail or hornwort), *Myriophyllum spicatum* (parrot feather), *Typha latifolia* (cattail), *Phragmites* (common reed) and *Scirpus* spp. (bulrush) (Dhir et al. 2009; Dhir 2013). Aquatic plants form an important component of constructed wetlands that remove many inorganic contaminants including metals, nitrates, phosphates, cyanides, as well as organic contaminants such as explosives and herbicides (Horne 2000; Jacobson et al. 2003; Dhir 2013).

5.5.1 Types of Phytoremediation

Plants remove contaminants by different processes such as phytoextraction/phytoaccumulation, phytodegradation/phytotransformation, phytovolatilization, rhizofiltration/phytofiltration and phytostabilization (Cunningham et al. 1995; Raskin et al. 1997; Salt et al. 1995a, 1998). Inorganic contaminants are removed by phytoextraction and/or phytostabilization processes, while organic contaminants are most commonly treated by phytodegradation and phytostimulation mechanisms.

In phytoextraction/phytoaccumulation process, contaminants are taken up by plants via roots followed by translocation to aboveground plant tissues, which are subsequently harvested (Salt et al. 1995a, b). It is used for removal of contaminants such as metals which cannot be degraded (Cd, Pb, Zn, Ni, Cr, Co, metalloids such as As, Se) and radionuclides (such as ^{90}Sr, ^{137}Cs, ^{238}U). It is also referred as phytoaccumulation, phytoabsorption, phytosequestration, phytomining, or biomining.

In phytodegradation/phytotransformation process, the metabolization and degradation of contaminants takes place within the plant with the help of enzymes produced and released by them. Phytodegradation is most suited for moderately hydrophobic organic chemicals (octanol-water partition coefficients, log Kow = 0.5 ~ 3.0). Plant enzymes such as dehalogenase, peroxidase, nitroreductase, laccase and nitrilase assist in degradation of organic pollutants, such as 2,4,6-trinitrotoluene (TNT) and polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), herbicides, pesticides, BTEX (benzene, toluene, ethylbenzene, and xylene), chlorinated solvents such as trichloroethylene (TCE) and short-chain aliphatic chemicals, explosives such as 2,4,6-trinitrotoluene (TNT), and inorganic nutrients.

In phytovolatilization process, plants take up contaminants through roots followed by their release as volatile chemicals by shoot or leaf surfaces. Biomethylated forms of metals such as Se, As and Hg form volatile molecules (less toxic), which are lost to atmosphere. Selenium is converted to methyl selenate, and the volatile form is released in the atmosphere (Meagher 2000).

In rhizofiltration/phytofiltration process, plant roots absorb, precipitate, and remove contaminants from water in either a hydroponic or a constructed wetland. This process is applicable for removal of inorganic contaminants such as metals (Pb, Cd, Cu, Fe, Ni, Mn, Zn, Cr), nutrients and radionuclide (^{90}Sr, ^{137}Cs, ^{238}U, ^{236}U) present in groundwater, surface water and wastewater (Dushenkov et al. 1995, 1997a, b).

In phytostabilization process, plants immobilize or stabilize contaminants in the soil through accumulation by plant roots or precipitation in the soil by root exudates, thereby reducing the bioavailability of contaminants in the environment. The contaminants are sequestered from the soil and the process is efficient in removing inorganic and organic contaminants from the soils, sediments, and sludges. Contaminants also bind to humic (organic) matter through the process of humification. Phytostabilization of organic contaminants or metabolic by-products is also achieved by attaching to plant components such as lignin which is referred to as "phytolignification" (Cunningham et al. 1995).

5.5.2 Mechanism of Removal of Contaminants

5.5.2.1 Inorganic

Metals and radionuclides are captured by root cells and subsequently translocated to plant parts (symplastic). Metal uptake in plants also involves cation exchange by

cell walls (apoplastic) (Williams et al. 2000; Pollard et al. 2000), or transport via symplastic pathway involves the role of membrane transport proteins (Blaylock and Huang 2000; Pollard et al. 2000). Intracellular high-affinity binding sites facilitate metal uptake across the plasma membrane (Dhankher et al. 2002; Hall 2002; Yang et al. 2005a, b). The natural resistance-associated macrophage (Nramp) family of proteins, cation diffusion facilitator (CDF) family proteins and zinc-iron permease (ZIP) family proteins (Williams et al. 2000) assist in metal transportation across the membranes. Metal chelate complexes are transported across the plasma membrane via specialized carriers. Cadmium is actively transported across the tonoplast of roots via a Cd/[H^+] antiport. CPx-type heavy metal ATPase transport proteins use ATP to pump variety of charged substrates along Cu and/or Cd across cell membranes (Williams et al. 2000). ZIP proteins mainly transport potentially toxic metals (Zn) as well as nutrients (Fe). These include the iron transporter 1 (*ITR1*) gene of *Arabidopsis* which is an iron (Fe [II]) transporter. Subsequent to uptake and translocation, heavy metals are stored in vacuole. Final sequestration of metal ions in chelated form or phytochelatins takes place in vacuole (Kramer et al. 1996). Metals are sequestered by bonding with organic sulfur (R-SH) on the cysteine residues by formation of metallothioneins (MTs) and phytochelatins (PCs). Organic acids, viz., citrate and phytosiderophores such as mugenic and avenic acid chelate metal ions, increase the efficiency for uptake and translocation of metals.

Radionuclides are translocated to the aboveground plant parts through the vascular system via high-affinity K^+ transporters. Translocation is followed by compartmentalization and complexation with ligands present in the cell including proteins, cysteine and glutathione. Radionuclides passively bind to negatively charged groups on the cell surface followed by transport to the cell wall. In active process, metabolically dependent penetration of ions through the cell membrane, movement inside cytoplasm and the bioaccumulation of the metal ions onto the protoplasts take place.

5.5.2.2 Organic

Organic contaminants (xenobiotic compounds) are subjected to partial or complete degradation within plants (Sandermann 1994). Plants absorb xenobiotics by simple diffusion primarily through roots and leaves (Wang and Liu 2007). Uptake and metabolism of hydrophobic organic contaminants is rapid. They are bound strongly to the surface of the roots especially by hemicellulose in the cell wall and the lipid bilayer of plant membranes; hence, their translocation within the plant is slow. They are actively transported through plant membranes (Meagher 2002; Pilon-Smits 2005). Several enzymes including monooxygenases, dioxygenases, dehydrogenases, hydrolases, peroxidases, nitroreductases, nitrilases, dehalogenases, phosphatases and carboxylesterases play an important role in degradation of xenobiotics (Dietz and Schnoor 2001; Pilon-Smits 2005). The detoxification of xenobiotic is carried out in three stages, namely, transformation, conjugation and sequestration.

Xenobiotics generally undergo transformation via chemical modification (oxidation, reduction, hydrolysis), conjugation (with glutathione, sugars, amino acids

resulting in soluble, polar compounds), and sequestration or compartmentalization (conjugants are converted to other conjugates and deposited in plant vacuoles or bound to the cell wall and lignin) (Cherian and Oliveira 2005). Oxygenation increases water solubility and provides site for conjugation via glycosidic bond formation. The reaction is catalyzed by enzymes such as P450 monooxygenases, carboxylesterases, cytochrome P450 and peroxidases. Oxidation reactions are followed by reduction and/or hydrolysis reactions after which conjugation with glutathione (GSH), sugars, or organic acids takes place. Enzymes such as glutathione S-transferases, carboxylesterases, O-glucosyltransferases, O-malonyltransferases, N-glucosyltransferases and N-malonyltransferases are associated with xenobiotic metabolism.

The conjugation-sequestration involve coupling of glucose or malonyl group to the organic compound followed by the transport of the conjugate to the vacuole or the apoplast. Conjugated xenobiotics are then sequestered as part of insoluble cell wall polymers or in cellular compartments such as vacuoles and further metabolized to form CO_2 (Pilon-Smits 2005). Cell compartmentation is mediated by a wide array of glutathione S-transferases (GSTs). ATP-binding cassette (ABC) transporters play a key role in the transfer of conjugates from the cytosol to either the vacuole or the apoplast (Klein et al. 2006).

The metabolism of certain nonagricultural contaminants such as PAHs, TCE, 2,4,6-trinitrotoluene (TNT), glyceryl trinitrate (GTN), and other chlorinated compounds has been well documented in literature (Macek et al. 2000; Alkorta and Garbisu 2001). Poplar trees have shown the potential of oxidizing alkanes, alkenes and methane and their halogenated analogues via dehalogenase enzyme. Dehalogenase(s) ultimately mineralize TCE to CO_2 via an oxidative pathway.

5.6 Factors Affecting Bioremediation Process

The bioremediation processes is regulated by many factors. These mainly include metabolic capacity of the organism, availability of contaminants, and the environmental factors such as type of soil, temperature, pH and the presence of oxygen and nutrients. The compounds either serve as primary or secondary substrate to the organism (Boopathy 2000). Type of contaminants, their concentration and the physicochemical bioavailability of pollutants critically regulate the biodegradation potential. The growth and activity of microbes is affected by pH, temperature and moisture. The rate of enzymatic reactions within microorganisms is also regulated by temperature. After every 10 °C rise in temperature, the rate of biochemical reactions gets doubled due to increase in enzymatic activity. Bacteria found in soil are mesophiles and degrade petroleum hydrocarbons at an optimum temperature ranging from 25 °C to 45 °C. Soil pH is one of the important factors because it affects survival of microbial species and also affects availability of nutrients. Biodegradation of organic contaminants is optimal at a pH range of pH 6–8. Moisture influences the rate of contaminant metabolism because it influences the

kind and amount of soluble materials that are available as well as the osmotic pressure and pH of terrestrial and aquatic systems.

Aerobic or anaerobic conditions also decide the rate and extent of biodegradation process. Hydrocarbons are readily degraded under aerobic conditions, whereas chlorinate compounds are degraded only in anaerobic ones. Stimulation of microorganisms is achieved by the addition of growth substances, nutrients, terminal electron acceptor/donors, or some combination, thereby resulting in an increase in organic pollutant degradation and biotransformation. The process of bioremediation can be enhanced by supplementing microorganisms with nutrients, carbon sources, or electron donors. Establishment of such microbial consortia can be done in several ways, e.g., by promoting growth through addition of nutrients, by adding terminal electron acceptor, or by controlling moisture and temperature conditions (Agarwal 1998). Addition of supplements such as fertilizers, oxygen, etc. assists in bioremediation as they act as biostimulants. Sufficient amount of nutrient and oxygen must be available in a usable form and in proper proportions for unrestricted microbial growth to occur.

Among the biological factors, metabolic ability of microorganisms affects the microbial degradation of organic compounds. The capacity of the plants to remove contaminants varies according to varieties, cultivars or genotypes and type of pollutant (Dipu et al. 2011). The selection of the plant species is very crucial. Plants with less maintenance, acclimatization to varied climate conditions, and increased biomass production are favored. The tolerance to contaminant also regulates the extent of contaminant removal capacity of plants.

5.7 Success Stories in Bioremediation

In situ bioremediation of U-contaminated sites has been conducted successfully with *Desulfosporosinus* spp. and *Closteridium* spp. (Bruschi and Florence 2006). Consortium of SRB (sulfate-reducing bacteria) has been used successfully to remove Zn and sulfate. The metals were precipitated as sulfides. Eight months after project implementation, 80% reduction in Site COC comprised a complex mixture of halogenated organic compound (mixture of brominated and chlorinated organic compounds). A company named TMPD technologies, Lafayette, LA, treated acres of land with multiple contaminants ranging from PCBs to hydrocarbons using microbes. It also removed oil spill from Lake Charles Refinery in Lake Charles, LA, via bioremediation techniques involving biostimulation and bioaugmentation. The Microbiological Resource Centers (MIRCENs) at Cairo, Egypt, is examining the use of microbes in degrading persistent pesticides pollutants.

The companies such as Edenspace Systems Corporation of the USA have successfully used Indian mustard plant to treat the soil contaminated with radionuclide strontium ($Sr^{89/90}$) at Fort Greely in Alaska, USA and Cs^{137} from the contaminated pond waters (Singh et al. 2006). Indian mustard plant was used with sunflower (*Helianthus annuus*) to phytoremediate the Pb-contaminated soil

at industrial facility in Connecticut, USA (Singh et al. 2006). Plants remove contamination by bioaccumulation in aerial parts. The Phytotech, Florida, USA, used the Indian mustard plant to remediate Pb and Cd from contaminated soil at the Czechowice Oil Refinery, Katowice, in Poland (Singh et al. 2006). In Milwaukee, Wisconsin, USA, the Ecolotree Inc. used the hybrid poplar trees to phytoremediate soil and groundwater contamination with petroleum-related organics, PAHs, and chlorinated organic compounds. In Illinois, USA, the Ecolotree Inc. used the hybrid poplar to treat soil contaminated with chemical fertilizer and pesticides. Hybrid polar was successfully used by Phytokinetics Inc., USA, to treat groundwater contaminated with chlorinated volatile organics including dichlorobenzidines and soils contaminated by gasoline and diesel compounds at old gas filling station at Axelved, Denmark, and cyanide, PAHs, oil, and BTEX (benzene, toluene, ethylbenzene, and xylene) in Denmark.

5.8 Genetic Engineering Approach for Improving Bioremediation

The genetic engineering technology has proved useful in improving the bioremediation process (Rugh et al. 1998; Bizily et al. 1999; Joutey et al. 2014). Recombinant DNA techniques enhance the capacity of organisms for degradation and breakdown of toxicants such as hydrocarbons and pesticides. Recombinant DNA techniques help to create organism with an ability to metabolize xenobiotics by detection of genes responsible for enzymes involved in degradation. Transgenic plants show improved metal tolerance, accumulation and enhanced capacity for degradation of organic compounds (Meagher 2000; Kramer and Chardonnens 2001; Pilon-Smits 2005). The genes encoding for biodegradative enzymes are present in chromosomal and extrachromosomal DNA of microbes. Plasmid exchange results in the production of novel microbial strains with a large number of degradative capabilities.

Inorganic contaminant removal is achieved via plants engineered to improve pollutant uptake by overexpression or knockdown of specific membrane transporter proteins or enzymes, root-shoot translocation abilities, sequestration and volatilization. The expression of the introduced gene is regulated by promoters. The protein may be directed to different cellular compartments, such as the chloroplast, the vacuole, or the cell wall. Various transgenic plants were created with metal tolerance and accumulation properties, either by overexpression of membrane transporter proteins (Hirschi et al. 2000; Song et al. 2003) or by overproduction of chelator molecules (Zhu et al. 1999a, b; Dhankher et al. 2002). Transgenic plants have been raised by transfer of metal hyperaccumulator genes to high-biomass, fast-growing species (Chaney et al. 2000; LeDuc et al. 2004). Synthesis of metal chelators leading to enhanced metal uptake, translocation, and sequestration has been overexpressed in plants (Cherian and Oliveira 2005; Pilon-Smits 2005). The biosynthesis of MTs is regulated at the transcriptional level and is induced by several factors, such as hormones, cytotoxic agents, and metals, including Cd, Zn, Hg, Cu, Au, Ag, Co, Ni, and Bi (Yang et al. 2005a, b). Phytochelatins are a class of

posttranslationally synthesized (cysteine-rich metal-chelating) peptides that play a pivotal role in heavy metal tolerance in plants by chelating these substances and decreasing their free concentrations (Vatamaniuk et al. 1999). Metal-tolerant tobacco (*Nicotiana tabacum*) has been developed by expressing a yeast metallothionein gene for higher tolerance to Cd. *Brassica juncea* was genetically engineered with *E. coli gshl gene* for increased glutathione and phytochelatin production for high Cd tolerance and high concentrations of phytochelatins (Fulekar et al. 2009). Overexpression of a bacterial glutathione synthetase (GS) for higher GSH and PC concentrations and increased Cd tolerance/accumulation by *Brassica juncea* has also been noted. Overexpression of plant phytochelatin synthase (PS) in transgenic yeast increases tolerance and accumulation of Cd. Manipulation of GSH and PC concentrations increases potential for increasing the accumulation of toxic metals by plants. Abhilash et al. (2009) reported the introduction of genes for enzyme glutathione S-transferase (GST) (responsible for GSH synthesis), by introduction of a [gamma]-glutathione synthetase into *Populus trichocarpa* (Gullner et al. 2001). For heavy metals, Sriprang et al. (2003) introduced *Arabidopsis thaliana* gene for phytochelatin synthase (PCS; PCSAt) into *Mesorhizobium huakuii* subsp. rengei strain B3 and then established the symbiosis between *M. huakuii* subsp. rengei strain B3 and *Astragalus sinicus*. The gene was expressed to produce phytochelatins and accumulate Cd^{2+}, under the control of bacteroid-specific promoter, the nifH gene.

Some genes for increased heavy metal (Cd) resistance and uptake, like *AtNramps* (Thomine et al. 2000), *AtPcrs* (Song et al. 2004) and *CAD1* (Ha et al. 1999) from *Arabidopsis thaliana*; *gshI*, *gshII* (Zhu et al. 1999a), and PCS cDNA clone (Heiss et al. 2003) from *Brassica juncea*, tobacco (Goto et al. 1998) and rice (Goto et al. 1998); *ferritin* from soybean for increased Fe accumulation; and *merA* from bacteria to *A. thaliana* and tobacco for resistance to Hg with gene (Bizily et al. 1999; Eapen and D'Souza 2005), have been introduced into plants. Transgenics have also been raised for Se tolerance with a bacterial glutathione reductase in the cytoplasm and chloroplast for Indian mustard. Transgenic *A. thaliana* plants expressing SRSIp/ArsC and ACT 2p/γ-ECS with high tolerance to As than wild plants and transgenic plants expressing γ-ECS or ArsC alone have also been reported (Dhankher et al. 2002; Mello-Farias and Chaves 2008). Studies also report overexpression of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase for an enhanced metal accumulation (Eapen and D'Souza 2005).

The genes for phytovolatilization have also been introduced into plants. Introduction of bacterial mercury reductase (*MerA*) and organomercurial lyase (*MerB*) genes into plants such as *Arabidopsis thaliana* increases plants' tolerance to Hg. Toxic organic mercuric compounds are converted into volatile elemental Hg (Rugh et al. 1996; Bizily et al. 2000; Dhankher et al. 2002; Eapen and D'Souza 2005). Overexpression of two key enzymes, cystathionine gamma-synthase and selenocysteine methyltransferase, which promote the conversion of selenocysteine to volatile Se has also been reported (van Huysen et al. 2003; LeDuc et al. 2004). Transgenic plants engineered to have enhanced sulfate/selenate reduction showed fivefold higher Se accumulation in the field (Bañuelos et al. 2005). Transgenic

Arabidopsis plants which could transport oxyanion arsenate to aboveground, reduce to arsenite, and sequester it to thiol peptide complexes by transfer of *Escherichia coli C* and *γ-ECS* genes have been developed (Eapen and D'Souza 2005).

The degradation of organic pollutant can be improved by overexpressing enzymes that facilitate degradation in plant tissue or rhizosphere. The genes procured from other organisms such as bacteria or mammals are introduced in plants. The transformed organisms possess the enzymatic machinery required to achieve a complete mineralization of organic molecules. Specific proteins or peptides for binding and transporting xenobiotics and enzymes involved in biodegradation have been introduced or overexpressed in plants to achieve compete degradation. The genetically transformed plants for degrading herbicides, organomercurials, phenolic compounds, PCBs and nitroaromatics (Bizily et al. 1999; Karavangeli et al. 2005; Rylott et al. 2006; Mohammadi et al. 2007) include *Arabidopsis*, tobacco (*Nicotiana tabacum*), Indian mustard (*Brassica juncea*), hybrid poplar (*Populus* sp.), and yellow poplar (*Liriodendron* sp.). Transgenic wetland species include *Spartina* spp., reeds and *Typha* spp. (Czako et al. 2005). Abhilash et al. (2009) reported the introduction of genes and enzymes such as mammalian cytochrome p450s gene into rice plant.

The genes coding for cytochrome P450 and GST for the enhanced degradation and remediation of herbicides, explosives, PCBs etc. have been overexpressed in plants. Increased expression of extracellular enzymes laccases, peroxidases, and cytochrome P450 has been proposed as an approach for remediation of small organic compounds (Doty 2008). *Pseudomonas putida* MHF 7109 isolated from cow dung has shown ability for biodegradation of petroleum hydrocarbon compounds – benzene, toluene and o-xylene (BTX). The bacterium *Deinococcus radiodurans* (the most radioresistant organism known) has been modified to consume and digest toluene and Hg from highly radioactive nuclear waste. Transgenic poplar trees and tobacco plants overexpressing a mammalian cytochrome P450 2E1 (CYP2E1) and human cytochrome P450 2E1 were developed with the capacity for metabolizing trichloroethylene (TCE). Rabbit cytochrome P450 has been introduced in *Atropa belladonna* to facilitate faster metabolism of TCE. Transgenic plants removed organic compounds as high as 79% of TCE, 49% of vinyl chloride, and 40% of benzene in comparison to 10–30% controls. Bacterial genes dhlAB from *Xanthobacter* improved removal and degradation of 1,2-dichlorethane in plants. Higher expression of genes responsible for root development has been targeted for effective remediation of atrazine and alachlor. The expression of bacterial genes atrazine chlorohydrolase (AtzA) and 1-aminocyclopropane-l-carboxylate deaminase has shown promising role in remediation of atrazine and alachlor (Wang et al. 2008). Hydrophilic organics cannot pass the hydrophobic interior of membranes passively as there is no suitable transporter available in the plant. Hydrophobic organic contaminants stick to soil particles, thereby reducing their bioavailability, or become stuck inside root membranes preventing their movement into the cell's interior. Rhizoremediation utilizes the capacity of plant-associated microbes that have been proposed for remediation of PCBs (Doty 2008; Rylott and Bruce 2009). The degradation of PCBs takes place in two steps. In the

first step, PCB degradation takes place by expressing the genes of first multicomponent enzyme biphenyl 2,3-dioxygenase in degradation pathway. The released intermediate compounds undergo further transformation by rhizospheric bacteria. In the second step, expression of 2,3-dihydroxybiphenyl dioxygenase enzyme harbors bphC and avoids plants' inability to cleave toxic dihydroxybiphenyls. These transgenic plants are more resistant to PCBs than wild type indicating the potential utility of plants for effective rhizoremediation of PCBs.

Shiota et al. (1994) made transgenic tobacco plants by fusing rat P450 1A1 to yeast NADPH P450 oxidoreductase for metabolizing the herbicide chlortoluron. *Helianthus tuberosus* CYP76B1 and *Glycine max* CYP71A10 were the first transgenic plant with enzymes to actively metabolize organic contaminants (Siminszky et al. 1999). Human P450s have been shown to significantly enhance herbicide tolerance in transgenic potato (*Solanum tuberosum* L.) (Inui et al. 2001), rice (*Oryza sativa* L.) (Kawahigashi et al. 2007), *Arabidopsis* and tobacco (*Nicotiana tabacum* L.) (Didierjean et al. 2002).

Transgenic plants have been developed by introducing genes that are able to degrade explosive nitrate esters and NACs by introducing the bacterial enzyme pentaerythritol tetranitrate reductase (French et al. 1999). Van Aken (2008) reported the development of transgenic plants for remediation of 2,4,6-trinitrotoluene, hexahydro-l,3,5-trinitro-l,3,5-triazine and glyceroltrinitrate by introducing and expressing bacterial nitroreductases and cytochrome P450s. Plants expressing these genes show significantly increased tolerance, uptake and detoxification of the targeted explosives. The introduction of the pnrA gene encoding for nitroreductase from *Pseudomonas putida* into a fast-growing tree aspen has shown promising results for remediation of explosives in contaminated field conditions (Rylott and Bruce 2009; James and Strand 2009). Transgenic approaches increased the ability of tobacco to degrade explosives such as GTN and TNT by overexpressing a bacterial NADPH-dependent nitroreductase (French et al. 1999). The genes encoding a nitroreductase from a bacterium have been inserted in tobacco, and the transformed species showed faster removal of TNT and enhanced resistance to the toxic effects of TNT.

Genetically engineered microorganisms (GEMs) have enhanced degrading capabilities of a wide range of chemical contaminants. The principles involved in the development of GEM plants include (1) modification of enzyme specificity and affinity; (2) pathway construction and regulation; (3) bioprocess development, monitoring, and control; and (4) bioaffinity bioreporter sensor applications for chemical sensing, toxicity reduction and end point analysis. Genes responsible for degradation of environmental pollutants, for example, toluene, chlorobenzene acids and other halogenated pesticides and toxic wastes, have been identified. For every compound, one separate plasmid is required. It is not like that one plasmid can degrade all the toxic compounds of different groups. The plasmids are grouped into four categories: (1) OCT plasmid which degrades octane, hexane and decane, (2) XYL plasmid which degrades xylene and toluenes, (3) CAM plasmid that decomposes camphor, and (4) NAH plasmid which degrades naphthalene. The potential for creating, through genetic manipulation, microbial strains able to

degrade a variety of different types of hydrocarbons has been demonstrated. They successfully produced a multiplasmid-containing *Pseudomonas* strain capable of oxidizing aliphatic, aromatic, terpenic, and polyaromatic hydrocarbons. *Pseudomonas putida* that contained the XYL and NAH plasmid as well as a hybrid plasmid derived by recombinating parts of CAM and OCT developed by conjugation could degrade camphor, octane, salicylate, and naphthalene and could grow rapidly on crude oil because it was capable of metabolizing hydrocarbons more efficiently than any other single plasmid. This product of genetic engineering was called as superbug (oil eating bug). The plasmids of *P. putida* degrading various chemical compounds are TOL (for toluene and xylene), RA500 (for 3,5-xylene), pAC 25 (for 3-cne chlorobenxoate), and pKF439 (for salicylate toluene). Plasmid WWO of *P. putida* is one member of a set of plasmids now termed as TOL plasmid. *Alcaligenes eutrophus* AE104 (pEBZ141) was used for chromium removal from industrial wastewater, and the recombinant photosynthetic bacterium, *Rhodopseudomonas palustris*, was constructed to simultaneously express mercury transport system and metallothionein for Hg^{2+} removal from heavy metal wastewater. For polychlorinated biphenyl degradation, chromosomally located PCB catabolic genes of *R. eutropha* A5, *Achromobacter* sp. LBS1C1, and *A. denitrificans* JB1 were transferred into a heavy metal-resistant strain *R. eutropha* CH_34 through natural conjugation.

5.9 Conclusions

Bioremediation is a natural process utilizing bacteria and fungi or plants to degrade or detoxify substances hazardous to human health and/or the environment. The microorganisms indigenous to a contaminated area or site aid in removal of contaminants. Biotechnology utilizes the application of genetic engineering to improve the efficiency of microorganisms to reduce the toxic substances. Bioremediation must be tailored to the site-specific conditions. More research is needed to develop and engineer bioremediation technologies that are appropriate for sites with complex mixtures of contaminants and are not evenly dispersed in the environment. This technology can be applied both in situ and ex situ for removing broad range of environmental contaminants, viz., organic and inorganic. Environmental conditions regulate the growth and degradation ability of organism. Resistance to degradation is some of the major concerns for bioremediation technology. A comprehensive understanding of the transport and sequestration mechanisms in plant cells is essential for formulating effective strategies to develop genetically engineered plants with higher phytoremediation efficiency. Genetic engineering of endophytic and rhizospheric bacteria can be used in plant-associated degradation of toxic compounds in soil and is considered one of the most promising new technologies for remediation of contaminated environmental sites.

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Bioremediation Technologies for Decolorization of Effluent

6

Anamika Pokharia and Sarabjeet Singh Ahluwalia

Abstract

Wastewater from the textile industry contains significant amounts of synthetic dyes that require treatment to prevent groundwater contamination. These synthetic dyes are stable and are highly persistent in nature. The search for innovative, cost-effective, and environment-friendly technologies has become the real challenge in recent years. In view of the need for a technical and economically satisfying treatment technology, a flurry of emerging technologies has been proposed and examined at different stages of commercialization. Appliance of biotechnological techniques in recent period emerged as a very promising area for decolorization of textile wastewater, i.e., targeted at breaking down the dye molecule to basic elements (mineralizing them), and has much less environmental impact than conventional methods. A lot of research in this field revealed the existence of a variety of microbial communities capable of decolorizing a wide group of dyes. This chapter reviews the usage of various microorganisms such as bacteria, fungi, algae, and microbial consortium as free cells or in immobilized form for the decolorization of different types of textile dyes. The performance and results of latest research studies with pure and mixed cultures in various reactors have been also compiled pertaining to the bioremediation of dyes and colorants from wastewater with the possible alternative emerging technologies.

Keywords

Bioremediation • Decolorization • Microbial consortia • Textile dyes

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6.1 Introduction

Due to rapid industrialization and urbanization, manufacturing and usage of synthetic dyes have been increased in various sectors. Dyes are the substances that impart color to the substrate. They adhere on compatible surfaces by mechanical retention, physical adsorption, and formation of covalent bond/complexes with metals. Dyes are used in the textile industry, leather tanning industry, paper production, food industry, photography, wood staining, biological and chemical research, pharmaceutical and medicine, light-harvesting arrays, photo-electrochemical cells, hair colorings, and cosmetics. The color of dye is combined effects of chromophores, delocalized electron system with conjugated double bonds, and auxochrome–electron-withdrawing or electron-donating substituent that enhance the color of chromophore by changing the overall energy of electron system. In addition to enhance the chromophore in production of color, auxochromes are also responsible for the solubility of dye and increase its reactivity toward fibers (Dos Santos et al. 2005).

Textile industry is one of the greatest consumers of water uses about 100 L of raw water per kg of textile materials in dying process. So during dyeing and finishing operations, approx. 200,000 tons of these textile dyes are lost to effluent every year (Jin et al. 2007). The discharge of highly colored synthetic dye effluent can be very damaging to the receiving water bodies since these dyes in the water strongly absorb sunlight that decrease the light intensity absorbed by the plants and phytoplankton-reducing photosynthesis and the oxygenation of water reservoir. Moreover, the presence of unnatural color is aesthetically unpleasant and tends to be associated with contamination. In addition, dyes used in the textile industries are toxic to aquatic organism and can be resistant to natural biological degradation.

During the last few years, stringent regulations coupled with increased enforcement concerning colored wastewater discharges have been established in many countries. Government legislation is becoming more and more stringent, especially in the more developed/developing countries, regarding the removal of dyes from industrial effluent (Robinson et al. 2001). Enforcement of this law will continue to ensure that textile and other dye-utilizing industries treat their dye-containing effluent to the required standards. So, traditional wastewater treatment technologies have proven to be markedly ineffective for handling wastewater of synthetic textile dyes because of the chemical stability of these pollutants. A wide range of methods has been developed for removal of synthetic dyes from aqueous solution to decrease their impact on the environment.

They are divided in following major categories:

- *Physical method*: Adsorption, (activated carbon, peat, bagasse, wood chips, fly ash and coal, silica gel), irradiation, ion exchange, electrokinetic coagulation
- *Chemical method*: Oxidative process, Fenton's reagent, ozonation, sodium hypochlorite photochemical
- *Biological method*: Activated sludge process, enzymatic treatment, anaerobic process
- *Emerging technologies*: Advanced oxidation process, membrane filtration, photocatalysis, sonication, redox mediators and engineered wetland systems, etc.

Biological and chemical methods involve the destruction of the dye molecule, while physical methods usually transfer the pollutant to another phase. Many of the conventional methods used for treating dye wastewater have not been widely applied on large scale as a result of the high operational cost and sludge disposal problems associated with them. Due to the complex nature of dye effluent, there is hardly any single method to treat the dye wastewater efficiently. So, combinations of different process are preferred to achieve the economical and desired level water quality (Saratale et al. 2010; Lonc˘ar et al. 2013; Karthik et al. 2014). Different combinations of treatment methods have been proposed in order to effectively manage the textile wastewater. Thus, chemical coagulation-flocculation, chemical oxidation, activated carbon adsorption, and anaerobic biological treatment usually combined with a activated sludge secondary treatment step are among the most well-known techniques (Hao et al. 2000; Robinson et al. 2001; Forgacs et al. 2004; Joshi et al. 2004).

6.2 Biological Methods

Bioremediation, or the use of microbial techniques to deal with pollution, is a key research area in the environmental sciences. Microbes acclimatize themselves to the toxic wastes, and new resistant strains develop naturally, which then transform various toxic chemicals into less harmful forms.

The ability of biological treatment for decolorizing of industrial effluent is ambiguous, different, and divergent. A number of biotechnological approaches have been suggested by recent research as of potential interest toward combating this pollution source in an eco-efficient manner, including the use of bacteria or fungi often in combination with physicochemical processes (Beydilli et al. 1998; Willmott et al. 1998; Borchert and Libra 2001; McMullan et al. 2001; Robinson et al. 2001; Zissi and Lyberatos 2001). Bioremediation of textile effluent using pure bacterial and fungal decolorization are represented in Table 6.1. Bioremediation systems were commonly applied in the treatment of industrial effluent using many microorganisms such as bacteria, yeasts, algae, and fungi that have capability to accumulate and degrade different pollutants. There were three principle advantages of biological technologies for the removal of pollutants; first, biological processes can be carried out in situ at the contaminated site; second, bioprocess technologies are usually environmentally benign (no secondary pollution); and third, ex situ method is cost-effective.

Recent fundamental work has revealed the existence of wide variety of microorganisms capable of decolorizing wide range of dyes. The use of microorganisms for the removal of synthetic dyes from industrial effluent offers considerable advantages, and the process was relatively inexpensive, running costs were low, and the end products were completely mineralized with no toxicity. Biodegradation is defined as biologically mediated breakdown of chemical compounds. When biodegradation is complete, the process is called mineralization, i.e., the total breakdown of organic molecules into water, carbon dioxide, and/or any other inorganic end products (Banat and Faison 1999). Degradation by mixed culture enhances the degradation process since individual strains attack the dye

Table 6.1 Decolorization of textile dyes with pure microbial cultures

S. no.	Microorganisms and source	Dye	Experimental conditions	Time of contact	Decolorization (%)	Reference
Bacteria						
1.	*Acinetobacter calcoaceticus* NCIM 2890 [NCIM, NCL-Pune]	Direct brown MR	Temp. 37 °C, pH 6.5–7.5, static anoxic conditions	24 h	91.3	Ghodake et al. (2009)
2.	*Agrobacterium radiobacter* MTCC 8161 [IMTECH, Chandigarh]	Crystal violet	Temp. 30 °C, anoxic conditions	24 h	100	Parshetti et al. (2011)
3.	*Bacillus thuringiensis* [Bacillus Stock Center, Ohio State University, UK]	Methylene blue	Temp. 37 °C, pH 7	2 days	98	El-Sersy (2007)
4.	*Halomonas variabilis* MTCC 3712 *Halomonas glaciei* MTCC 4321 [IMTECH, Chandigarh]	Reactive red 2	pH 5–11, temp. 25–40 °C, anaerobic	2 days	–	Balamurugan et al. (2011)
5.	*Micrococcus glutamicus* NCIM 2168 [NCIM, NCL, Pune]	Green HE4BD, golden yellow HE4R, orange 3R	pH 8, temp. 37 °C, static, dye conc. 50 mg/L	24 h	100	Saratale et al. (2010)
6.	*Pseudomonas desmolyticum* NCIM 2112 [NCIM, NCL, Pune]	Direct blue 6	Temp. 30 °C, aerobic	24 h	92	Kalme et al. (2007)
Fungi						
7.	*Alternaria alternata* CMERI F6	Congo red dye	pH 5.0, 25 °C, aerobic (150 rpm)	48 h	100	Chakraborty et al. (2013)
8.	*Aspergillus ochraceus* NCIM 1146	Malachite green	pH 7.4, temp. 37 °C, aerobic (150 rpm)	24 h	98	Saratale et al. (2013)
		Cotton blue			92	
		Crystal violet			61	
		Congo red			57	
9.	*Galactomyces geotrichum* MTCC 1360	Scarlet RR (disperse dye)	–	–	100	Jadhav et al. (2008)

10.	*Ganoderma lucidum*	Remazol black 5 Remazol brilliant blue 5	–	–	95	Murugesan et al. (2007)
11.	*Ganoderma* sp.	Reactive blue 19	–	–	75.4	Mohammadian et al. (2010)
12.	*Phanerochaete chrysosporium* ATCC 24725	Direct blue 15	Temp. 39 °C, stationary	6 days	95	Pazarlioglu et al. (2005)
13.	*Trametes versicolor* ATCC 20869	Amaranth	pH 4.5, temp. 26 °C, aerobic	7 days	58	Ramsay et al. (2006)

molecule at different positions or uses decomposed products produced by one strain will be further decomposed by another strain (Mohana et al. 2008). However, it was stressed that the composition of mixed cultures may change during the decomposition process, which interferes with the control of technologies using mixed cultures. Moreover, the efficacy of decomposition considerably depends on the chemical character of the synthetic dye and biodegradation capacity of the microorganism consortium (Schliephake et al. 2000). Decolorization of dyes with pure culture was found to be impractical, as the isolated culture would be dye specific, and their application in large-scale wastewater treatment plants with a variety of contaminant dyes was not feasible (Murugesan and Kalaichelvan 2003). Efficient biodegradation of dyes can be accomplished when catabolic activity of individual strain was complement with each other in a mixed culture community. The other biological treatment method that includes bioaccumulation was defined as the accumulation of pollutants by actively growing cells by metabolism and temperature-independent and metabolism-dependent mechanism steps (Nigam et al. 2000; Robinson et al. 2001; Ola et al. 2010; Tan et al. 2013; Saratale et al. 2013). These processes have potential to mineralize dyes to harmless inorganic compounds like carbon dioxide, water, and the formation of relatively insignificant amount of sludge.

6.3 Microbial Decolorization

The application of microorganisms for the biodegradation of synthetic dyes is an attractive and simple method by operation. However, the biological mechanisms can be complex. The large number of species has been tested for decolorization and mineralization of various dyes. Besides the traditional wastewater cleaning technologies, other methods have been employed in the microbial decolorization of dyes. The effectiveness of microbial decolorization depends on the adaptability and the activity of selected microorganisms including bacteria, actinomycetes, fungi, yeasts, and algae capable of degrading azo dyes (Chen et al. 2003; Daneshvar et al. 2007; Kalyani et al. 2009).

6.3.1 Bacterial

Numerous bacterial strains isolated from the contaminated sites of textile dyes, having the ability to decolorize dyes, have been reported by various researchers (Table 6.2). Bacterial cultures capable of degrading azo dyes are *Bacillus subtilis* (Mabrouk and Yusef 2008), *Aeromonas hydrophila* (Ogugbue and Sawidis 2011), and *Bacillus cereus* (Ola et al. 2010). *Klebsiella pneumoniae* RS-13 and *Acetobacter liquefaciens* S-1 having the ability to decolorizing textile industrial effluent containing methyl red have been reported for bioremediation of azo dye (Wong and Yuen 1996). An efficient isolated species of *Pseudomonas* from soil degraded and decolorized dyes belonging to triphenylmethane and azo group. Malachite green, fast green, brilliant green, Congo red, and methylene blue were decolorized in the range of 30–70% under aerobic condition (pH 6–8 and temp.

Table 6.2 Decolorization of dyes with bacteria isolated from contaminated sites

S. no.	Microorganisms	Dye	Source of isolation	Experimental conditions	Time of contact	Decolorization (%)	Reference
Bacteria							
1.	*Acinetobacter calcoaceticus* YC210	Victoria blue R	Isolated from the soil near a wastewater sewage treatment plant in Southern Taiwan	pH 7.0, temp. 30 °C, static anoxic conditions	24 h	95	Chen et al. (2011)
2.	*Acinetobacter radioresistens*	Acid red 37	Isolated from soil samples of textile industry, Chennai	pH 6–7, temp. 37 °C	24 h	95	Ramya et al. (2010)
3.	*Acinetobacter junii* FA10	Reactive red 120	Isolated from Paharang drain wastewater, Pakistan	pH 7, temp. 30 °C, static	48 h	94	Anwar et al. (2014)
4.	*Aeromonas hydrophila*	Triarylmethane dyes	Textile wastewater treatment plant in Greece	pH 7–8, 35 °C, and culture agitation	24 h	72–96	Ogugbue and Sawidis (2011)
5.	*Alcaligenes faecalis*	Red orange 13	Balaji Industries, Vatva Ahmedabad, India	Anoxic conditions	24 h	90	Shah et al. (2012)
6.	*Alcaligenes* sp. AA09	Azo dye, reactive red BL	Textile printing wastewater treatment plant of Perundurai, Chennai (India)	pH 7.0 and temperature 25 °C with 50–200 mg/L dye	24 h	92–95	Pandey and Dubey (2012)
7.	*Bacillus* sp.	Mordant black 9	Isolated out of the wastewater drain of a textile finishing company, Portugal	Temp. 65 °C, pH 9.5, aerobic	24 h	96	Maier et al. (2004)
		Mordant black 96					
		Acid blue 225					
		Disperse red 86					
8.	*Bacillus cereus*	Cibacron black PSG	Isolated from effluents sites, Abeokuta textile mill, Nigeria	pH 7.0, temp. 35 ° C	5 days	68	Ola et al. (2010)
		Cibacron Red P4B				88	

(continued)

Table 6.2 (continued)

S. no.	Microorganisms	Dye	Source of isolation	Experimental conditions	Time of contact	Decolorization (%)	Reference
9.	*Bacillus endophyticus strain* VITABR 13	Acid red 128 (azo dyes)	Isolate contaminated site at Coimbatore, Tamil Nadu, India	37 °C, pH 8.0	24 h	90	Prasad and Bhaskara Rao (2011)
10.	*Bacillus* sp.	Methyl orange	Textile effluent containing serilene black BNFS (C.I. Disp. Blk. Mix) disperse dye	30 °C, aerobic (140 rpm)	48 h	98	Pourbabaee et al. (2006)
11.	*Bacillus* sp. 3	Acid orange 7	–	Temp. 37 °C	3 days	73	Abraham and Kurup (2014)
12.	*Bacillus subtilis*	Fast red dye	Dye contaminated samples of Dyestuffs and Chemicals Company (DCC) at Kafr El Dawwar, Egypt	pH (5–9) and temperatures (25–40 °C), static	12 h	80	Mabrouk and Yusef (2008)
13.	*Bacillus subtilis* N4A *Bacillus megaterium*	Drimarene blue Sulfur black Acid red	Textile mill of Kohinoor textile mill effluent Islamabad, Pakistan	37 °C	8 days	89	Ali et al. (2009)
14.	*Bacillus megaterium*	Turquoise blue dye (remazol blue BB)	Isolated from contaminated site of dye industries, Gujrat	pH 7.0, temp. 37 °C addition of carbon sources	48 h	95	Joshi et al. (2013)
15.	*Bacterial isolates*	Azo dyes, triarylmethane dyes	Pali, Rajasthan, India	30 °C, pH 7, aerobic (180 rpm)	24 h	80	Kaushik and Malik (2009)
16.	*Brevibacterium* sp.	Azo dyes reactive yellow 107, reactive black 5, reactive red 198, direct blue 71	Activated sludge obtained from the Vicunha Textile Company, Itatiba, Brazil	pH 7.0, temp. 30 °C static	96 h	99	Franciscon et al. (2012)

17.	*Brevibacillus laterosporus*	Disperse brown 118	–	pH 7.0, temp. 40 °C, static	48 h	77	Kurade et al. (2011)
18.	*Burkholderia cepacia*-TN5	Azo dye, acid orange 7, and direct blue 75	Sludge samples of ETP, China Eldwakhlia-Bassiun	–	–	80	Alalewi and Jiang (2012)
19.	*Citrobacter* sp.	Reactive red 180	Isolate, textile mill Xiamen, China	pH 7.0, temp. 32 °C, anaerobic	36 h	96	Wang et al. (2009)
20.	*Comamonas acidovorans* TN	Azo dyes, acid orange 7, and direct blue 75	Sludge samples of ETP, Eldwakhlia-Bassiun, China	–	–	80	Alalewi and Jiang (2012)
21.	*Comamonas* sp.	Direct red 5	Isolated from dye-contaminated site around Manpasand textile industry, Kolhapur, India	pH 7.0, temp. 40 °C, static	24 h	100	Jadhav et al. (2008)
22.	*Enterobacter GY-1*	Reactive black 5	Activated sludge from textile industry, China	pH 7, temp. 35 °C, dye conc. 100 mg/L	24 h	86	Chen et al. (2011)
23.	*Enterobacter agglomerans*	Azo dye methyl red	Isolated from Casablanca city, Morocco.	pH 5.0, 37 °C, aerobic	6 h	92	Moutaouakkil et al. (2003)
24.	*Klebsiella* sp. strain VN-31	Azo dyes, reactive yellow 107, reactive black 5, reactive red 198, direct blue 71	Activated sludge produced by the Vicunha textile company, Itatiba, Brazil	pH 7, temp. 30 °C, dye conc. 100 mg/L microaerophilic, and aerobic conditions	168 h	98	Franciscon et al. (2009)
25.	*Listeria denitrificans*	Blue FNR, orange W3R, red FNR, and navy WB	Isolated from textile effluent Chittagong, Bangladesh	–	–	70–80	Hussain et al. (2013)

(continued)

Table 6.2 (continued)

S. no.	Microorganisms	Dye	Source of isolation	Experimental conditions	Time of contact	Decolorization (%)	Reference
26.	*Lysinibacillus* sp. RGS	Remazol red	Isolated from soil samples collected from the textile effluent disposal site of Mahalaxmi textile processing plant, Ichalkaranji	pH 7.0, temp. 30 °C, under static condition	48 h	87	Saratale et al. (2013)
27.	*Marinobacter gudaonensis* AY-13	Azo dye acid yellow 25	Isolated from natural marine environment	pH 7.0, temp. 35 °C,	24 h	94	Shertate and Thorat (2013)
28.	*Micrococcus luteus,*	Orange W3R, red FNR	Isolated from textile effluent Chittagong, Bangladesh	–	–	85–90	Hussain et al. (2013)
29.	*Micrococcus* sp.	Reactive azo dyes such as reactive yellow 42, reactive blue 3, reactive red 58	Isolated from contaminated sites of textile industry, Oshodi, Lagos, Nigeria	pH 7.0, temp. 37 °C, anoxic conditions	24 h	95	Olukanni et al. (2009)
30.	*Nocardia atlantica*	Blue FNR and red FNR	Isolated from textile effluent Chittagong, Bangladesh	–	–	100	Hussain et al. (2013)
31.	*Pleurotus pulmonarius*	Bleu BF-R, Red BF-5G	Isolate in Brazil	–	–	–	Santos et al. (2007)
32.	*Polyporus rubidus*	Reactive blue	Isolated from suburbs of Mumbai	–	–	–	Dayaram and Dasgupta (2008)
		Reactive orange					
		Remazol black					
		Congo red					

33.	*Pandoraea pulmonicola* YC32	Malachite green	Isolated from contaminated sites around a textile plant in southern Taiwan	pH 7.0, Temp. 35 °C, aerobic		95	Chen et al. (2009)
34.	*Proteus mirabilis* LAG	Reactive blue 13	Isolated from a municipal dump site soil near Lagos, Nigeria	pH 7.0, temp. 35 °C, anoxic state	5 h	8	Olukanni et al. (2010)
35.	*Pseudomonas aeruginosa* CR-25	Remazol black 5	Isolated from activated sludge of the common effluent treatment plant, Jetpur, Rajkot, (Gujarat, India)	pH 7.0 temp. 37 °C, dye conc. 150 mg/L, static condition	24 h	86	Joe et al. (2011)
36.	*Pseudomonas* sp.	Methyl orange dye	Contaminated soil	Dye conc. (50–200 mg/L), pH 6–10, temp. 30–40 °C	4 days	90	Shah et al. (2013)
37.	*Pseudomonas* sp. RA20	Reactive black 5	Isolated from Paharang drain effluents in Pakistan	pH 8 and 25 °C static conditions	48 h	98	Hussain et al. (2013)
38.	*Pseudomonas putida* SKG-1 (MTCC 10510)	Orange II	Isolated from dairy sludge, (India)	pH 8.0, 30 °C, static	96 h	92	Garg et al. (2012)
39.	*Rhizobium radiobacter* MTCC 8161	Reactive red 141	Isolated, contaminated sites of textile industry, Ichalkaranji, India	–	–	–	Telke et al. (2008)
40.	*Shewanella* strain J18 143	–	Isolated from soil that had been contaminated with textile wastewater, China	pH 6.8, temp. 30 °C static	48 h	100	Li and Guthrie (2010)

(continued)

Table 6.2 (continued)

S. no.	Microorganisms	Dye	Source of isolation	Experimental conditions	Time of contact	Decolorization (%)	Reference
41.	*Staphylococcus epidermidis*	Black WNN	Isolated from the contaminated soil of Nahar Oswal Denim, Lalru (India)	pH 9.0, temp. 35 °C, dye conc. 100 mg/L static	72 h	97	Pokharia and Ahluwalia (2012)
42.	*Sphingomonas paucimobilis*, *Bacillus* sp., and *Staphylococcus epidermidis*	Azo and triphenylmethane dyes (Congo red, methyl red, methyl orange, malachite green, phenol red, fuchsin, methyl green, and crystal violet)	Isolated from textile wastewater plant in Ksar Hellal, Tunisia	pH 7.0, temp. 37 °C, aerobic	24 h	~100	Ayed et al. (2011)
43.	*Staphylococcus hominis* RMLRT03	Acid orange	Isolated from textile effluent contaminated soil of Tanda, Ambedkar Nagar, Uttar Pradesh (India)	pH 7.0 and 35 °C, static	60 h	85	Singh et al. (2014)
44.	*Tsukamurella* sp. J8025	Methyl orange	–	Temperature 30 °C	48 h	98	Wen-Tung and Ming-Der (2012)

30–40 °C) (Mali et al. 2000). Decolorization of Congo red and direct black 38 were carried out using *E. coli* and *Pseudomonas* sp. under anaerobic, aerobic, and microaerophilic conditions (Isik and Sponza 2003). Color of the Congo red and direct black 38 was removed up to 98 and 72%, respectively, by *E. coli* at the end of anaerobic incubation, while no color was observed under aerobic incubation, whereas under microaerophilic condition, the azo dyes such as Congo red and direct black 38 were decolorized by *E. coli* up to 39 and 75%, respectively, 5-day incubation with anaerobic *Pseudomonas* sp., and showed 100% color removal. Further, out of the six bacterial strains which were isolated from sludge samples and mud lakes having ability of degrading textile dyes, *Aeromonas hydrophila* exhibited the higher color removal efficiency with various dyes (Chen et al. 2003) under optimal conditions (pH 5.5–10, temp. 20–35.8 °C). More than 90% of decolorization of red RBN was examined within 8 days at a dye concentration of 3000 mg/L. Pearce et al. (2003) presented an excellent review on the color removal from textile wastewater using whole bacterial cells. Mixed cultures of bacteria from a wide variety of habitats have also been shown to decolorize the diazo-linked chromophore of dye molecule in 15 days (Nagarathnamma et al. 1999). Ramya et al. (2010) reported 100% and 92% effective decolorization of indigo carmine by *Paenibacillus larvae* under shaking condition. Moosvi et al. (2007) also reported decolorization of Reactive Violet 5R (100 mg/L) by a microbial consortium consisted *Paenibacillus polymyxa* and *Micrococcus* sp. within 36 h, whereas individual isolates could not show decolorization even on extended incubation. Similarly, Palamthodi et al. (2011) also revealed effective decolorization of green and blue dye by *Paenibacillus* azoreducers and further investigated 70% decolorization of textile wastewater by microbial flocs consisted *Bacillus* sp., *Paenibacillus* sp., *Achromobacter* sp., etc. and their ability to accumulate heavy metals present in the textile wastewater. Cetin and Donmez (2006) revealed that *E. coli* and *Pseudomonas luteola* had the ability to decolorize reactive black B, remazol Blue, and reactive red RB at pH 7.0 with constant decolorization rates up to pH 9.5. In contrast *Klebsiella pneumoniae* RS-13 completely degrades methyl red in pH range from 6.0 to 8.0 (Wong and Yuen 1996; Mali et al. 2000). A pH from 7 to 8.5 has been reported as the optimum pH for the decolorization of reactive red 5 (Moosvi et al. 2005) and reactive violet 5R (Moosvi et al. 2007).

6.3.2 Fungi

Fungi have proved to be a suitable organism for treatment of textile effluent and dye removal. Fungal mycelia have an additive advantage over single cell organisms by solubilizing the insoluble substrates by producing extracellular enzymes. Due to an increased cell-to-surface ratio, fungi have a greater physical and enzymatic contact with the environment. The extracellular nature of the fungal enzymes is also advantageous in tolerating high concentrations of the toxicants. Many genera of fungi have been reported for the various types of textile dye decolorization (Table 6.3). White rot fungi are most efficient in breaking down synthetic dyes as

Table 6.3 Decolorization of dyes with fungi isolated from contaminated sites

S. No	Fungi						
1.	*Aspergillus* sp.	Reactive blue	Isolated from soil sample of textile industry , Kumbakonam, Tamil Nadu, India	pH 3.0, 30 °C, aerobic	24 h	75	Ramya et al. (2007)
2.	*Aspergillus allhabadii*,	Reactive blue MR	–	Temp. 25 °C	10 days	95	Namdhari et al. (2012)
	Aspergillus niger					83	
	Aspergillus sulphureus					93	
3.	*Aspergillus fumigates* XC6	Reactive black, Reactive yellow, Reactive blue	Isolated from mildew rice straw, from the suburb of Wuxi, China	pH 3.0, temp. 30 °C, aerobic	24 h	90	Jin et al. (2007)
4.	*Coprinus plicatilis*	Reactive blue 19	Isolated from university fungus research Laboratory	Temp. 26 °C and pH 5.5–7.5	15 days	99	Akdogan et al. (2014)
5.	*Candida tropicalis* TL-F1	Acid brilliant scarlet GR	Isolated from the sea mud, harbor industrial zone in Dalian, China	pH 7.0, temp. 35 °C, aerobic 160 rpm	24 h	97	Tan et al. (2013)
6.	*Coriolus versicolor*	Synthetic dyes	Chiang Mai, Thailand	Temp. 37 °C, pH 5.5–7.5, aeration	4 days	94	Srikanlayanukul et al. (2008)
7.	*Crepidotus variabilis*	Remazol brilliant blue R	Isolated from mangrove forests of coastal, Tanzania	pH 4.5, temp. 30 °C, stationary	14 days	92%	Mtui (2007)
8.	*Irpex lacteus* KACC 43353	Reactive levafix blue E-RA granulate dye	Korean Agricultural Culture Collection	pH 6.0, 25 °C, aerobic	4 days	100	Kalpana et al. (2012)

9.	*Penicillium simplicissimum*	Reactive red 198	Isolated from contaminated sites, Brazil	pH 5.5, temp. 28 ± 2 °C, aerobic (140 rpm); dye Concr. 100 mg/L	7 days	100	Bergsten-Torralba et al. (2009)
		Reactive blue 214			7 days		
		Reactive blue 21			2 days		
10.	*Polyporus rubidus*	Reactive blue	Isolated from suburbs of Mumbai	pH 5.5, temp. 28 ± 2 °C	5 days	70	Dayaram and Dasgupta (2008)
		Reactive orange			2 days	80	
		Remazol black			3 days	90	
		Congo red			3 days	100	
11.	*Scytalidium thermophilum*	Phenol red	Isolated from a locally prepared compost in the north of Tunisia	pH 5.5, temp. 45 °C	5 h	26	Younes et al. (2012)
		Congo red				72	
		Malachite green				82	
		Bromocresol Green				98	
12.	*Schizophyllum commune IBL-06*	Solar brilliant red 80	–	pH 4.5 and temp. 30 °C	7 days	84.8	Asgher et al. (2013)
13.	*Trametes trogii*	Anthraquinonic dyes (remazol brilliant blue R , reactive blue 4, acid blue 129) azo dyes (acid red 1, reactive black 5)	–	Temp. 30 °C, aerobic	2 days	60–90	Zeng et al. (2012)
14.	*White rot fungi (Pleurotus florida) C*	Crystal violet	–	pH 5.5 temp. 37 °C	24 h	100	Krishnaveni (2011)
		Orange G					
		Malachite green					
15.	*Thelephora* sp.	Orange G	Isolated from stumps of a burnt tree in the Western Ghats region of Tamil Nadu, India	pH 6.5, temp. 30 °C, aerobic	9 days	33	Selvam et al. (2003a, b)
		Congo red			8 h	97	
		Amido black 10B			24 h	98	

these constitute a diverse ecophysiological group comprising mostly basidiomycetous fungi capable of extensive aerobic lignin depolymerization and mineralization. This property is based on the WRF's capacity to produce one or more extracellular lignin-modifying enzymes (LME), which is due to their lack of substrate specificity are also capable of degrading a wide range of xenobiotics.

Extracellular production of ligninolytic enzymes by mycelium growing on solid malt extract/glucose medium supplemented with different dyes (malachite green, azure B, poly R-478, anthraquinone blue, Congo red, and xylidine), dye decolorization, and the relationship between these two processes were studied with 26 white rot fungi from Argentina (Levin et al. 2004). Only ten strains decolorized all the dyes and produced laccase, lignin peroxidase, and manganese peroxidase on solid medium. Comparing the isolates with the well-known dye degrader *Phanerochaete chrysosporium*, a new fungus, *Coriolus versicolor*, is potentially a candidate for use in biodecolorization processes. Eighteen-day-old cultures of *P. chrysosporium* were able to decolorize within hour up to 28, 30, 43, 88, and 98% of xylidine (24 mg/L), poly R-478 (75 mg/L), remazol brilliant blue R (9 mg/L), malachite green (6 mg/L), and indigo carmine (23 mg/L), respectively. Moreover, five species of white rot fungi were further evaluated for their ability to decolorize amaranth, remazol black B, remazol orange, remazol brilliant blue, reactive blue, and tropaeolin O in agar plates, *Bjerkandera* sp. BOS55, *Phanerochaete chrysosporium*, and *Trametes versicolor*. In static aqueous culture, the three cultures form fungal mats, which did not decolorize any dye beyond some mycelial sorption. When agitated at 200 rpm, the biomass grew as mycelial pellets. *Bjerkandera* sp. BOS55 pellets decolorized only amaranth, remazol black B, and remazol orange dye. Batch cultures of *Bjerkandera* sp. BOS55 and *P. chrysosporium* had a limited ability to decolorize repeated dye additions; however, *T. versicolor* rapidly decolorized repeated additions of the different dyes and dye mixtures without any visual sorption of any dye to the pellets (Swamy and Ramsay 1999). Similarly, decolorization and biodegradation of orange II, tropaeolin O, Congo red, and azure B with white rot fungus, *Phanerochaete chrysosporium*, were demonstrated by Cripps et al. (1990). Nyanhongo et al. (2002) examined the four ligninolytic fungi, namely, *Trametes modesta*, *Trametes hirsuta*, *Trametes versicolor*, and *Sclerotium rolfsii*, having the ability to produce fungal laccases which were screened for their ability to decolorize dyes such as anthraquinone, azo, indigo, and triarylmethane. The decolorization rate of this laccase increased with the rise in temperature to 50–60 °C. The decolorization efficiency of *T. modesta* laccase was improved remarkably in the presence of mediators like 1-hydroxybenzotriazole and 2-methoxyphenothiazine.

The strain *Aspergillus fumigatus* XC6 isolated from mildewing rice straw was found to be capable of decolorizing dyes effluent over a pH range 3.0–8.0 with the dyes as sole carbon and nitrogen sources (Jin et al. 2007). The optimum pH was 3.0; however, supplemented with either appropriate nitrogen sources (0.2% NH_4Cl or $(NH_4)_2SO_4$) or carbon sources (1.0% sucrose or potato starch), the strain decolorized the effluent completely at the original pH of the dyes effluent. A new azo dye-decolorizing fungi strain identified as *Penicillium* sp. based on 26S rRNA

gene sequence analysis was isolated from activated sludge (Gou et al. 2009). *Penicillium* sp. could aerobically decolorize 70% of reactive brilliant red X-3B at optimum pH 4–5, up to salinity 6% by the way of bioadsorption, and nutrient-poor medium was more beneficial for adsorption. Furthermore, the decolorization of azo dyes by fungal-bacterial co-cultures demonstrated that *Penicillium* sp. and *Sphingomonas xenophaga* QYY co-cultures performed better than any single strain.

6.3.3 Algae

Algae have become significant organisms for biological purification of wastewater since they are able to accumulate plant nutrients, heavy metals, pesticides, organic, inorganic toxic substances, and radioactive matters in their cells. Biological wastewater treatment systems with microalgae have particularly gained importance in the last 50 years, and it is now widely accepted that algal wastewater treatment systems are as effective as conventional treatment systems. These specific features have made algal wastewater treatment systems significant low-cost alternatives to complex expensive treatment systems particularly for purification of municipal wastewater (Table 6.4). The microalgae biomass production from textile waste effluent is a possible solution for the environmental impact generated by the effluent discharge into water sources. Pure and mixed algal cultures removed 50–70% of color within 3 months of incubation, and color reduction pattern showed a rapid removal rate phase followed by declining removal rate phase. Color removal by algae was due to three intrinsically different mechanisms of assimilative utilization of chromophores for production of algal biomass, CO_2 and H_2O transformation of colored molecules to noncolored molecules, and adsorption of chromophore on algal biomass. A report of algae capable of degrading azo dyes, through an induced form of an azo reductase, showed good color removal (Jinqui and Houtian 1992). Several species of *Chlorella* and *Oscillatoria* were capable of degrading azo dyes to their aromatic amines and to further metabolize the aromatic amines to simpler organic compounds. Some were even capable of utilizing azo dyes as their sole carbon and nitrogen source. Use of such algae in stabilization ponds was proposed by Banat et al. (1996) as they play an important role in aromatic amine removal. The biodegradation of azo dyes by the algae (*Chlorella pyrenoidosa*, *C. vulgaris*, and *Oscillatoria tenuis*) has also been assessed (Liu and Liu 1992). In addition, the algae can play a direct role in degradation of azo dyes. *Chlorella vulgaris* which have biosorption capacity for several reactive dyes were reported by Aksu (2005). Dried *Spirogyra rhizopus* have the ability to decolorize acid red 274 dye by both biosorption and biocoagulation process, and the removal amounts decreased, while the removed concentration of AR 274 dye increased with increasing *S. rhizopus* concentration (Ozer et al. 2006). The potential of *Cosmarium* sp. belonging to green algae was investigated as a viable biomaterial for biological treatment of triphenylmethane dye and malachite green (Daneshvar et al. 2007). Immobilized thermophilic cyanobacterial strain *Phormidium* sp. has good decolorization activity under thermophilic condition (Ertugrul et al. 2008). Agitated batch sorption performed on

Table 6.4 Decolorization of dyes with algae isolated from contaminated sites

S. No	Algae						
1.	Algal biomass	Malachite green	–	pH 4–6, temp. 50 °C	45 min	85	Gajare and Menghani (2012)
2.	*Chlorella* sp.	Basic green 4	Isolated from natural lake, Iran	pH 7.0, temp. 25 °C		95	Khataee et al. (2009)
3.	*Cosmarium* sp.	Malachite green	Acquired from natural lake, Iran	pH 9.0, temp. 25 °C, static	24 h	92.4	Daneshvar et al. (2007)
4.	Green algae	Monazo and diazo dyes	–	Temp. 25 °C	2 days	68	Omar 2008
5.	Green algae	Indigo	–	pH 8, temp. 25 °C, and salinity at 15 g L^{-1}	5 days	89.3	Elisangela et al. (2009)
		Direct blue				79	
		Remazol brilliant orange				75.3	
		Crystal violet				72.5	
6.	*Lyngbya* sp. BDU 9001 with coir	Pith textile dye	–	pH 7 and the temp. 29 °C	15 days	73	Henciya et al. (2013)
7.	*Shewanella aquimarina*	Azo dyes including acid red 27, methyl orange, acid orange 7, reactive red 120, direct blue 71	–	Temp. 30 °C, aerobic, 200 rpm	–	–	Meng et al. (2012)
8.	*Shewanella putrefaciens*	Chrysophenine red 3BN	–	pH 4.39–8, temp. 30 °C	24 h	63.15	Hema and Suresha (2014)
						89.4	
9.	*Vaucheria* sp.	Malachite green	Acquired from Azna lake in North of Iran	pH 8.5, temp. 25 °C, static	7 h	100	Khataee et al. (2011)

algae *Spirogyra* 102 revealed the ability of test biosorbent to remove azo dye from the aqueous phase at acidic pH 2 at optimized temperature 30 °C and dye concentration 5 mg/L (Verkata Mohan et al. 2008). *C. vulgaris* culture in the textile waste effluent demonstrated the possibility of using this microalga for the color and COD removal and for biomass production. The cultivation of *C. vulgaris* presented maximum cellular concentrations C_{max} and maximum specific growth rate μ_{max} in the wastewater concentration of 5.0% and 17.5%, respectively (El-Kassas and Mohamed 2014).

6.4 Enzymatic Treatment

Enzymes are able to break apart large sludge particles, creating more surface area for microbes to attack. This allows for a more complete and more efficient degradation of the sludge particles. Such particles are held together by extracellular polymeric substances that come from cell autolysis, bacterial metabolic reactions, and wastewater itself. Researcher has recognized the potential for enzymatic treatment systems. Hence, enzymes are the ultimate molecules which deal with the dye compounds and bring about cleavage and successive degradation. The initial step in degrading the azo dye is to cleave the electrophilic azo linkage, which immediately causes decolorization. Azoreductase brings about the cleavage of azo linkages in compounds containing azo bond to produce aromatic amines. A large number of enzymes from different plants and microorganisms have been reported to play an important role in array of waste treatment applications. The enzymatic decolorization of industrial dyes was a big challenge due to large diversity of chemical structures (Wesenberg et al. 2003; Akhtar et al. 2005). Enzymes can act on specific recalcitrant pollutants to remove them by precipitation or transformation to other products (Akhtar and Husain 2006). Enzymatic approach has attracted much interest in the removal of phenolic pollutants from aqueous solutions (Duran and Esposito 2000). Oxidoreductive enzymes, polyphenol oxidases, and peroxidases are participating in the degradation/removal of aromatic pollutants from various contaminated sites (Husain and Jan 2000; Bhunia et al. 2001). Polyphenol oxidases can act on a broad range of substrates such as substituted polyphenols, aromatic amines, benzene thiols, and a series of other easily oxidizable compounds. Thus, they can catalyze the decolorization and decontamination of organic pollutants. White rot fungi were able to degrade dyes using lignin peroxidase (LiP) and manganese-dependent peroxidase (MnP) (Murugesan et al. 2007). Other enzymes used for this purpose include H_2O_2-producing enzymes, such as glucose-2-oxidase along with laccase and phenol oxidase enzyme (Husain 2010).

Decolorization of eight synthetic dyes including azo, anthraquinone metal complex, and indigo was examined in white rot fungi by peroxidase-catalyzed oxidation (Young and Yu 1997). The dyes were not decolorized by manganese-dependent peroxidase (MnP), and while above 80% color was removed by ligninase-catalyzed oxidation, further dye decolorization rate increased linearly with ligninase dosage

(Lip). Some azo and heterocyclic dyes were almost completely degraded by *P. chrysosporium* in ligninolytic solution but decolorized to different extent (0–80%) by crude ligninase (Cripps et al. 1990). Peralta-Zamora et al. (1999) reported that enzymatic process promotes quick decolorization of the dye; nevertheless, maximum decolorization degree of about 30% is insignificant in relation to the decolorization degree achieved by the other processes. The enzymatic activity of four white rot fungi, viz., *P. tremellosa*, *P. ostreatus*, *B. adusta*, and *C. versicolor*, has the ability to produce ligninolytic enzymes, which decolorize dyes in artificial effluent (Robinson et al. 2001). Recently enzyme membrane reactors are emerging for wastewater treatment more specifically for dye decolorization (Lopez et al. 2002). In view of the potential of the enzymes in treating the phenolic compounds, several microbial and plant oxidoreductases have been employed for the treatment of dyes, but none of them has been exploited at large scale due to low enzymatic activity in biological materials and high cost of enzyme purification. In order to improve polyphenol oxidases activity and stability, enzyme immobilization technology has been applied. This technology is an effective means to make enzymes reusable and to improve its stability, which is considered as a promising method for the effective decolorization of dye effluents.

Further, immobilization of microbial biomass for dye removal in growth-restricted conditions is advantageous when the effluent has toxicity and does not promote cellular growth. Also, inactivated biomass does not require a continuous supply of nutrients and can be regenerated and reused in many cycles (Prigione et al. 2008). Immobilization can be of two types: entrapment and attachment. Entrapment means entrapment of microorganisms in the interstices of fibrous or porous material, whereas attachment means adherence of microorganisms on surfaces due to chemical bonding or self-adhesion (Couto and Toca Herrera 2007). Prigione et al. (2008) immobilized the conidial suspension of *Cunninghamella elegans* in calcium alginate beads. This immobilized biomass (300 g beads corresponding to 50 g biomass on wet weight basis) was then inactivated through autoclaving and packed in a glass column for treatment of synthetic dye-containing effluent. After 30 min, 70% decolorization and nearly complete decolorization were obtained after 6 h.

6.5 Reactors Studied in Dye Decolorization

The widely used systems were stirred tank reactor (Linko 1988), airlift and bubble column, fixed-bed bioreactor, rotating disk reactor (Kirk et al. 1986), packed-bed reactors (Feijoo et al. 1995), and silicone membrane reactor. Researcher also investigated continuous decolorization of an azo dye, orange II, in a packed-bed reactor (Zhang et al. 2009) and pulsed flow bioreactor packed (Mielgo et al. 2001), achieving high 97% and 90% decolorization efficiency, respectively, and up to 80% decolorization of a disperse dye (red-553) in a continuous (10–20 days) fixed-film bioreactor (Yang and Yu 2003).

Textile wastewater was treated by means of a fluidized-bed loop reactor and immobilized anaerobic bacteria at low hydraulic residence time of 6 h for a period of 3 months and achieved complete decolorization of the wastewater in addition to the production of methane-rich biogas. Furthermore, the effluent proved to be highly biodegradable by aerobic microbes (activated sludge), whereas Shah et al. (2012) reported the overall color, COD, and BOD removal in the stirred tank bioreactor system were 49.67%, 37.45%, and 33.89%, respectively, with 50 mg/L dye concentration, pH 6.6 ± 1, and HRT of 24 h in a reactor with 2 L capacity. The color removal efficiency in activated sludge process was 75 ± 10%, and around 50–70% of removed color was adsorbed on biomass or precipitated within the reactor. The color rejection of nano-filtration after biological treatment was almost complete and permeates color was always lower than 10 Pt–Co (Ayed et al. 2011). The performance of a bench-scale submerged microfiltration bioreactor using the white rot fungus *Coriolus versicolor* NBRC 9791 for treatment of textile dye wastewater was investigated with an average flux of 0.05 m/d (HRT = 15 h) for a month at controlled temperature and pH of 29 ± 1 °C and 4.5 ± 2.0, respectively (Hai et al. 2006)

Upflow Anaerobic Sludge Blanket (UASB) reactor, considered as high rate reactor, are generally more resistant to toxic compounds as a result of structure of formed granular sludge with good settling velocities and mechanical strength, and suitable for the treatment of wastewater containing xenobiotic and recalcitrant compounds, and it promotes adaptation of bacteria to the presence of toxic compounds, and as well as it can be used for treatment of wastewater previously considered unsuitable for anaerobic treatment (Jantsch et al. 2002; Harada et al. 1996; VanLier et al. 2001; Donlon et al. 1997). Synthetic textile wastewater containing three acid dyes was treated in UASB reactor system and achieved decolorization up to 89 ± 1.86% at 300 mg/L dye concentration.

Immobilized *Phanerochaete chrysosporium* decolorized 94% of the maxilon red dye in the trickle-bed reactor over a period of 4–5 days (Afzal et al. 2009), using the basal nitrogen-limited growth medium. Moreover, it continuously decolorized three different mixed azo dye effluents by greater than 90% in rotating tube bioreactor system over the 38-day operating period (Alleman et al. 1995; Kirby 1999) to investigate the remediation of actual textile effluent by *P. chrysosporium*. However, *Phanerochaete sordida* decolorized 80% of the phthalocyanine dye basic blue 22 in a rotating disk reactor operating with a retention time of 48 h (Yang et al. 2004) and 90.3% in 72 h for an initial reactive black 5 concentration of 100 mg/L on nylon sponge and sunflower seed shells (SS) in laboratory-scale bioreactors (Enayatizamir et al. 2011). Similarly, Blánquez et al. (2004) reported the biodegradation of Grey Lanaset G, a mixture of metal complex dyes, was studied in a reactor with the fungus *Trametes versicolor*. The ability to achieve 80% decolorization with *Irpex lacteus* to decolorize the remazol brilliant blue R and reactive orange 16 was reported by Pocedic et al. (2009).

Moreover, a fixed-bed bioreactor packed with *Trametes pubescens* was able to decolorize, for four successive cycles, 200 ml of a solution of the dye reactive black 5 at a concentration of 60 mg/L (Enayatzamir et al. 2009), and Casieri et al. (2008)

have shown the efficiency of degradation of *Bacillus adusta* and *Pseudomonas ostreatus* against successive cycles of solutions containing 200, 1000, and 2000 mg/L of one model and two industrial dyes. Sponza and Isik (2005) reported the 96% color removal efficiencies of direct red 28 azo dye in a sequential upflow anaerobic sludge blanket reactor systems. The decolorization efficiency for malachite green was found to be 85.2% at pH in the range of 7–10, with increasing initial MG concentration up to 100 mg/L with immobilized *P. pulmonicola* YC32 continuous column system (Chen et al. 2009).

6.6 Microbial Consortia for Treatment of Textile Wastewater

In nature there is a diverse range of microorganisms and energy sources that makes it possible to break down a large number of different organic chemicals. Basically, microorganisms cannot mineralize most hazardous substances individually. So, the target pollutant being a complex molecule/mixture of compounds can only be broken down by a very specific combination of microorganisms (a "consortium") and pathways. Therefore, the use of microbial consortia offers considerable advantages over the use of pure cultures in the degradation and decolorization of synthetic dyes. The individual strains may attack the dye molecule at different positions or may use the decomposition products produced by another strain for further decomposition. However, the composition of mixed cultures may change during the decomposition process interfering with the control of the system. The most commonly used consortium in activated sludge system is mainly constituted by bacteria in addition to the presence of fungi and protozoa.

Various researchers have examined the microbial consortium JW-2 (Moosvi et al. 2005) consisting of *Paenibacillus polymyxa*, *Micrococcus luteus*, and *Micrococcus* sp. completely decolorizes reactive violet 5R (100 mg L^{-1}) within 36 h, and aerobic bacterial consortium SKB-II [Tony et al. 2009] comprised of *Bacillus* sp. decolorizes the azo dyes such as Congo red, Bordeaux, Ranocid fast blue, and blue BCC of 10 mg/L concentration each. Joshi et al. (2008) have investigated bacterial consortium that decolorizes acid orange 7 and consists of *A. caviae P. mirabilis* and *R. globerulus*. Further, a microbial consortium (comprising of two spp. of *Bacillus* and six spp. of fungi, viz., *Aspergillus flavus*, *A. niger*, *A. fumigates*, *Cladosporium cladosporioides*, *Trichoderma harzianum*, *Fusarium oxysporum*) isolated from the textile wastewater polluted habitats of Sanganer showed the 95% decolorization of 100 mg/L methyl red (Kumar et al. 2006). Similarly, significantly a higher reduction in color (90.14%) and COD removal (77.47%) from textile wastewater in less time (96 h) were achieved with the consortium comprises of *Sphingomonas paucimobilis*, *Bacillus* sp., and *Staphylococcus epidermidis* (Ayed et al. 2011). The variance in the microbial communities in these consortia might involve different mechanisms for dye decolorization.

Waghmode et al. (2012) reported the enhanced decolorization and degradation of azo dye rubine GFL (50 mg/l within 30 h) using defined consortium GG-BL of *Galactomyces geotrichum* MTCC 1360 yeast and *Brevibacillus laterosporus*

MTCC 2298 bacterium, whereas individual cultures fail to completely decolorize the dye. The rate of decolorization of consortium AP was significantly higher than that of individual cultures. The increased decolorization rate might be due to the synergistic enzymes actions of both the organisms in the consortium. As researcher reported, the degradation of intermediates metabolites by bacteria could decline the fungal inhibition and thus enhances the decolorization efficiency of consortium (Gou et al. 2009). It is also known that the degradation products of one culture in the consortium may act as inducer for another co-culture, which results in the further mineralization of dye and metabolites (Chang et al. 2001; Forgacs et al. 2004). Similar findings were reported by Kurade et al. (2011), who observed higher decolorization rate of azo dye navy blue HE2R in solid state fermentation by developed consortium PA of *Aspergillus ochraceus* NCIM-1146 and *Pseudomonas* sp. SUK1.

6.7 Conclusions

This chapter concluded the effective decolorization of a wide variety of commercial textile dyes from simulated and real textile wastewater by the utilization of various types of microorganisms (bacteria, fungi, algae, and yeast) isolated from textile wastewater, sludge, and soil contaminated with textile effluent. Such microbes acclimatized themselves against the highly toxic dyes and make use of them for their growth with or without supplementing additional nutrients. This was also observed that a combination of different microorganisms (viz., bacterial-fungal, bacterial-algal and bacterial yeast, etc.) were more potent decolorizer than single pure cultures. Decolorization performance in reactor studies also confirmed the complete decolorization and degradation of toxic synthetic dyes with a small hydraulic residence time (HRT) and enhancing the efficiency of continuous reactor system. No doubt, bioremediation is considered to be one of the green approaches to clean the planet. Although advances in bioremediation techniques seem to be highly attractive, these technologies need scale-up trials in order to increase its market potential. Broader validation of these techniques and integration of different methods in the current treatment schemes will most likely, in the near future, render both efficient and economical viability.

In most cases, single technology fails to work in field due to various environmental factors associated and the toxicity of targeted compounds. Hence there is a need to develop hybrid technologies which can fit to ever-changing environmental conditions and toxicity of the compound. Researchers and scientists have been trying to develop a single and economical method for the treatment of dyes in the textile wastewater, but economical removal of color from effluent remains a big challenge. Thus, there is a necessity to develop better integrated technique to decolorize and completely mineralize the textile industrial effluent in spite of various successful systems of physicochemical techniques.

Most of the microbes are unculturable in laboratory condition, but their role in the particular niche cannot be neglected particularly in the process like

bioremediation. In order to trace the microbial population and their role in the environment, molecular techniques prove to be a boon in this field. The genes involved in the bioremediation of the targeted compound and their respective enzymes can be traced, and hence in turn the microbial floras involved in the bioremediation process are traceable. It also helps to record the metabolic pathway followed by the organism to degrade the particular compounds.

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Bioremediation of Tannery Wastewater

7

Prachi Chaudhary, Vinod Chhokar, Anil Kumar, and Vikas Beniwal

Abstract

Tannery effluent is a serious environmental threat due to its high chemical levels which include salinity, organic load (chemical oxygen load or demand, biological oxygen demand), inorganic matter, dissolved and suspended solids, ammonia, total Kjeldahl nitrogen, sulfide, chromium, chloride, sodium and other salt residues, heavy metals, etc. These components present in the effluent affect agriculture, human beings and livestock. Exposure to chromium and other pollutants in tannery effluent increases the risk of dermatitis, ulcer, nasal septum perforation and lung cancer. The environmental protection regulations stipulate that industries are not allowed to emit sulfide and chromium in the wastewater. Thus, removal of these high-strength toxic chemicals from the wastewater is very important. Treatment of tannery wastewater is carried out by physical, chemical, biological, or combination of these methods. Biological treatment of wastewater is more favorable and cost effective as compared to other physiochemical methods. A number of bioremediation strategies have been reported in the recent past showing their potential in the treatment of tannery effluent. The present review summarizes the recent advances in bioremediation of tannery effluent.

Keywords

Tannery • Chromium • Effluent • Wastewater

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7.1 Introduction

Water is the important natural resource for all living forms. This natural resource is being polluted by rapid growth of population, metropolitanization and mechanization that ultimately pollute the environment (Singanan et al. 2007). Increasing population and sophisticated lifestyle increase the demand of quality industrial products at an exceptional rate. An extensive volume of wastewater originated from industries which are released into channels either untreated or inadequately treated causing water pollution. Industrialization leads to several environmental problems like water, land and air pollution.

Tanning is an ancient trade of India and has been followed for many centuries at the village level. In tanning process putrefiable animal hides are preserved from decomposing and are converted into an enduring material, known as leather. India is the third largest producer and exporter of leather. Leather industry is one of the greatest contributors toward the economy of India. In India there are about 3000 tanneries spread across Tamil Nadu, West Bengal, Uttar Pradesh, Andhra Pradesh, Karnataka, Maharashtra, Rajasthan and Punjab (Mullick 2012). Increasing demand of leather led to the founding of large commercial tanneries (Durai and Rajasimman 2011; Midha and Dey 2008) (Fig. 7.1). Every year 314 million kilograms of skin are processed, approximately 30 l of effluent are generated from every kilogram of hides processed and total quantity of effluent discharged is about 50,000 m^3/day (Subramani and Haribalaji 2012). Long-term disposal of the tannery wastes has resulted in wide contamination of agricultural land and water sources. When tannery waste is discharged in agricultural lands or used to irrigate the lands, it affects the fertility of the soil (Mohan and Devi 2015). Tannery effluents are highly complex, foul smelling, dark brown in color and basic in nature and have high organic content that varies according to the chemicals used. The pH value of tannery effluent is between 7.5 and 10 (Durai and Rajasimman 2011). Based on the leather process, tanneries generate a large amount of wastewater containing ammonium, chromium, sulfates, surfactants, acids, dyes, sulfonated oils and organic substances, including natural or synthetic tannins (Hasegawa et al. 2011) (Table 7.1).

It is divided into four main categories: beamhouse operations, tanyard operations, post-tanning operations, and finishing operations (conditioning, staking, dry milling, buffing, spray finishing, and plating). Tanning process involves chemical, physical, and bacteriological principles. Chemicals used in tanning are sodium carbonate, lime, sodium sulfate, sodium bicarbonate, common salt, fat liquors, vegetable oils, dyes and chrome sulfate (Fig. 7.2). Vegetable tanning and chrome tanning are applied for tanning of raw hide/skin. About 90% of the world's leather is produced by chrome tanning process (Garg et al. 2012).

Vegetable tanning is the oldest process used in the leather industries. It utilizes hydrolysable tannins and condensed tannins. Hydrolysable tannins are derivatives of pyrogallols and condensed tannins are derivatives from catechol. Phenolic groups of vegetable tannins form hydrogen bonding to the peptide bonds (Ockerman and Hansen 1988). First of all the hides are trimmed and permeated

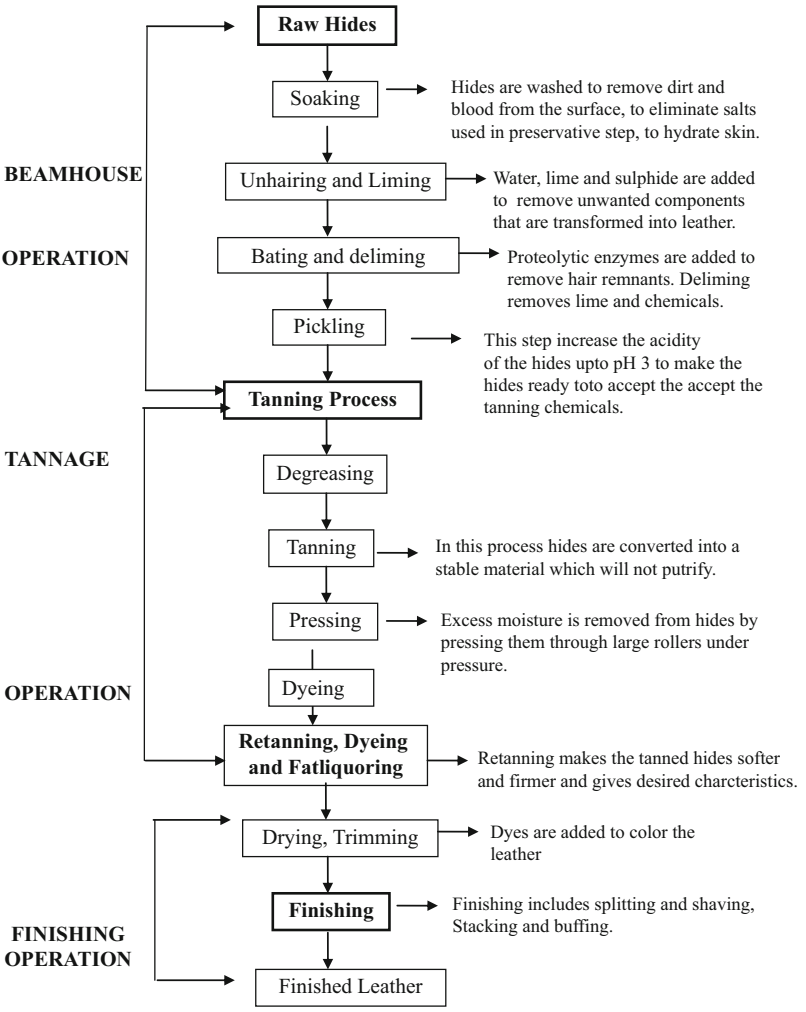

Fig. 7.1 Process of leather production

to remove solids and salt and to restore moisture lost during curing process. Hides are then unhaired in the liming process other than liming, oxidative, thermal and chemical methods that also exist. Deliming process makes the skin susceptible to vegetable tanning. After liming, bating and enzymatic actions are performed to remove undesirable hide components and to relate smoothness, elasticity and stretchability to the leather. The process of bating and deliming is carried out together by insertion of hides in an aqueous solution of proteolytic enzymes and ammonium salt. This process takes 3 weeks to pierce the tanning material to the center of the hide, and then hides are dipped in drums containing sulfuric acid or sodium bicarbonate for bleaching and removal of surface tannins. The leather is

Table 7.1 Characteristics of tannery effluent

pH	TDS	Suspended solids	Total solids	COD	BOD	TKN	Chromium	Sulfide	Reference
7.5–10	37,000	5300	–	6200	–	273	23.3–42.5	466.3–794	Durai and Rajasimman (2011)
3.5	–	–	–	–	–	–	570	–	Benazir et al. (2010)
1–13	–	–	–	180–27,000	210–4300	90–630	3–350	1–500	Midha and Dey (2008)
6–8.2	–	6000–31,000	–	12,000–23,000	800–4000	–	–	30–130	Mannucci et al. (2010)
7.08 ± 0.28	–	2820 ± 140	10,265 ± 1460	4800 ± 350	–	225 ± 18	95 ± 55	–	Ganesh et al. 2006
2.8–3.7	–	–	–	13,000–14,000	5008–8498	–	–	55–190	El-Sheikh et al. (2011)
7.9 ± 0.11	930 ± 50	–	–	–	–	–	100 ± 3	32 ± 0.4	Masood and Malik (2011)
7.7–11.8	–	–	120–390	–	–	–	798–22,200	13–15	Nakatani et al. (2011)
8.0–8.5	–	–	–	3500–4000	2000–2400	300–500	–	–	Iaconi et al. (2002)
8	–	–	–	520 ± 22.4	325 ± 18.6	818 ± 92.6	–	–	Sharma and Adholeya (2011)
7.7	–	–	–	2426	–	–	29.3	286	Szpyrkowicz et al. (2005)
11.4 ± 1.7	6814.0 ± 280.3	–	–	3598.3 ± 76.3	344.3 ± 29.3	–			Murugan and Al-Sohaibani (2010)

Except pH, each value is in mg/l

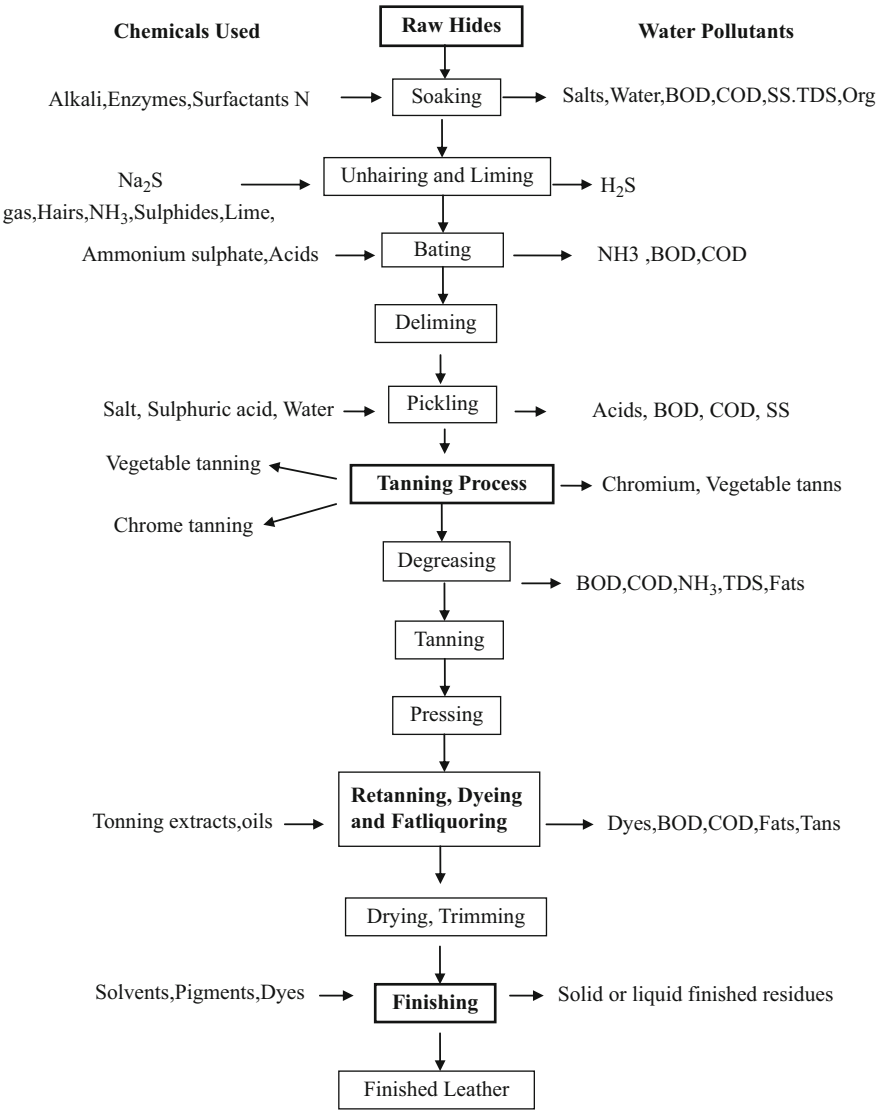

Fig. 7.2 Chemicals used in leather production and released water pollutants

then set out to smoothen and dry. However, most of the time there is no need to do coloring, retanning, fat liquoring, or finishing of vegetable leather. Effluent from vegetable tannery is dark in color, and it persists for a long time. Discharge of effluent into water course increases the turbidity of water reducing light penetration and impairing the photosynthetic activity of aquatic plants.

Leather generated by chrome tanning has a tendency to be more bendable and softer than vegetable-tanned leather. Chrome-tanned leather has properties of higher thermal stability and more stability in water, and the whole process of chrome tanning takes less time than vegetable tanning to produce leather. In chrome tanning process, steps are basically the same as vegetable tanning process. Though, further process of dyeing, retanning, and fatliquoring is generally carried out in chrome tanning. The chromium-tanned leather is piled down, squeezed, evaluated for the thickness and quality, separated into grain and flesh layers, and cut to the desired thickness. In fatliquoring process oil is introduced into the skin to restore the natural oil lost in beamhouse and tanyard processes. After fatliquoring, the leather is squeezed, set out, dried, and finished. Chromium salts used in tanning process generate two forms of chrome, hexavalent chromium Cr(VI) and trivalent chromium Cr(III). Hexavalent chromium compounds cause health risk to humans, plants, animals, and fishes. In the Indian standard, Cr(VI) discharge limit in domestic water is 0.1 mg/L. In India, the release limit of chromium for direct ejection to water bodies ranges from 1 to 5 mg/l and 1 to 20 mg/l for discharge to sewage systems (Tadesse et al. 2006).

7.2 Major Pollutants of Tannery Effluent

(a) Chromium Chromium is widely used in industries because of its hardness and stability (Benazir et al. 2010; Panda and Sarkar 2012). Chromium salt is used in tanning process and generates two forms of chrome, hexavalent chromium Cr (VI) and trivalent chromium Cr(III). Cr(VI) is the most toxic and carcinogenic as a result of its high solubility in water, permeability through biological membranes, and successive interaction with intracellular proteins and nucleic acid (Samrithi and Usha 2012). In an acidic environment, hexavalent chromium is present as $Cr_2O_7^{-2}$ and in alkaline environment as CrO_4^{-2}. Due to their stability in the environment, trivalent chromium and hexavalent chromium are of major significance. Tanneries release hexavalent chromium Cr(VI) ranging from 40 to 25,000 mg/l. As per the Indian standard, the maximum tolerance of total chromium has been fixed to 0.05 mg/l for public water supply. According to environmental protection agency in drinking water, maximum permissible level of Cr(VI) is 3 μg/dm^3 (Benazir et al. 2010).

When humans are exposed to Cr(VI), they may suffer from several health hazards such as nasal irritation, allergic dermatitis, ulceration, skin irritation, eardrum perforation, lung carcinoma, renal tubular necrosis, and epidermal dermatitis and increase risk of respiratory tract cancer and cytotoxic and genotoxic effects (cell death, cell transformation, and gene mutation). Accumulation of Cr takes place mainly in the liver, kidneys, spleen and bone marrow. At high levels Cr damage cell membranes, modify enzyme specificity, disrupt cellular function and damage the structure of DNA (Farag and Zaki 2010; Samrithi and Usha 2012).

(b) Tannins Tannins are water-soluble polymeric polyphenols having different molecular weights (Murugan and Al-Sohaibani 2010; Pepi et al. 2010). They are naturally found in leaves of plants, seeds and flowers. Tannins have two major classes: hydrolysable tannins and condensed tannins. Hydrolysable tannins are of esters of gallic acid (gallotannins) or ellagic acid and are hydrolyzed by acids or enzymes into monomers. Condensed tannins are flavonoid monomers. Their molecular weight ranges from 500 to 3000 g/mol. Tannins are used in leather industry for tanning animal hides and skins. Tannins have astringency property, i.e., they precipitate phospholipids in the cell membrane because this cell wall becomes impermeable from solutions that increase its stability to water, bacteria and heat (Chowdhury et al. 2004). Samanta et al. (2004) studied the impact of tannic acid on the gastrointestinal microflora. According to Samantha tannic acid inhibits the activity of enzymes of rumen microbes. If large amounts of tannin-containing plant material, such as leaves of oak (*Quercus* sp.) and yellowwood (*Terminalia oblongata*), are consumed, hydrolysable tannins are toxic and cause poisoning in animals. Despite of its antimicrobial activity, a number of bacteria, fungi and yeasts show resistance to tannins (Bhat et al. 1998; Jadhav et al. 2011; Hernandez et al. 2005).

Tannins are toxic to aquatic organisms and microorganisms and are recalcitrant to biodegradation (Nitiema et al. 2010). It is also toxic to plants and animals, if consumed in large amount. It inhibits degradation and absorption of nutrients in the gastrointestinal tract and also causes liver and kidney necrosis, jaundice, photosensitization and death in severe cases. It causes stunting of growth in plants, chlorosis, and reduction in yield (Subramani and Haribalaji 2012; Jadhav et al. 2011; Bhat et al. 1998). On the other hand, medical grade tannic acid cures many diseases such as tonsillitis, laryngitis, hemorrhoids and skin diseases. Tannic acid can be extracted from plants such as Chinese and Turkish galls and used in wool dyeing and also in treatment of burns and fever.

(c) Sulfide It is major component of tannery effluent. At low levels of exposure, sulfides can cause headache and nausea and affect the central nervous system. At concentrations of only 800–1000 mg/L, it causes death within 30 min and immediate death at higher concentration. For human consumption, the upper concentration limit of sulfide in water is 250 mg/L (Midha and Dey 2008). There is high risk of dehydration from diarrhea that may be caused by high levels of sulfate in drinking water (Mullick 2012). The acceptable H_2S concentration in clean water is between 0.025 and 0.25 μg/l (Balasubramanian and Pugalenthi 2000).

(d) Others In tanning industry, azo dyes, cadmium compounds, cobalt, copper, antimony, barium, lead, selenium, mercury, zinc, arsenic, polychlorinated biphenyls (PCB), nickel, formaldehyde resins and pesticide residues are also found (Mwinyihija 2010). Concentration of Zn over 400 mg/kg (dry weight) would be phytotoxic. Toxicosis of Zn in plants is rarely observed until plant tissue levels reach to 1000 potential mg/kg. Copper (Cu) is essential for plants and animals. Cu phytotoxicity occurs when biosolids with very high Cu concentrations (2000 mg/kg)

are applied to strongly acidic soils in sensitive crops. Lead (Pb) is not essential for plants or animals and it is toxic to both. Cadmium (Cd) is also not essential for plants or animals, although it is phytotoxic when added to acidic soils (Haroun et al. 2007). If cadmium is present in drinking water, it can induce weakness in bones (Bosnic et al. 2000).

(e) Effect of Tannery Effluent on Water Quality The total discharge of tannery effluent in India is estimated to be 9.42×10^6 L annually (Srivastava and Pathak 1997). Expeditious industrialization in India has resulted in considerable increase in the liquid waste which is usually discharged into open land or into nearby water bodies, causing a number of environmental problems. Tannery effluent wastes are ranked as primary pollutants among all industrial wastes (Eye and Liu 1971). Hussain (1976) reported heavy fish mortality in lake Hussain Sagar, Hyderabad, due to the discharge of effluents without being properly diluted and treated. Rao and Kumar (1983) studied the ecological danger of tannery effluent on water resources in North Arcot district of Tamil Nadu. The salts of the discharged effluent find their way into irrigation reservoirs, contaminating the water by a canal extending from the industries to the reservoir mouth, stretching for a distance of about 5 km. Verma et al. (1977) studied the characteristics and disposal problem of tannery industrial effluent and its effect on streams and land. Baskaran (1977) reported that the arsenic, chloride and chromium present in tannery effluent would render the water unsuitable for drinking and lead to heavy chloride pollution in well water. Bose (1994) studied the quality of drinking water around the leather industries concentrated in Kanpur area and reported that the drinking water contains high concentration of sulfate and sulfide and that it affects plants, animals and human beings. Discharge of tannery effluent affects surface and groundwater sources, making them unsuitable for drinking and irrigation. In turn the BOD and COD of the water body are altered, resulting in eutrophication. Ahamed et al. (1977) conducted a survey of the tannery-affected areas of North Arcot district and observed that the total soluble salt and chloride values of surface water in the river at Vaniyambadi were about 2000 and 800 mg/1, respectively. Dikshit and Shukla (1989) reported that tannery effluent affects the groundwater and that the chromium in the tannery effluent is highly toxic to fishes and other aquatic lives. Reynolds and Varger (1995) reported that the nitrate from tanneries and textile dyeing industries contaminates the groundwater sources and agricultural lands of the central valley of India. Apparao and Karthikeyan (1990) pointed out the characteristics of tannery effluent let into the land. The discharged effluents have created severe land pollution in Dindigul. Tannery wastewater results from a variety of processes using an array of different chemicals. Tannery effluent has changed the quality and composition of water, rendering it harmful for drinking and domestic and agricultural purposes (Chauhan and Kumar 1997). Thabaraj et al. (1964) stated that though the nitrogen content in the tannery effluent could supply nitrogen to the plants, the yield was not increased. He also reported that the excessive amount of sodium chloride, in addition to nitrogen, in the tannery effluent inhibited the uptake of other elements like magnesium, potassium, and

phosphorous. Varadharajan et al. (1970) analyzed tannery effluent and reported the presence of considerable amounts of chlorides, carbonates, bicarbonates, sulfates, sodium and magnesium, which were harmful to plant growth. Rao and Mariappan (1972) reported that sulfides, ammonia and tannin were present in the vegetables grown in soil irrigated with tannery wastewater. Sastry and Prasad (1990) reported that inorganic pollutants, viz., chlorides, trivalent chromium, nitrogen, phosphate, sulfate and lime were present in significant quantities in tannery effluent.

(f) Effect of Tannery Effluent on Land Tannery effluent also contains large amounts of organic and inorganic compounds, which are toxic to aquatic plants or organisms (Banerjea and Motwani 1960). Rajagopalan and Davies (1967) reported that the productivity of soil decreased when tannery wastewater was applied, and the land became infertile. Ahamed et al. (1977) studied the pollution effects of tannery water and reported that the salts in tannery effluent percolate through the soil, thereby causing severe salinity of the land. About 800 acres of agricultural land in and around Dindigul have been seriously polluted due to tannery effluent, and fertile land has now become barren land (Paul Bhaskar 1992). Rao and Kumar (1983) reported that about 532 acres of agricultural land was contaminated by tannery effluent drawn from 45 tanneries of Ranipet. About 10,000 acres of agricultural land was seriously affected by the tanneries of North Arcot district, and 30,000 acres of land was moderately affected in Vaniyambadi, Dindigul, Ranipet, Vishram and Timur township areas. Apparao and Karthikeyan (1990) reported that most of the agricultural land in and around Dindigul became highly saline due to the waste from tanneries. Further, they reported that tannery wastewater, when allowed to stagnate without treatment, gives rise to odor, nuisance and unsightly appearance, besides causing groundwater and surface water pollution. Chromium and arsenic, which are the major components of tannery effluent, affect the health of the ecosystem (De 1990, Dara 1990). Trivedi and Gurudeepraj reported that wastes of the tanning industry pose a serious problem to mankind. When discharged into the soil, they affect the ecosystem (Trivedi and Raj 1992).

7.3 Treatment Methods

Treatment of tannery wastewater is carried out by physical, chemical or biological, or combination of these methods (Durai and Rajasimman 2011).

7.3.1 Physicochemical Method

A number of physicochemical methods such as sedimentation (Song et al. 2000), electro-floatation (Murugananthan et al. 2004), filtration (Tiglyene et al. 2008), membrane filtration, precipitation and coagulation (Şengil et al. 2009), adsorption

(Santosa et al. 2008; Covarrubias et al. 2008) and ion exchange (Tiravanti et al. 1997; Kabir and Ogbeide 2009) have been used for treatment of tannery effluent.

Esmaeili et al. (2005) reported that chromium precipitation is a simplest technique in which chromium and other metals are precipitated as highly insoluble hydroxides. Song et al. (2000) reported that COD and TSS are reduced by using chemical precipitation. Aluminum sulfate and ferric chloride were used as a coagulant material. Poulopoulou et al. (1998) removed chromium from tanned leather waste by physical (irradiation) and chemical methods. Rajalo and Petrovskaya (Rajalo and Petrovskaya 1996) applied soft electrochemical oxidation method for the removal of sulfide compounds from tannery wastes.

Electrochemical oxidation was investigated for final tannery wastewater treatment showing complete mineralization of vegetable tannery wastewater (Panizza and Cerisola 2004). Pretreatment of tannery wastewater using two systems, electrolytic and physicochemical systems, showed poor efficiencies for the electrolytic system and significant removal of pollutants with the physicochemical system (El-Sheikh et al. 2011).

7.3.2 Biological Method

Biological treatment of wastewater is more advantageous and cost effective as compared to other physiochemical methods. Microbes decompose waste into harmless inorganic solids by aerobic or anaerobic process. In anaerobic process, longer detention period is required and gives unpleasant odors, whereas aerobic process does not have unpleasant odors. For biological treatment of tannery waste, mostly activated sludge process (ASP) and upflow anaerobic sludge blanket (UASB) process are used (Fig. 7.3). ASP-based treatment is regarded to be energy intensive and expensive from an operation and maintenance point of view (Midha and Dey 2008).

Microbes are the most important sustainable agents for the degradation and detoxification of industrial pollutants (Mullick 2012). Various microorganisms *Pseudomonas aeruginosa*, *Acinetobacter* sp., *Bacillus* sp., *Desulfovibrio desulfuricans*, *D. vulgaris*, *D. gigas*, *Flavobacterium* sp., *Escherichia coli*, *Lactobacillus* and *Micrococcus yunnanensis* etc. are able to reduce the content of pollutants significantly by utilizing them as energy and nutrient source in the presence or absence of oxygen (Mullick 2012; Samrithi and Usha 2012; Selvi et al. 2012; Midha and Dey 2008) (Table 7.2). In nature, microorganisms do not exist in isolated form; sometime and somewhere they coexist with different microorganisms and established relationships that have an effect in the biological competence of all interacting species (Cardenas-Manriquez et al. 2007). Microbial consortia are universal in nature. They are associated in environmental remediation and wastewater treatment. Microbial consortia are more vigorous to environmental variations and are able to survive in nutrient limitation better because members of the consortium correspond with one another by exchanging metabolites or by trading molecular signals; each population or individual identifies and acts in

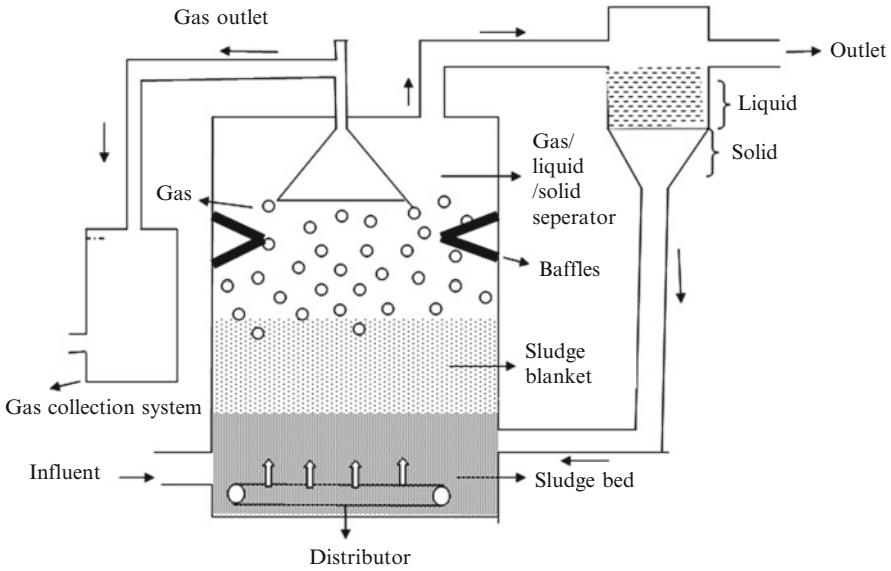

Fig. 7.3 Upflow anaerobic sludge blanket reactor

response to the presence of others in the consortium. Due to this property, microbial consortium can serve obscure functions rather than individual population. This communication empowers the important feature, the division of labor among consortium population (Brenner et al. 2008).

7.3.2.1 Aerobic Biological Treatment

In the effluent organic carbon is used by aerobic microorganisms and converts it to biomass and carbon dioxide. Along with high energy utilization, large amount of sludge is also generated in the process (Midha and Dey 2008).

Activated sludge treatment is most widely used with extended aeration. It is an aerobic biological process, in which microorganisms convert oxygen-demanding organic compounds into environmentally more acceptable forms. Ganesh et al. (2006) degraded tannery effluent by using sequencing batch reactor. The reactors contain two exits: one for sludge withdrawal and the other for cleaning and emptying the reactor. Sludge was introverted directly from mixed liquid at the end of the aerobic phase.

7.3.2.2 Anaerobic Biological Treatment

Anaerobic treatment of tannery wastewaters will be noticed in the future due to the warm climate of emerging countries. Song et al. (2004) used upflow anaerobic fixed biofilm reactor (UAFBR) for treatment of tannery effluent (Song et al. 2004). El-Sheikh et al. treated tannery effluent with the help of upflow anaerobic sludge blanket reactor (El-Sheikh et al. 2011). Zupancic and Jemec (2010) used anaerobic sequencing batch reactor. Primarily, UASB reactors, upflow anaerobic filters

Table 7.2 Microbial sources for the removal of chromium, tannic acid, and sulfide

S. No.	Biodegrading agent	Reference
Chromium		
1.	*E.coli*, *P. aeruginosa*, *Acinetobacter* sp.	Srivastava et al. (2007)
2.	*Bacillus subtilis*, *Pseudomonas aeruginosa*, *Saccharomyces cerevisiae*	Benazir et al. (2010)
3.	*Enterobacter aerogenes*, *Acinetobacter* sp.	Panda and Sarkar (2012)
4.	*Spirogyra condensate*, *Rhizoclonium hieroglyphicum*	Onyancha et al. (2008)
5.	*Acidithiobacillus thiooxidans*	Wang et al. (2007)
6.	*Pseudomonas* sp.	Poornima et al. (2010)
7.	Free and immobilized spores of *A. niger* and *A. parasiticus*	Shugaba et al. (2010)
8.	*Serratia* sp.	Srivastava and Thakur 2012
9.	Crustacean shell (ground shrimp shell)	Fabbricino and Gallo (2010)
10.	Paecilomyces lilacinus	Sharma and Adholeya (2011)
11.	*Bacillus subtilis* P 13	Pillai and Archana (2012)
12.	Sulfur-oxidizing bacteria	Fang et al. (2007)
13.	*Calymperes dessertii* (moss)	Low et al. (1997)
14.	*Pseudomonas aeruginosa* and *Micrococcus yunnanensis*	Mullick (2012)
15.	*Bacillus* spp., *Staphylococcus* spp.	Mythili and Karthikeyan (2011)
16.	*Bacillus firmus* TE7	Bachate et al. (2013)
17.	*Pseudomonas aeruginosa*, *Bacillus flexus*, *Exiguobacterium homiense*, and *Staphylococcus aureus*	Sivaprakasam et al. (2008)
Tannic acid		
1.	*Klebsiella* sp. NACASA1	Jadhav et al. (2011)
2.	*Aspergillus* sp. and *Penicillium* sp.	Hernandez et al. (2005)
3.	*Enterococcus faecalis*	Goel et al. (2011)
4.	Black *Aspergillus* species	Diepeningen et al. 2004
5.	*Rhodococcus* NCIM 2891	Nadaf and Ghosh (2011)
6.	O3/H2O2	Ji-min et al. (2008)
7.	Ligninolytic soil fungi	Silva et al. (2010)
8.	*Bacillus* sp. AB1	Ilori et al. (2007)
9.	*Streptococcus* sp.	Nitiema et al. (2010)
10.	*Pseudomonas citronellolis*	Chowdhury et al. (2004)
11.	*Serratia* sp. and *Pantoea* sp.	Pepi et al. (2010)

(continued)

Table 7.2 (continued)

S. No.	Biodegrading agent	Reference
Sulfate/sulfide		
1.	*Acinetobacter*, *Alcaligenes*, *Ochrobactrum*, and *Pseudomonas*	Aguilar et al. (2008)
2.	*Desulfovibrio desulfuricans*, *D. vulgaris*, and *D. gigas*	Mullick (2012)
3.	SRB	McCauley et al. (2009)
4.	SRB	Kieu et al. (2011)
5.	SRB	Jong and Parry (2003)

(UAF), and downflow anaerobic filters (DAF) are used in laboratory or pilot scale for anaerobic treatment (Table 7.3).

Midha and Dey (2008) removed sulfide from tannery effluent by aerobic and anaerobic treatment. They used *Thiobacillus*, *Pseudomonas*, *Beggiatoa*, and *Thiothrix* for sulfide removal by oxidation processes. Song et al. (2004) also developed an upflow anaerobic fixed biofilm reactor (UAFBR) to treat tannery wastewater and obtained good COD and TSS removals even under conditions of temperature shock. Lefebvre et al. (2006) used upflow anaerobic sludge blanket reactor to study anaerobic digestion of tannery soak liquor and achieved 78% COD removal at hydraulic retention time (HRT) of 5 days and a total dissolved solid (TDS) concentration of 71 g/l. Genschow et al. (1996) also removed sulfide from tannery wastewater by using two-stage anaerobic treatment. Goltara et al. (2003) reported a membrane sequencing batch reactor (MSBR) to treat sulfide compounds in tannery wastewater. Mullick (2012) isolated *Pseudomonas aeruginosa* and *Micrococcus yunnanensis* from tannery waste and carried out sulfate removal in benchtop fermenter. A number of researchers reported biogas production by digested tannery waste in anaerobic sequencing batch reactor (Farabegoli et al. 2004, Ganesh et al. 2006; Zupancic and Jemec 2010). Iaconi et al. (2002) treated tannery wastewater by combining discontinuous biological degradation in a sequencing batch biofilm reactor (SBBR), with chemical oxidation, by using ozone. Szpyrkowicz et al. (2005) used a combination of electrochemical and biological processes for tannery wastewater treatment. Mannucci et al. (2010) and El-Sheikh et al. (2011) studied biological tannery wastewater treatment by applying two-stage UASB (upflow anaerobic sludge blanket) reactors. Banu et al. (2007) made an attempt to treat the tannery wastewater by using hybrid upflow anaerobic sludge blanket reactor. Subramani and Haribalaji (2012) used microorganisms particularly *Bacillus* sp., *Pseudomonas aeruginosa*, and *Aspergillus niger* to reduce pollution load of tannery effluents by activated sludge process.

Srivastava et al. (2007) achieved 90 and 67% removal of chromium and PCP from tannery effluents by sequential bioreactor where they treated effluents by

Table 7.3 Reactors used in treatment of tannery effluent

Authors	Reactors	Details
Pillai and Archana (2012)	Solid substrate column reactor using chrome shavings	A glass column of 30 cm height was developed as a reactor with silicon tubing attached to the bottom with a stopper
Srivastava et al. (2007), Ganesh et al. (2006), Farabegoli et al. (2004)	Sequential bioreactor	The reactor had two exits: one for sludge draw and the other one for discharge. 2–1 glass column connected with another 2-liter glass fractionation column in a sequential way. pH was maintained at 7.0. A PC controlled the reactor by means of a dedicated software
Zupancic and Jemec (2010)	Anaerobic sequencing batch reactor	This is a batch process with duration of 7-day cycle. Four treatment phases (feed, react, settle, and draw) are completed one by one in a vessel
El-Sheikh et al. (2011)	Two-stage upflow anaerobic sludge blanket bioreactor	UASB consists of tanks manufactured from PVC material. The tanks include sedimentation tank, pH equalization tank, storage tank, first-stage UASB reactor, and second-stage UASB reactor (all are connected in series)
Di Iaconi et al. (2002)	Combined chemical and biological degradation of tannery wastewater by sequencing batch biofilm reactor (SBBR)	This treatment is carried out in a sequencing batch biofilm reactor, with chemical oxidation, performed by ozone. SBBR is made of a closed upflow cylindrical reactor (geometric volume of 30 L, working liquid volume of 16 L) maintained at a constant temperature of 20 °C, with a peristaltic pump
Song et al. (2004)	Upflow anaerobic fixed biofilm reactor (UAFBR)	UAFBR retains a high concentration of accumulated biomass in the form of biofilm supported by a carrier

Pseudomonas aeruginosa, *E. coli* and *Acinetobacter* sp. Abskharon et al. (2009) studied the reduction of hexavalent chromium to trivalent chromium by using four resistant strains of *E. coli* ASU, 3, 7, and 8 isolated from wastewater. Panda and Sarkar (2012) examined bioremediation potential of *Acinetobacter* sp. PD 12 and *Enterobacter aerogenes* and used them to uptake chromium from tannery effluents. Pillai and Archana (2012) and Samrithi and Usha (2012) isolated *Bacillus subtilis* P13 and *Bacillus* sp., respectively, from tannery effluent which reduced 85.9% of

chromium. Benazir et al. (2010) studied the ability of *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Saccharomyces cerevisiae* in accumulation, detoxification, degradation and absorption of chromium in tannery effluents and found that all strains are able to remove 99% of chromium. Chen et al. (2012) isolated *Marinobacter*, *Pseudochrobactrum*, *Shewanella*, *Psychrobacter*, *Microbacterium* and *Agrococcus* strains from tannery waste which showed significant Cr (VI) removal (1.2%–99.1%) competence and good potential for Cr(VI) pollution treatment. Poornima et al. (2010) isolated chromium-degrading bacteria, *Pseudomonas putida* and *Pseudomonas plecoglossicida*, from rhizosphere soil of Amirthi forest region, Tamil Nadu, by enrichment method. Sau et al. (2008) reported a highly chromium-resistant *Bacillus firmus* strain in soil samples with chromium effluents. This strain was able to remove 80% of Cr. Selvi et al. (2012) were evident that various bacterial strains such as *Pseudomonas*, *E.coli*, *Alcaligenes*, *Flavobacterium* and *Bacillus* species isolated from tannery effluent collected in and around Chennai, South India, showed tolerance to chromium up to 70%. Goumghar et al. (2005) recovered *D. hansenii*, a yeast species from tannery wastes which was resistant to chromium. Onyancha et al. (2008) demonstrated that *Spirogyra condensata* and *Rhizoclonium hieroglyphicum*, algae biosorbents, removed chromium from tannery wastes. Wang et al. (2007) illustrated the ability of using the indigenous sulfur-oxidizing *A. thiooxidans* as a potential aspirant for microbial removal of chromium from tannery sludge because of its high chromium solubilization efficacy. Masood and Malik (2011) investigated 99% biosorption of hexavalent chromium by *Bacillus* sp. FM1, isolated from soil irrigated with tannery waste. Fabbricino and Galo (Fabbricino and Gallo 2010) investigated the use of crustacean shells for the removal of chromium from tannery wastewater. Fadali et al. (2004) removed chromium from tannery effluent by using synthetic chromium salts (chromium chloride) as adsorbate and cement kiln dust (a waste from white cement industry) as adsorbent.

Jadhav et al. (2011) isolated *Klebsiella* sp. NACASA1 from the garden soil of botanical garden of N.A.C. & S. College, Ahmednagar, India, which was able to rapidly degrade tannic acid at 15 °C. Hernandez et al. (2005) evaluated and isolated tannin-degrading fungal strains *Penicillium commune*, *Aspergillus niger*, *Aspergillus rugulosa*, *Aspergillus terricola*, *Aspergillus ornatus* and *Aspergillus fumigates* from Mexican desert. Nitiema et al. (2010) isolated a tannic acid-degrading *streptococcus* sp. from anaerobic shea cake digester. Chowdhury et al. (2004) isolated *Pseudomonas citronellolis* from tannery soil samples, which is capable to degrade tannic acid and also studied molecular diversity of isolate. Murugan and Al-Sohaibani (Murugan and Al-Sohaibani 2010) isolated *Aspergillus candidus* MTCC 9628 from the biomass of mango industry solid waste. They isolated tannase enzyme from *Aspergillus candidus* which was found to degrade the tannin content of the tannery effluent. Mahadevan and Muthukumar (1980) studied tannin degradation with reference to aquatic microorganisms; according to them, tannins inhibit microbial growth, metabolism, and respiration. Bhat et al. (1998) reported that species of *Bacillus*, *Klebsiella*, *Pseudomonas*, *Aspergillus*, *Penicillium* and *Tricoderma* degrade tannins.

7.4 Conclusion

Bioremediation offers a real and practicable alternative as a means to clean Cr, not only from tannery waste but also to clean other heavy metals from several industrial effluents. Four types of bioremedial processes, at present, seem particularly applicable for treating tannery waste, viz., (a) biosorption, (b) bioaccumulation, (c) bioreduction, and (d) immobilization of microbial cells for bioremediation. The application of combined processes of physical and chemical with biological process to treat tannery wastewater would give satisfactory results compared to individual treatment process.

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Sustainable Environmental Biotechnology

8

Harmanjit Kaur

Abstract

In today's era of rapid globalization, sustainability in the environment has become a priority for world leaders; they affirm their intent to pursue broad range of technologies with the potential to reach the goal of sustainability. Human interferences in terms of their increasing anthropogenic activities, destructive behavior, resource exploitation fueled by growing consumption, and swiftly eroding natural ecosystems are driving us toward an environmental precipice. Today's environmentalists remain alarmed at the inefficient use of technologies by mankind. Therefore, to cope up with this alarming situation, the recent advances in "biotechnology" played an important role. Some of the defining technologies of modern biotechnology with the probability of attaining the goal of sustainability are included in fields like food production, various industrial and agricultural practices, capturing valuable products from renewable raw materials, energy sources, waste management, and bioremediation. This chapter addresses the challenges ahead and various strategies that can be dealt with to achieve a sustainable environment.

Keywords

Sustainable environment • Biotechnology • Industrial sustainability • Waste management

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Abbreviations

BOD Biochemical oxygen demand
COD Chemical oxygen demand
EPA Environmental protection agency
IPM Integrated pest management
PAH Polyaromatic hydrocarbon

8.1 Introduction

The biggest challenge in today's world is to develop a sustainable and stable environment, which can be achieved through a stable global economy. To attain sustainability, billions of dollars are used in the form of services, products, and technologies. Today, we look in for zero environmental impacts, whereas for tomorrow we need to build a positive impact. To think past greening and to reach until sustainability, numbers of technical and economic problems need to be addressed, and a multifaceted set of global issues must be unraveled. Various human activities having deep impacts on the environment and sustainability include industrialization, agriculture, forestry and fishing, and mineral extractions. So, there is a need to improvise methods for managing resources, controlling wastes, and preventing pollution (Fig. 8.1).

Some areas of biotechnological impact are listed below:

8.2 Sustainable Agricultural Practices

Current agricultural practices favor increased agriculture production at the expenditure of ecosystem services. It's the need of the hour to aspire "sustainable agriculture" through the implications of various farming techniques that lead to a balanced agricultural system. Sustainability in agriculture tends to achieve profitability in farm produce by improving soil texture, quality, environment profitability and stability through reduced dependence on nonrenewable resources and also adds to community health and prosperity by minimizing ill environmental impacts. A shift is needed toward an optimal biological system that will help in enhanced production of food and fiber, bio-based products with minimum wastage. Taking into consideration, the speedily mounting world population and ill effects of agricultural systems on the surroundings, it is of utmost importance to develop a sustainable form of food production. However, the job seems to be tedious as nearly half of the crop varieties are nearing their limits of productivity in struggle for survival with weeds, pests and pathogens (Zechendorf 1999). To remedy this situation, biotechnology offers numerous potential key contributions, such as:

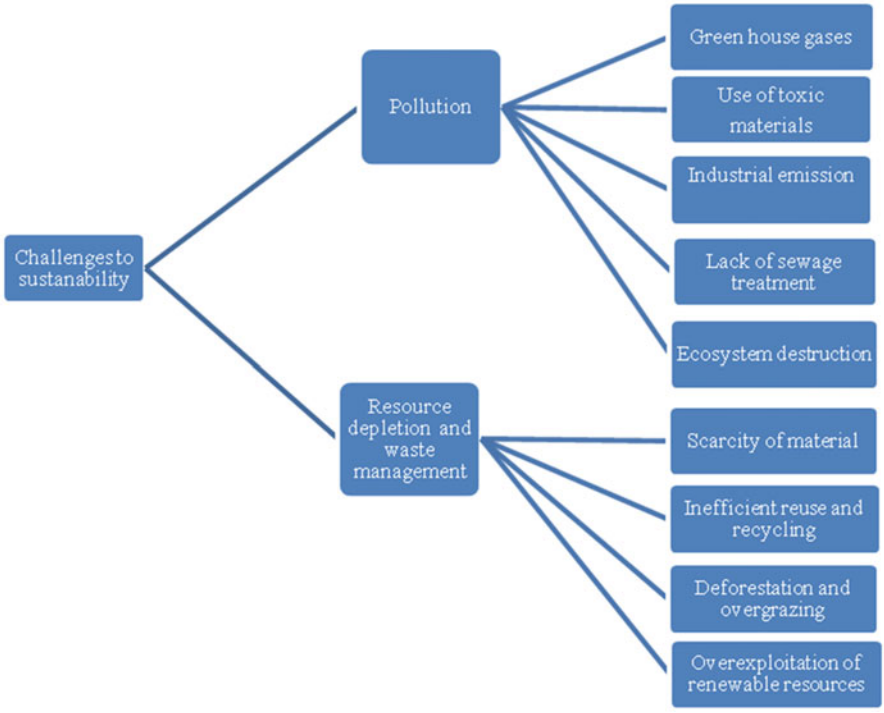

Fig. 8.1 Some major challenges faced in order to attain environmental sustainability

- Supporting the concept of biodiversity and vital ecosystems – using same area of land for producing more food thereby realizing the nutritional yield per acre; reducing the pressure to expand into the wilderness, rain forests, marginal lands, and also avoiding soil erosion
- Introducing the idea of "integrated pest management" (IPM) which brings alterations in traditional methods by means of crop rotations, clubbing varietal crop species together with minimum dependence on agrochemicals, introducing refuge varieties and environment-friendly crops, which are insect resistant and herbicide tolerant and can fix nitrogen
- Relocating energy-intensive means like fertilizers/pesticides and fuels, thereby reducing inadvertent environmental influences and also preserving resources for the future use
- Encouraging lesser environmentally damaging agricultural practices and the adoption of sustainable practices, such as conservation tillage, precision agriculture, and integrated crop managements
- Making a cordial use of nonrenewable resources, on-farm produces to attain sustainability toward biological systems

With the advent of newer technologies, agriculture has also undergone tremendous changes. Different improved means are flourishing day by day for improving and enhancing agricultural production which in turn helps develop improved varieties. New breeding programs have helped selective breeding of crops, which help raise plants through selective cross- and induced-breeding programs and resulted in the development of new beneficial traits which are more eco-friendly. The role of genetic engineering programs toward sustainable agricultural development has become an interesting topic for future studies. Crops developed through advanced biotechnological approaches are much more sustainable and better adapted toward our environment. Such plants need lesser pesticide applications, use farming techniques with lesser impacts on soil health and water retention capacity, as well as are suitable for less-plowed soils. Biotechnology also enables agriculturist to adopt no- or reduced-till techniques, which result in maintaining the soil texture, health, and quality through reduced erosion and also result in lesser consumption of fuel on farms which in turn leads to higher carbon sequestration. Erosion damages soil quality, and eroded soil will lead to decrease in cultivated land, retarded plant growth, and decline in overall crop yields.

Biotechnology prevents loss of crop production and yield by building resistance against various pests and disease. Integrated pest management (IPM) is another important concept which looks for minimizing crop losses by implementing improved and lesser harmful methods. IPM includes the incorporation of suitable measures that keep check on the development of pest populations and also maintain a safe level for the use of pesticides and decrease overdependence on conventional insecticides and other agrochemicals so that they offer minimum health and environmental impacts (Way and Emden 2000).

8.3 Agrochemicals Replacement

A market survey for the use of agrochemicals clearly figures out that use of pesticides is expanding at a faster rate of 10% per year. By 2010, global sales touched the $1 billion mark with nearly 4.2% percent of pesticide usage. However, experts estimate that much of this pesticide use declines at a rate of 1.3% per year due to increased health and environmental concerns, which clearly show the growing awareness among the farmers and the community (Wilson and Tisdell 2001). To enhance the awareness drive, we need to apply the concept of biotechnology in a sustainable way that comes in the form of green chemistry. Green chemistry is required for sustainable agriculture as both the farmers and green chemists are dependent on each other. Agriculturists need chemists to make safe and sound agricultural inputs, while chemists need farmers to provide bio-based raw materials for processing new products. Different principles have been employed that eliminate use of harmful substances, thereby taking a step toward innovative use of renewable feedstocks.

Abrupt use of agrochemicals in routine conventional agricultural practices is clearly associated with detrimental environmental impacts. From human's health

issues to ecological impacts, concerns are on the rise about how and what to farm. Potential benefits of biotechnology toward the environment include utilization of technique to grow plants that offer herbicide and pesticide resistance and show lesser dependence on these. This helps in the decline in carbon emissions and soil erosion, thus dropping the overall impacts that can be caused. Excessive pesticide utilization is harmful toward environments because higher resistance levels demand continuous higher inputs of pesticides which can ultimately lead to extinction in useful predators. It becomes more adverse when application of pesticides is done through foliar sprays from aircrafts.

The use of various alternatives to fertilizers, such as fermentation sludge, cyanobacteria, chitosan, plant product-based biopesticides, insect-derived pheromones, and repellents has been employed to combat the ill effects caused by pesticides. Similarly, biological fertilizers like BIOFIX, a rhizobium inoculant used at a concentration level of 100 g, could replace 90 kg of chemical nitrogen, which not only cuts down the costs by ten times but also showed promising results. Likewise, nitrogen fixation rates could be enhanced by nearly 20% along with the yields by inoculating rice field with mycorrhizal fungi and *Alcaligenes faecalis* (Pimentel and Peshin 2014). Herbicide-tolerant crops that make lesser herbicide use especially during early growing season have been developed. Moreover, herbicide resistance genes already exist in many wild plants; hence, an immense use of herbicide will build pressure toward the dispersal of resistance genes throughout the plant population. Similar advantages are being offered by other organic bioinsecticides, plant-based biopesticides, pheromones, and repellents.

8.4 Industrial Sustainability

The concept of industrial sustainability is a correlation between natural ecological systems and industrial systems, which are in turn responsible for bringing sustainability in the environment. With the advent of biotechnology, various efficient industrial processes have been formulated which offer cleaner and sustainable environment by reducing pollution and waste, for the same production levels. Since 1990s, emphasis is not toward the removal of pollutants and waste gases from the environment but is on to redesign different industrial processes so that pollution can be prevented at the source level. The important sectors that have been examined in terms of generating industrial pollution and environmental impacts include paper & pulp industry, food processing, leather & textile industry, mineral & metal processing industry, etc. To cope up with the ill effects generated by these industrial processes, it becomes vital to introduce the concept of clean technology. Continued technical innovation, through biotechnological approach in terms of introduction of biological catalysts, viable organisms, could play a pivotal role as they generate products through cleaner processes with minimum wastage and lesser by-products. Processes of combining genetic potential, such as BioConsortia, metabolic engineering, rDNA technology, and bioinformatics offer strong methods for culturing organisms with new capabilities. Researchers are continuing studies for exploring

the hidden microbial diversity which apparently holds the great biocatalytic potential. It is even expected that microorganisms which are living under extreme conditions of temperature, salinity, acidity, etc. have their enzymes which show improved catalytic performance in industrial conditions.

Making use of plants for various industrial processes, such as extracting metabolites, oils, fats, and fibers is surely not a new concept because living organisms are much more efficient and offer fewer waste products, which in turn are also recyclable and biodegradable. In many developing countries, nonfood plants are considered as substitutes for the use of uncovered land. For example, in Europe nearly 18% of molasses, 6% of maize and oil plants, and 2% of milk proteins are used for nonfood purposes (Grommen and Verstraete 2002). Various other plant-derived products like oil, acids, enzymes, proteins, etc. have multiple applications in cosmetics, as additives, detergents, solvents, lubricants, inks, and many other products. Uses of such by-products and other biotechnology-based products have offered considerable environmental advantages. For example, for energy generation, the most commonly used raw material is fossil carbon while the process emits CO_2 which alarms the situation as enhanced levels of CO_2 in the atmosphere would result in global warming. However, it may be kept in mind that widespread use of biotechnological processes and products, which consume lesser CO_2 contributes to reduce fossil carbon consumption and thus are zero net contributors to atmospheric greenhouse gases.

The use of biotechnological techniques, such as bioleaching, bioremediation, and biooxidation for mining and metal recovery offers superior cleanliness and economic profitability. Uses of natural material are of utmost importance toward sustainability because they can be renewed in contrast to nonorganic materials (metal, minerals). Various other processes undertaken under the thrust area include biofuel production (biodiesel and bioethanol), which seeks to conserve natural resources, cut the production cost, and reduce greenhouse gas emissions and is environment-friendly. To meet the global challenges, use of biotechnology for biofuel production has been promoted all around the globe (Sexton et al. 2009).

8.5 Waste Management

Today, environmental pollution is the most important problem worldwide. Since the introduction of the concept of industrialization, pollution is continuously growing; therefore, it is of utmost importance to take precautionary steps to help reduce and prevent pollution. Open dumping of waste without any pretreatment releases various obnoxious gases, which heat and pollute the surroundings. To avoid urban waste problems, integrated waste recycling practices should be adopted. Biotechnology employs various biological methods and techniques for dumping and disposing wastewater and solid wastes. Environmental engineering is one such alternative scheme of environmental management wherein all the consequences of treating a particular waste are considered. So far, wastes are believed to be of no use or value, and hence, their management was kept at the minimum, wherein,

nowadays the theory has changed and management of waste has gained utmost importance to attain a state of equilibrium (Hanife and Levent 2009). Several contributions have been made through biotechnology to waste treatment and environmental management. Some of the highlights include:

1. Introduction of technologies for conversion of readily biodegradable wastes
2. Cleaner technologies of production which in turn dissipate less number of pollutants and harmful by-products
3. Biological alternatives for conventional products which are much safer
4. More effective environmental management through the introduction of sensitive and rapid detection techniques
5. Isolation of microbial strains with novel capabilities of degradation of harmful chemicals

Biotechnological applications used for waste treatment are listed under bioprocessing, biopulping, phytoremediation, bioleaching, biobleaching, biodesulfurization, bioremediation, biofiltration, and fermentation using bioreactors.

8.6 Microbial Ecology in Waste Management

The pollutants, despised by human activities, are what effective microorganisms consume and that is why microbial ecology helps monitoring environmental pollution. The growth of the microorganisms in flocs is responsible for metabolism of both solid and liquid wastes (Aktan 1983). The possible impact of these microorganisms is basically due to zymogenic microorganisms (antioxidants producing microorganisms) and synthesizing microorganisms (facultative anaerobic microbes that can decompose under extreme temperature and pH conditions).

Different biotechnology methods employed for wastewater treatment include anaerobic treatment and biological treatment including activated sludge, composting techniques, trickling filters, oxidation ponds, and biofilters. In all these methods, suitable microorganisms thus required will degrade organic substances. Effective microorganisms have become the most powerful and acceptable tool to carry out effective environmental management due to the following reasons (Rittmann 2006):

1. It reduces the organic pollutants, biochemical oxygen demand (BOD), chemical oxygen demand (COD), etc. in the waste effluent.
2. Microbes immediately feed on the organic matter and multiply creating in the process the enzymes that curb pollution and make the compost suitable for use.
3. Microbial ecology developed in waste/polluted water decreases the algal growth and algal pollution.
4. It suppresses the total coli and *E. coli* count.

5. It also reduces the foul odor and presence of toxic gases in treated water, is eco-friendly, and makes the environment healthy and fresh.
6. It reduces sludge volume, no further treatment is required for sludge obtained, and it makes the effluent disposable/reusable.

Bioremediation is a most common term used for the waste management that makes use of natural organisms to combat problem of pollutant. It is broadly classified as *in situ* and *ex situ* bioremediation. The former provides the treatment at the contaminated sites and avoids any kind of excavation and transport, while in the latter, the contaminated soil excavates and is treated at another place. *In situ* bioremediation further involves techniques like bioventing, biosparging, and bioaugmentation, whereas *ex situ* includes biological techniques like biopiling, land forming, and composting (Charcosset 2006). Bioremediation is an effective way of removing pollutants, organic wastes, polyaromatic hydrocarbon (PAH)-containing waste, halogenated hydrocarbon waste, pesticides, and waste from environment, though some of these are more easily biodegraded than others. However, other pollutants which are not easily treated by bioremediation using microbial cultures include heavy metals like Cd, Cu, Ar, Zn, Pb, etc. not only contaminate soil but also groundwater through leaching. Plants grown on such contaminated lands absorb and accumulate heavy metals in the form of mobile ions in their roots, stems, fruits, grains, leaves, and other plant parts (Fatoki 2000 and Madejon et al. 2003). These accumulated metals in different plant parts end up entering the food chain with adverse impacts on human and animal health. So, it is of utmost importance to clean up this heavy metal pollution, and hence, phytoremediation has emerged as a cost-effective technology in this regard. These metals can also be removed with the help of various biological agents (such as yeast, fungi, bacteria, algae, etc.), which act as biosorbent for sequestering the metals from complex solutions very quickly and, hence, are ideal candidates. These metals can be further concentrated by incinerations or recycle for further use.

Nearly 38 billion metric tons of organic waste is generated through different agricultural practices worldwide; therefore, attention needs to be paid to develop effective technologies to develop value-added products from wastes. Vermicompost is a venture for degrading organic matter through the process of feeding by earthworms and other epigenetic worms which potentially minimize the waste from different sources (Aalok et al. 2008). With the rapid advances now occurring in the field of molecular biology and biotechnology to the study of microbial ecology, it is important to orient some of this activity to biological biotechnology systems. Biotechnological tools could supplement the understanding of a particular operation, or a process includes genetic engineering, nucleic acid probes, immunological assays, microbial diversity measures, gene expression, stress promoter activation, stable isotopes, and reporter genes. With the mounting awareness that engineered organisms offer, least environmental impact will obviously necessitate overcoming of various issues.

8.7 Biotechnology and a Safe Environment

With the advent of new technologies, it becomes important to access the risk factors and their impacts on the environment. Likewise, with the introduction of genetically engineered crops, herbicides and pest-resistant crops and crops with increased yield and nutritional contents are some of the areas which need to be critically evaluated. Influence of biotechnology crops varies regionally and according to the crop type. The Environmental Protection Agency (EPA) is putting a check over the quality and health of the environment. Biotechnology crops undergo vigorous testing over the years before they are put in the farmer's fields, and also farmers are well trained about the concerns associated with the crops. Therefore, various-regulating agencies ensure that the biotechnological products that are released into the environment do not have any detrimental impacts on the environment. The role of biotechnology toward environmental sustainability is a combined effort of various other technologies like biochemistry, environmental molecular biotechnology, environmental engineering, ecology, etc. Taking into consideration the effects of all the technologies, biotechnology acts as a driving force toward environmental protection.

8.8 Conclusions

Biotechnology is an emerging field that offers new possibilities for developing and building a safe, eco-friendly, and sustainable ecosystem. Agricultural biotechnology focuses toward improved agricultural practices and enhanced productivity through newly engineered crops, monitoring bioindicators, reduced greenhouse emissions, thinning the use of pesticides, production of renewable materials and energy, cleaner water and reduced use of fuel, which are the main sectors supporting sustainable development. Biotechnological methods redesign processes to replace and minimize harmful impacts of various products released into the environment.

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Application of Nanotechnology in the Environment Biotechnology

9

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Abstract

With the increasing industrialization and urbanization, the environment is getting polluted. Conventional techniques such as filtration, centrifugation and biological treatment are expensive and not efficient one. Therefore, there is a need for the development of recent and efficient techniques for environmental monitoring and treatment. Nanotechnology is the solution to the abovesaid problems. Nanoparticles, nanomembranes, nanofilters, and nanocatalysts have been developed for wastewater treatment. These have smaller size (1–100 nm) and higher surface area to volume ratio. Due to these properties, they provide more reaction surface which results in increased efficiency and selectivity. With the some issues solved, nanotechnology will answer all the environmental problems.

Keywords

Nanotechnology • Nanoparticles • Environment • Contaminants

9.1 Introduction

With increase in industrialization, the pollution level in the environment is also increasing. The environment is being contaminated using heavy metals (cadmium, zinc, arsenic, mercury and lead), sulfur dioxide, carbon monoxide, nitrogen oxide, chlorofluorocarbons (CFCs), dioxins, volatile organic carbons (VOCs) etc.

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(Environmental Defence Fund 2006). Due to the excessive burning of coal, oil, and gas, the amounts of nitrogen and sulfur oxide have increased in the environment which lead to acid rain. Water pollution is caused by a lot of factors including the leakage of herbicides, pesticides, oil spills in the water bodies, and also the release of by-products of industrial processes and fossil fuel extraction and combustion (Krantzberg et al. 2010).

The contaminants are mostly found in water, air and soil. The toxicity of contaminants is defined as toxicity level and measured in ppm (parts per million) or ppb (parts per billion). The toxicity level for mercury in water is 0.002 ppm and that for arsenic in soil is 10 ppm. The contaminants are present in the environment in mixtures as well as in very low concentration. Therefore, it is necessary to develop some technique that could detect the contaminants present even in low concentrations. The answer to this alarming question is nanotechnology. "Nano" is derived from a Greek word meaning dwarf. One billionth of a meter (10^{-9}) represented by length of 10 "H" atoms lined up in a row makes a nanometer. Naturally, nanotechnology had emerged a billion of years ago from the point where the molecules began to arrange themselves in form of a complex structure. Various tools for measuring nanotechnology are scanning tunneling microscope (STM), atomic force microscope (AFM), molecular beam epitaxy (MBE) and scanning probe microscopes (SPMs) (Roco et al. 1999). Nanotechnology has an ability to control the matter at nanoscale and thus creates some materials with specific properties and functions. Nanotechnology is the advanced technology which uses materials with dimensions between 1 and 100 nm and produces the devices using these nanomaterials (Guzmán et al. 2006). The small size and larger surface area to volume ratio of nanoparticles help in developing sensitive, accurate and miniature devices (nanosensors) for the monitoring of pollution levels in the environment (Formoso et al. 2016). Moreover, using nanotechnology, the harmful pollutants could be degraded into the less toxic form and also reduce the amount of pollutants by minimizing the quantity of material used in the manufacturing processes. Therefore, nanotechnology not only helps in the detection of pollutants but also in their treatment.

Nanotechnology helps in lowering down the pollutant generation by applying industrial processes and material technology. Therefore, it has three major applications in the field of environment:

- Pollution detection (sensing and detection)
- Prevention of pollution
- Purification and remediation of contamination

9.2 Detection of Contaminants Using Nanotechnology

For the efficient treatment of pollutants, it is very necessary to develop the detection methods which will help in the fast and precise detection. The sensors need to be portable and remote so that detection could be carried over large field areas. Sensor

is a device that produces a digital electronic signal upon interaction with the compound (biological or chemical) that has to be detected. Using the conventional sensors, the pollutants in biological samples, soil, water, air, chemical substances and industrial products can be detected in low levels up to ppm and ppb. These detection levels could be improved using nanoparticles for the development of sensors. The developed sensors using nanoparticles are highly selective and accurate. They help in the detection of heavy metals, microbial pathogens, organic compounds etc. even at very low concentration (Formoso et al. 2016). With the increased surface area to volume ratio, the reactivity increases and thus its sensitivity. Moreover, the small size also increases the number of reactive sites on the sensor which help in the detection of multiple compounds (multiplex sensors).

Nanosensors in the form of nanowires and nanotubes have been developed for environmental monitoring. Single-walled carbon nanotubes (SWCNTs) have been used for the detection of NH_3 and NO_2 which prove to give faster response as compared to solid-state sensors (Kong et al. 2000). Moreover, the SWCNTs can be operated at room temperature, whereas solid-state sensors are operated at 200–600 °C. This enhanced reactivity is based on the fact that gaseous atoms bind directly to the SWCNTs resulting in increase or decrease of electrically generated signal. In addition to SWCNTs, boron-doped silicon nanowires have been used for the detection of glucose in water (Shao et al. 2005), calcium, antibodies and proteins (Cui et al. 2001; Patolsky and Lieber 2005). The real-time sensing using nanowires is used for the detection of pathogens and biological and chemical agents in food, water, and air.

9.3 Treating Pollutants Using Nanotechnology

The contamination of places in the vicinity of manufacturing plants such as abandoned mines, lakes, rivers, underground storage leakages and landfills is a great matter of concern. The contaminants in these areas could be organic compounds such as creosote, chlorinated solvents, benzene etc. and heavy metals like arsenic, lead, mercury and cadmium. The conventional methods used for the waste treatment are expensive, time-consuming and laborious. Therefore, nanotechnology can be used to develop cost-effective and specific methods. The treatment technologies involve the pretreatment and removal of contaminated site, i.e., pump and treat method. This method disturbs the ecosystem as contaminants are removed from the site and carried to a different site for the treatment purposes (Filipponi and Sutherland 2007). This problem could also be solved using nanotechnology as it can help in the development of in situ treatment technologies which are specific and efficient for a particular pollutant. The organic dyes (indigo carmine and methylene blue) can also be removed from the wastewater using nanocomposites made from surface-functionalized TiO_2 nanoparticles, CNT (carbon nanotubes), and PAN (polyacrylonitrile) (Mohamed et al. 2016).

9.3.1 Nano-traps (Nanofilters and Nanomembranes)

For the treatment of wastewater and waste air, nanomaterials such as nanomembranes, nano-adsorbents, and nanofilters have also been developed (Filipponi and Sutherland 2007). Like nanoparticles, these nanomaterials have properties such as large surface area, high surface-volume ratio and specificity. These are also known as nano-traps. One such membrane was developed by Rice's CBEN (Center for Biological and Environmental Nanotechnology) for the treatment of wastewater, i.e., ferroxane membrane (iron oxide ceramic membrane) (Cortalezzi et al. 2003, 2005). Researchers from the University of Tennessee have been trying to develop a nano-trap that will not only specifically detect the contaminant but also convert it into nontoxic form upon reaction with it. One other advancement in the use of nanomembranes is developed by UCLA (University of California, Los Angeles) which is a reverse osmosis (RO) membrane and is used for the treatment of wastewater and desalinization of seawater. The membrane allows the inward movement of water molecules and the repulsion of contaminants (organic matter and bacteria). This semipermeable property of the membrane is due to the design of engineered nanoparticles and cross-linked matrix of polymers. This design results in the formation of nano-sized holes which act as tunnels for the entry of water. These membranes also serve the advantage of increased shelf life as these are not prone to clogging as in conventional RO membranes (Shon et al. 2013). Heavy metals such as Cu (II), Ni (II) and Pb (II) could be easily adsorbed on the surface of carbonaceous nanofibers (CNFs) with Pb (II) showing the highest affinity for CNFs (Ding et al. 2016). Similarly radionuclides [Eu (III) and U (VI)] could also be removed from the environment using CNFs. The advantage of CNFs is that they are readily available and cheap source (Sun et al. 2016).

9.3.2 Nanoparticles

9.3.2.1 Iron Nanoparticles

The most suited example of the treatment of the environment using nanotechnology is the use of iron nanoparticles (Fe^0) for the remediation of soil and groundwater (Zhang 2003). Iron is the nontoxic compound present in soil, rocks and water; therefore, it is used by many industries as "iron powders" for the treatment of their fresh industrial wastes. But this iron powder cannot be used for the treatment of waste that has been previously dumped into the soil or water. In addition, the reduction using iron powders is partial, i.e., TCE (trichloroethylene) and PCE (perchloroethylene) are partially reduced to DCE (dichloroethylene). The products of partial reduction are much toxic than the parent compounds. Further, the iron powders could be used only for a limited period of time due to the formation of passivation layers on their surfaces. Therefore, the technology has shifted from the use of iron powders to iron nanoparticles. The nanoparticles (1–100 nm) are 10–1000 times effective as compared to iron powders. They are easily transported with the groundwater as they have small size and larger surface area. Further, the

nanoparticles serve the advantage that they do not change with the change in nutrient availability, pH, or temperature of soil. Thus, they remain in suspension creating an in situ treatment zone. Both the *in vitro* and in vivo studies show that the iron nanoparticles are able to degrade completely a number of contaminants such as PCBs, organochlorine pesticides and chlorinated organic solvents. When compared with the iron powders, the nanoparticles were able to degrade the contaminants completely without the formation of toxic by-products due to higher stability and reactivity of iron nanoparticles (Wang and Zhang 1997). The nanoparticle suspension is prepared and injected to the contaminated site. The concentration of nanoparticles at the injection site remains for 6–8 weeks and then slowly gets eliminated into the groundwater, whereas the concentration of contaminants is nearly eliminated within few days (Zhang 2003).

In addition to the zerovalent iron nanoparticles, the bivalent iron nanoparticles (Fe-Pd) have also been used for the bioremediation (Elliott and Zhang 2001). They have proved to be much efficient than zerovalent iron nanoparticles as they could be used for the ex situ wastewater treatment by immobilizing them on silica or activated carbon.

"Pump and Treat" system has been commonly used in the remediation of water (Tratnyek and Johnson 2006). In this system the water is pumped from the soil to the surface, is treated and then injected back to the ground. This method was applicable till 1999. And then another way called permeable reactive barrier (PRB) was devised which was used to clean subsurface groundwater without bringing it to the surface. It can be used to clean pesticides, polychlorinated biphenyls (PCBs), hydrocarbons, aromatic nitro compounds and chromate compounds. In order to overcome the cost of this method, zerovalent iron (ZVI) has been employed. ZVI is of two types:

- Nanoscale ZVI (nZVI)
- Reactive nanoscale iron product (RNIP)

nZVI has 100–200 nm of diameter composed of iron of valency zero, whereas RNIP has 50/50 weight of Fe and Fe_3O_4. ZVI has a higher reactivity toward Cu^{2+}, No^{3-}, chlorinated hydrocarbons and Cr_2^{2-}. The nano-iron is also used directly in soil, solid waste, and sediments, or the nanoparticles are directly attached to the solid matrix like activated carbon for use. Other metals like zinc, palladium, cobalt, copper and gold can also be used in place of iron to reduce the contaminants. Two alloys of iron, i.e., nickel-copper, have also been used to degrade and remove trichloroethane and trichloroethylene (O'Carroll et al. 2013).

The pollution of textile industries due to the release of inorganic salts and water-soluble dyes is also a major problem. nZVI can be used for the degradation of textile dyes and have proven to be much efficient due to their nontoxicity, low cost and higher reactivity toward the contaminant. Moreover their stability could also be increased using various supports such as bentonite, nickel, nickel-montmorillonite, kaolin, rectorite, cellulose and graphene (Raman and Kanmani 2016).

9.3.2.2 Titanium and Zinc Nanoparticles

The ZnO and TiO_2 are the semiconductors and are widely used for the remediation purposes as they are cheap and readily available. When these are used in the nano-size form, the results of the remediation are far more effective as the surface area for the interaction is larger. The main purpose of using ZnO and TiO_2 nanoparticles is the construction of solar photocatalysis remediation systems. In these the contaminants such as chlorinated benzene are converted into benign products by solar radiation. These semiconductors can degrade a number of toxic compounds (Oyama et al. 2002), but still there is a need to increase their efficiency as they absorb only ultraviolet (UV) light which is only 5% of the total solar spectrum. This could be overcome by coating the nanoparticles using inorganic or organic dyes which will shift the absorbance from UV region to visible region (Subramanian et al. 2001). The efficiency of TiO_2 can be enhanced by conjugating them with gold nanoparticles. The ZnO nanoparticles are known to act on the principle of "sense and shoot" as they can detect the chlorinated phenols and also help in their treatment (Kamat et al. 2002). ZnO nanoparticles doped with vanadium could be used for the removal of malachite green dye (Khezami et al. 2016). The bacterial enzymes could also be used for the biosynthesis of TiO_2 nanoparticles and then used for the treatment processes. In one study amylase from *Bacillus amyloliquefaciens* was used for the biosynthesis of TiO_2 nanoparticles and then used for the removal of reactive red 31 (RR31) dye from the contaminated sites (Khan and Fulekar 2016).

9.3.2.3 Magnetic Nanoparticles

The magnetic nanoparticles are the nanoparticles of rust, and they help in purification of water by the removal of arsenic under magnetic effect (Yavuz et al. 2006). The nanoparticles of rust have larger surface area and 10 nm diameter and act as small magnets. This helps in the attachment of arsenic to the nanoparticles which can then be removed with the help of magnets. Thus, the water is treated and becomes arsenic-free. As compared to the conventional techniques (filtration and centrifugation), the use of magnetic nanoparticles serves the advantage of being simple, requires less amount of sample, improves efficiency and requires no electricity (Gu et al. 2003). The arsenic-contaminated water is mostly present in remote areas where power is a major problem. In these areas the best method of treating wastewater is the use of magnetic nanoparticles (Gu et al. 2006). FS@IDA magnetic solid chelator powder composed of Fe_3O_4@SiO_2 nanoparticles coated with iminodiacetic acid can be used for the removal of Cd (84.9%) and Pb (72.2%). The advantage of the use of FS@IDA is easy removal of heavy metals through efficient chelation and simple magnetic separation (Fan et al. 2016).

9.3.2.4 Ferritin Nanoparticles

Ferritin is an iron-containing 24 polypeptide cage-like protein that controls the formation and functioning of mineralized structures and also stores iron in animals and plants (Theil 1987). Ferritin remediates chlorocarbon and toxic metals under the effect of solar radiations and visible light (Moretz 2004). The stability of ferritin

and its nonreactiveness under photoreductive conditions have proved to be advantageous over other ferrous catalysts. An important application of ferritin is that it changes Cr(VI) which is a carcinogenic pollutant to Cr(III) which is less poisonous and insoluble in water (Watlington 2005). Alcohols present in the water could be oxidized using hybrid formed by Pd in nanometallic form and ferrtin isolated from *Pyrococcus furiosus* (Kanbak-Aksu et al. 2012).

9.3.2.5 Polymeric Nanoparticles

Polymeric nanoparticles have been used in the treatment of water. These behave as amphiphilic molecules which have both hydrophobic and hydrophilic character. In the availability of water, a polymer cell with a diameter of several nanometers is formed with hydrophilic part inside and surrounded by the hydrophobic part. In order to stabilize these nanoparticles, cross-linking is done before aggregation. Amphiphilic polyurethane (APU) nanoparticles are used as a remediation agent. The traces of TNT (trinitrotoluene) were detected using electrochemical sensor developed on poly(styrene-co-acrylic acid) PSA/SiO_2/Fe_3O_4/AuNPs/lignin (L-MMS)-modified GCE (glassy carbon electrode). TNT was preconcentrated on the surface of electrode due to the presence of Fe_3O_4/AuNPs and lignin film which resulted into fast response time (3 sec). The electrode serves the advantage of being used repeatedly for five adsorption/desorption cycles (Mahmoud et al. 2015).

9.3.2.6 Bioactive Nanoparticles

Germfree and germ reduction in water or environment is provided by nanotechnology. But the increasing population and pollution has made the situation worse. So, an alternative which is offered is antimicrobial nanotechnology. Strong antimicrobial activity has been shown by various nanomaterials via different mechanisms (Dizaj et al. 2014). Some of which are listed below:

- Fullerol, ZnO, and TiO_2 are used in the production of reactive oxygen species (ROS) by photocatalysis and thus damage the viruses and cells.
- Silver and aqueous fullerene nanoparticles interrupt the energy transduction in the cell.
- Chitosan inhibits enzyme activity and synthesis of DNA.
- Peptides, chitosan, carbon nanotubes, ZnO, carboxy-fyllerene and fullerol interfere with bacterial envelope and disrupt it.

  For the prevention and control of fungal diseases in agriculture, various fungicides such as TBZ (tebuconazole) and carbendazim MBC (methyl-2-benzimidazole carbamate) are used. These can cause harm to the environment and human on leakage. They can be removed from the contaminated sites by the use of polymeric nanocapsules and solid lipid nanoparticles as the fungicides are encapsulated in these nanoparticles with >99% association efficiency (Campos et al. 2015).

9.4 Dendrimers

Dendrimers have nanoscale dimensions, controlled composition, and highly branched polymers used for the removal of metal contaminants (Diallo et al. 2005). They can bind to the appropriate surface and are soluble in nature as they form the cages to trap the zerovalent metals and metal ions. The research is under process to use the dendrimers in ultrafiltration systems as the nano-chelating agents.

9.5 Nanocatalysts

The compound that increases the speed of a reaction without being utilized in the reaction is called catalyst. The reactivity of a catalyst depends on its active site at which the reaction occurs. As the size of the compound decreases to nano level, the reaction surface area increases due to which its efficiency is enhanced (Gemming and Seifert 2007). Nanocatalysts are used for the generation of sulfur-free fuels. During the refining processes of fuels, sulfur remains in the fuel which upon combustion produces sulfuric acid. This could be reduced by treating the fuels with nanocatalysts (nano-sized cerium oxide). The organic dyes such as anionic monoazo dye, cationic phenothiazine dye and cationic fluorescent dye can be degraded efficiently in the presence of gold and silver nanoparticles synthesized using the juice of *Punica granatum* (Kumari and Philip 2015).

9.6 Problems to Be Solved

With the advancements in technology, a number of sensors have been developed for the continuous monitoring of environment. The microorganisms have been used for the development of sensors for environmental monitoring since the past, but their genomic analysis for better understanding has recently come to be known (Feldman and Harris 2000). The microbes could be studied and identified at their genetic levels to develop the efficient sensors for environmental monitoring and treatment. This could be only possible with the help of new advancements in nanotechnology. Further there is little knowledge about the nature, transformation process and chemical composition of the nanoparticles. In addition, it is difficult to identify the nanoparticles in soil, air and water. The conventional methods used for the detection of nanoparticles are nonquantitative, slow and not precise for the data collection. Therefore, the detection methods should be improved so that the nanoparticles can be detected easily and efficiently. Thus, the refinement of the recently used nanotechniques is required for an efficient detection and treatment of environmental contaminants.

9.7 Conclusion

With the globalization, pollution levels are increasing at alarming rates. A number of techniques such as filtration, centrifugation, and chromatography have been used for the monitoring and treatment of pollution levels, but these techniques have many loop holes. Nanotechnology can overcome these gaps and develop new and efficient means of detecting and treating pollution levels. Nanoparticles, nanocatalysts and nanocomposites have been the boons of nanotechnology for healing of the environment. Various nanoparticles such as iron, magnetic, TiO2, ZnO, ferritin and polymeric and bioactive nanoparticles give rapid response and also help in eradication of the pollutants. Some of the gaps such as study and use of microbes at genetic levels for the remediation of environmental resources need to be overcome to get the best results from nanotechnology in the environment.

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Biofertilizers and Biopesticides: Eco-friendly Biological Agents

10

Bhupinder Dhir

Abstract

Chemicals in the form of fertilizers and pesticides have been used in boosting agricultural productivity and crop protection since years. The adverse effects such as environmental toxicity and long residual action resulting from excessive use of these chemicals have prompted the search for nontoxic eco-friendly biological agents. Microbes have emerged as eco-friendly alternate to achieve enhanced plant productivity and protection. Microorganisms colonize rhizosphere/interior of the plant, thereby promoting growth of plants by increasing the availability of essential nutrients such as nitrogen and phosphorus and providing growth regulators. Microbes and their supplements also provide protection against various pests and pathogens. Biofertilizers and biopesticides serve as an eco-friendly substitute to toxic chemicals and form an important component of integrated nutrient management system. Efficiency of both biopesticides and biofertilizers can be increased by molecular approaches. The present chapter highlights the role of biofertilizers and biopesticides in crop improvement and hence achievement of sustainable agriculture.

Keywords

Microbes • Mycorrhizae • Nitrogen • Phosphorus

10.1 Biofertilizers

Biofertilizers are preparations containing strains of microorganisms which are efficient in providing nutrients to plants through rhizospheric interactions. Microbes as biofertilizers improve soil properties, help in expansion of the root

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Table 10.1 Use of various bacteria as fertilizer to improve crop plants

Bacteria	Dose	Crops
Rhizobium	50 to 300 kg N ha^{-1}	Groundnut, soybean, red gram, green gram, black gram, lentil, cowpea, bengal gram, and fodder legumes
Azotobacter	0.026 to 20 kg N ha^{-1}	Cotton, vegetables, mulberry, plantation crop, rice, wheat, barley, ragi, jowar, mustard, safflower, niger, sunflower, tobacco, fruit, spices, condiment, and ornamental flower
Azospirillum	10–20 kg N ha^{-1}	Sugarcane, vegetables, maize, pearl millet, rice, wheat, fodders, oil seeds, fruit, and flower

system, and increase availability of micro- and macronutrients via nitrogen fixation, phosphate and potassium solubilization or mineralization, release of plant growth-regulating substances, production of antibiotics and biodegradation of organic matter in the soil (Arun 2007; Singh and Prasad 2011; Yosefi et al. 2011; Mohammadi and Sohrabi 2012; Kawalekar 2013; Mishra et al. 2013; Raja 2013; Deepak et al. 2014; Patel et al. 2014; Seiber et al. 2014). The use of biofertilizers has proven effective in promoting growth of crop plants such as rice, pulses, millets, cotton, sugarcane, and vegetables crops (Rajasekaran and Sundaramoorthy 2010; Singh et al. 2014) (Table 10.1).

Microbes are applied to seed and plant surfaces (specifically roots) as inoculants (Chen 2006; Khan et al. 2011a, b, c; Mazid et al. 2011a; Raghuwanshi 2012; Gupta and Sen 2013). The supplementation of microbes increases the organic matter of the soil, thereby improving the exchange capacity of nutrients, increasing soil water retention, and buffers the soil against acidity, alkalinity, salinity, pesticides and toxic heavy metals. Longer shelf life (12–24 months), no contamination, easy storage without loss of properties (up to 45 °C), and effective dosage of biofertilizers provide optimum nutrients to plants for their growth and yield (Thakore 2004).

Commercialization of biofertilizers has been promoted by various organizations in different countries. NifTAL (USA) promoted the popularization of *Rhizobium* inoculants. Some of the commercially available biofertilizers include:

1. Rhizonik (*Rhizobium*) – inoculation groups available for legume crops
2. Azonik (*Azotobacter chroococcum*) – nonsymbiotic bacteria used for all cereals
3. Spironik (*Azospirillum brasilense*) – used for grasses and similar type of crops
4. Phosphonive (phosphate-solubilizing inoculant) – bacteria as well as fungi and used for solubilizing P
5. Sulphonik (sulfur-oxidizing inoculant) – bacteria and fungi that enhance the availability of sulfur
6. Phospho-sulphonik – contains equal mixtures of phosphonive and sulphonive
7. Niku-2000 (decomposing culture) – inoculant degrades all cellulolytic and lignolutic organic matter, thereby releasing nutrients to plants
8. Trichonik (*Trichoderma viride*) – restricts the growth of disease-producing organism
9. Vermiculture – mixed with N-fixing inoculant and P solubilizers so that nutrient value is increased

Table 10.2 Biofertilizer use in various countries for improving crop plants

Country	Biofertilizer	Crop	References
Bangladesh	*Bradyrhizobium*	Soybean	
India	*Azospirillum*	*Stevia rebaudiana*	Das and Dang (2010)
	Vesicular arbuscular mycorrhiza (VAM)		
	Phosphorus-solubilizing bacteria (PSB)		
	Azotobacter and PSB 25	*Brassica campestris*	Mondal et al. (2015)
Vietnam	*Rhizobium*	Peanut 1	Nguyen (2006)
Mexico	*Azospirillum* sp.	Corn seed	Caballero-Mellado et al. (1992)
Iran	*Azotobacter* and *Azospirillum*	Canola 21	Yasari and Patwardhan (2007)
	Azotobacter 25	Black cumin	Valadabadi and Farahani (2011)
Turkey	*Azospirillum brasilense* sp. 246	Wheat	Ozturk et al. (2003)
		Barley	
Colombia	*Azospirillum brasilense*, *A. amazonense*	Rice	Moreno-Sarmiento et al. (2007)
	Azotobacter	Cotton	
Egypt	*Rhizobium* (Rh)	Sweet fennel	Zaki et al. (2010)
	Bacillus megaterium (BM3)75		Gharib et al. (2008)
	Azospirillum	Snap bean	Shaheen et al. (2007)
	Azotobacter		
	Azospirillum sp.		
	Bacillus sp.	Flax	Naseriad et al. (2011)
	50 *Azotobacter*	Maize	
	Azospirillum		
Kenya	*Rhizobia*	Soybean	Majengo et al. (2011)
Thailand	*Bacillus cereus strain RS87*	Rice	Jetiyanon and Plianbangchang (2011)
Pakistan	*Bacillus mucilaginous*	Maize	Jilani et al. (2007)
	Azotobacter		
	Azospirillum		
Colombia	*Azospirillum brasilense*	Cotton, rice	Moreno-Sarmiento et al. (2007)
	A. amazonense		
	Azotobacter		

Besides several positive aspects of biofertilizers, their application in agriculture is restricted because of the variable response of plant species or genotypes to inoculation depending on the bacterial strain used. The success of a bacterial strain usage depends upon the saprophytic competence and competitive ability (Khan and Naeem 2011; Mazid et al. 2012a) (Table 10.2).

10.2 Types of Biofertilizers

10.2.1 Nitrogen Fixers

Many bacterial species are symbiotic in nature and fix atmospheric nitrogen. These mainly include *Rhizobium* (*Rhizobiaceae*) which fix nitrogen in legumes at 50–100 kg ha^{-1}. They colonize the roots of legumes to form tumor-like growths called root nodules. It is useful for pulse legumes and forage legumes. Inoculation of *Rhizobium* significantly increases the yields in crop plants such as pulses; legumes, viz., chickpea, red gram, bengal gram, lentil and black gram; oilseed plants like pea, lentil, soybean, and groundnut; and vegetables such as pea, alfalfa and sugar beet (Ramchandran et al. 2011; Sharma et al. 2011). The yield enhancement was noted as increased number of pods $plant^{-1}$ and number of seed pod^{-1} and 1000 seed weight (g).

Azotobacter (*Azotobacteraceae*) is a genus of nonsymbiotic, aerobic, free-living heterotrophic N-fixing bacteria. The bacterium colonizes the roots and fixes N at level 25 kg ha^{-1}. The N fixation increases the yield (up to 50%). *Azotobacter vinelandii*, *A. beijerinckii*, *A. insignis*, *A. nigricans*, *A. armeniacus*, *A. paspali*, *A. chroococcum* and *A. macrocytogenes* are the species commonly used as biofertilizers. The strain also produces antifungal antibiotics that inhibit the growth of several pathogens present in the root region and prevent seedling mortality. They improve seed germination and plant growth by producing vitamins such as thiamine and riboflavin and plant hormones such as indole acetic acid (IAA), naphthylacetic acid (NAA), gibberellins (GA) and cytokinins (CK) (Mazid et al. 2011a, b). Improvement in cereal and millet crops such as rice, wheat, sorghum, maize, pearl, millet, cotton, sesame, vegetables, cotton and sugarcane has been achieved using *Azotobacter* (Mazid et al. 2012a; Khan et al. 2012a; Sahoo et al. 2013a; Wani et al. 2013).

Azolla (*Cyanobacteria* or Blue-green algae) are phototrophic bacteria and are used as green manure. Reports suggest that one kg of *Azolla* fixes about 40–55 kg N ha^{-1}, 15–20 P ha^{-1}, and 20–25 kg K ha^{-1} (Sahu et al. 2012). It also produces phytohormones such as auxin, indole acetic acid and gibberellic acid. Blue-green alga (BGA) assists in biological nitrogen fixation (BNF) (Chianu et al. 2011; Olivares et al. 2013; Santi et al. 2013). Application of *Azolla* improves the physical and chemical properties of the soil such as N, organic matter, and cations such as Mg, Ca and Na (Carrapico et al. 2000; Bhuvaneshwari and Kumar 2013). The paddy crop has shown an increase in yield (21–34%) after application of *Azolla* as a biofertilizer. The number of pods $plant^{-1}$, number of seed pod^{-1}, and 1000 seed weight increased after application of *Azolla* (Yadav et al. 2014).

Azospirillum (*Spirilaceae*) are heterotrophic microbes. They are associative in nature and possess N-fixing ability (20–40 kg ha^{-1}). Inoculations of *A. amazonense, A. halopraeferens* and *A. brasilense* have been proven beneficial to crop plants via improvement in leaf area index and yield attributes. Inoculation with *Azospirillum* changes the root morphology by producing plant growth-regulating substances via siderophore production. It also increases the number of lateral roots and enhances

root hair formation to provide more root surface area for absorption of sufficient nutrients (Saikia et al. 2013). This improves the water status of the plant and aids the nutrient profile in the advancement of plant growth and development. High yield of maize, sugarcane, sorghum (*Sorghum bicolor* L.), pearl millet etc. has been reported after use of *Azospirillum*.

Herbaspirillum is a symbiont and N-fixing bacteria. It enhances the availability of nutrients such as N, K and P and production of growth-promoting hormones (kinetin, gibberellic acid, and auxin) (Khan et al. 2011a, b, c). The N fixation capacity of 15 kg ha^{-1} $year^{-1}$ has been reported. The synthesis of phytohormones such as IAA triggered by this biofertilizer enhances germination and root development which assist in the absorption of plant nutrients. Other nitrogen-fixing cyanobacteria used as biofertilizers include *Aulosira*, *Tolypothrix*, *Scytonema*, *Nostoc*, *Anabaena*, and *Plectonema*.

10.2.2 Phosphate Solubilizers

Bacterial species (both aerobic and anaerobic) possess the capacity to solubilize insoluble inorganic phosphate compounds, such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite, and rock phosphate. The organic acids produced by these strains convert the insoluble phosphorous compounds such as tricalcium phosphate to di- and monobasic phosphates that can be easily taken up by the plant. The phosphate-solubilizing bacteria include *Pseudomonas*, *Bacillus*, *Rhizobium*, *Burkholderia*, *Achromobacter*, *Agrobacterium*, *Micrococcus*, *Aereobacter*, *Flavobacterium* and *Erwinia* (Karpagam and Nagalakshmi 2014; Rowchoudhury et al. 2015).

10.2.2.1 Zinc Solubilizers

The microorganisms such as *Bacillus subtilis*, *Thiobacillus thiooxidans*, and *Saccharomyces* sp. assist in the solubilization of Zn from the compounds like zinc oxide (ZnO), zinc carbonate ($ZnCO_3$) and zinc sulfide (ZnS) (Mishra et al. 2013).

10.2.2.2 K-Solubilizing Bacteria

Bacteria such as *Frateuria aurantia* have shown capacity to solubilize K into a usable form. Potassium-solubilizing microorganisms (KSM) include *Aspergillus*, *Bacillus*, *Clostridium*, *Azotobacter*, *Azospirillum*, *Phosphobacteria* and *Rhizobacteria*. Co-inoculation of *Azospirillum brasilense* and *Rhizobium meliloti* produces a positive effect on grain yield and N, P and K content in *Triticum aestivum* (Mohammadi and Sohrabi 2012; Mazid and Khan 2014).

10.2.2.3 Silicate-Solubilizing Bacteria (SSB)

Microbes degrade aluminum silicates via organic acids produced by them. Hydrogen ions supplied by organic acids promote hydrolysis. These mainly include *Bacillus globisporus* Q12 and *Bacillus* sp. (Sheng et al. 2008; Kalaiselvi and Anthoniraj 2009; Ghouse et al. 2015).

10.2.3 Plant Growth-Promoting Rhizobacteria (PGPR)

Rhizospheric bacteria that exert a beneficial effect on plant growth are referred as PGPRs. These include *Actinoplanes*, *Agrobacterium*, *Alcaligenes*, *Amorphosporangium*, *Arthrobacter*, *Azotobacter*, *Bacillus*, *Cellulomonas*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Pseudomonas*, *Rhizobium*, *Bradyrhizobium*, *Streptomyces* and *Xanthomonas*. These inoculants promote growth via different mechanisms which include suppression of plant disease (bioprotectants), improved nutrient acquisition (biofertilizers), or phytohormone production (biostimulants). The growth hormones such as indole acetic acid, cytokinins, and gibberellins produced by PGPR strains act as biostimulants and promote growth by increasing the absorptive surface for uptake of water and nutrients (Adesemoye et al. 2009; Gholami et al. 2009).

10.2.4 Vesicular Arbuscular Mycorrhizae (VAM)

The symbiotic association between plant roots and fungal mycelia is termed as mycorrhiza. VAM fungi infect the plant primarily through roots. Hyphae absorb nutrients such as phosphate and transfer it to internal cortical root cells. The fungal partner is benefited by obtaining its carbon requirements from the photosynthates of the host, and the host in turn is benefited by obtaining nutrients especially P, Ca, Cu and Zn. They possess special structures known as vesicles and arbuscules. The arbuscules help in the transfer of nutrients from the fungus to the root system, and the vesicles (saclike structures) store P as phospholipids. The organic acids create acidic conditions that facilitate mineralization of the nutrients (Smith et al. 2011). The phytohormones such as indole acetic acid (IAA), gibberellins (GA) and cytokinins (CK) produced by them improve photosynthesis performance, confer tolerance to stress and increase resistance to pathogens. Seed inoculation with fungal species of *Glomus mosseae* mycorrhiza improved corn yield via increased plant height, leaf width, number of grains per ear, and 100 seed weight.

10.3 Application of Biofertilizers and Crop Improvement

Biofertilizers are cheaper than chemical fertilizers (cost-benefit ratio of more than 1:10) (Tiwari et al. 2004), and their use results in fewer nutrient losses, economic savings and environmental protection. The effect of biofertilizers on crop plants depends upon several factors such as crop genotype, the microbial strain, and environmental conditions (soil and weather) (Şahin et al. 2004; Cakmakci et al. 2006; Dhanasekar and Dhandapani 2012). Treatment with biofertilizers particularly N fixers, PGPR, co-inoculants of PGPR and AMF increases the crop yield and productivity by enhanced nutrient use efficiency (Bhardwaj et al. 2014).

The positive effect of combined treatments of *Azolla* and BGA on rice yield has been reported (Askary et al. 2009). The application of BGA+ *Azospirillum*

improved leaf area index (LAI) and all yield attributes in rice. Pusa Basmati 1 showed good yield with the application of four amendments (*Azolla*, BGA, vermicompost, and FYM). Use of BNF enhanced soybean (2000 kg ha^{-1}) and other crop yields (4000–6000 kg ha^{-1}) in Brazil, Argentina and Zimbabwe (Hungria et al. 2010; Yadav et al. 2014). Vegetables like cauliflower, broccoli, cabbage, and carrot also recorded high productivity with the use of biofertilizers (Naderifar and Daneshian 2012). Increase in maize yield has been achieved with the application of half the recommended N rate and biofertilizer, i.e., *Azospirillum*, in Egypt. The application of P fertilizers in combination with biofertilizers increased soybean yields by ≈47% (Woomer et al. 2014). Inoculation of *Rhizobium* with crop plants reduced the need for N fertilizers leading to cost saving of US$ 3 billion per cropping season in Brazil (Nicolas et al. 2006).

10.4 Role of Biofertilizers in Curtailing Stress

Mycorrhizae and other biofertilizers benefit plants exposed to drought and saline conditions. AM fungi along with N_2-fixing bacteria such as *Pseudomonas putida* or *Bacillus megaterium* have shown potential in combating drought stress in legume plants. In Sudan, inoculation of *Rhizobia* improved yield of alfalfa, fenugreek, cluster bean, field pea and common bean grown under drought conditions (Hussain et al. 2002; Abdelgani et al. 2003). Photosynthetic efficiency and the antioxidative capacity noted an increase after inoculation of arbuscular mycorrhiza in rice plants subjected to drought stress. Inoculation of PGPR alone or along with AM-like *Glomus intraradices* and *G. mosseae* resulted in the better nutrient uptake and physiological processes. *A. brasilense* and AM combination improved plant tolerance to various abiotic stresses (Aroca et al. 2013). *Pseudomonas inoculation* improved the seedling growth and seed germination in *A. officinalis* L. under water stress. *Rhizobium trifolii* inoculated with *Trifolium alexandrinum* showed higher biomass and increased nodulation under salinity stress (Yang et al. 2009). *P. fluorescens* MSP-393 assisted in producing osmolytes and proteins that help plants in overcoming the negative effects of salt stress. *P. putida* Rs-198 enhance germination rate and other growth parameters such as plant height, fresh weight, and dry weight of cotton under alkaline and high-salt conditions via increasing the rate of uptake of K^+, Mg^{2+}, and Ca^{2+} and decreasing the absorption of Na^+. *Pseudomonas* strains conferred tolerance in plants via 2,4-diacetylphloroglucinol (DAPG). Calcisol produced by PGPRs, viz., *Pseudomonas alcaligenes* PsA15, *Bacillus polymyxa BcP26* and *Mycobacterium phlei* MbP18, provides tolerance to high temperatures and salinity stress. A root endophytic fungus *Piriformospora indica* was found to defend plants against salt stress (Ilyas et al. 2012).

Application of biofertilizers provides protection to plants against disease and pathogens (Gao et al. 2012; Youssef and Eissa 2014). *Rhizobium* control seed-borne fungal infection of *Colletotrichum*, *Ascochyta*, and *Helminthosporium* in legume seeds. PGPR strains are found to be effective in managing the spotted wilt viruses and cucumber mosaic virus in tomato and pepper and bunchy top virus in banana.

B. amyloliquefaciens 937b and *B. pumilus* SE-34 provide immunity against tomato mottle virus, *B. megaterium* IISRBP 17 acts against *Phytophthora capsici*, *Bacillus subtilis* N11 was found effective against *Fusarium* infestation, *B. subtilis* (UFLA285) was found to provide resistance against *R. solani* in cotton plants, *Paenibacillus polymyxa* SQR- 21 used for the biocontrol of *Fusarium* wilt in watermelon, and *Glomus mosseae* was effective against *Fusarium oxysporum* sp. causing root rot disease in basil plants (Zhang et al. 2011). *Medicago truncatula* also showed induction of various defense-related genes with mycorrhizal colonization. Addition of arbuscular mycorrhizal fungi and *Pseudomonas fluorescens* to the soil reduce development of root rot disease in *Phaseolus vulgaris* L. (Aravind et al. 2009; Joe et al. 2009; Kohler and Caravaca 2010;Yao et al. 2010).

10.5 Molecular Approaches

Genes responsible for synthesis of certain factors/proteins involved in the functioning of biofertilizers such as mycorrhizae have been identified and expressed to develop symbiotic association with plants and achieve improvement in plant growth. Genome sequencing of two EM fungi (ectomycorrhizae), *L. bicolor* 13 and *T. melanosporum* (black truffle), provides means for identification of factors that regulate the development of mycorrhiza and its function in the plant cell. Genes upregulated during symbiosis were identified as putative hexose transporters in *L. bicolor*. The upregulation of transporter genes during symbiosis indicated the transport of useful compounds like amino acids, oligopeptides and polyamines through the symbiotic interface from one organism to other. Cysteine-rich proteins of fungus play an important role as effectors and facilitators in the formation of symbiotic interfaces. Genes related to auxin biosynthesis and root morphogenesis show upregulation during mycorrhizal colonization. PGPR produce IAA which induces the production of nitric oxide (NO), which acts as a second messenger to trigger complex signaling network leading to improved root growth and developmental processes. Expression of ENOD11 and defense-related genes and root remodeling genes are upregulated, and genes including subtilisin protease, phosphate transporter, or two ABC transporters involved in arbuscule formation are also overexpressed. Sugarcane plantlet inoculated with a wild strain of *G. diazotrophicus* shows N fixation, while *G. diazotrophicus* mutant, i.e., without nif D gene, lacked N fixation fixing capacity.

Many disease resistance genes such as jasmonate/ethylene signaling as well as osmotic regulation via proline synthesis genes are differentially expressed in microbes such as *B. subtilis* (UFLA285). Metallothionein-like protein type 1, a NOD26-like membrane integral protein, ZmNIP2–1, a thionin family protein, an oryzain gamma chain precursor, stress-associated protein 1 (OsISAP1), probenazole-inducible protein PBZ1, and auxin and ethylene-responsive genes are expressed and identified. Gene encoding glucose dehydrogenase (gcd) involved in the direct oxidation pathway was cloned and characterized from *Acinetobacter calcoaceticus*, *E. coli*, and *Enterobacter asburiae*.

Constitutive expression of certain proteins such as HetR driven by gene hetR gene improves nitrogenase activity in *Anabaena* sp. strain PCC7120. *G. versiforme* possesses inorganic phosphate (Pi) transporters on the hyphae that help in the direct absorption of phosphate from the soil**.** Bioactive compounds called Myc factors similar to Nod factors of *Rhizobium* are suggested to be secreted by mycorrhiza and *Rhizobium* and perceived by host roots for the activation of signal transduction pathway or common symbiosis (SYM) pathway (Roberts et al. 2013). The common SYM pathway prepares the host plant to bring about changes at molecular and anatomical level with the contact of fungal hyphae. Calcium acts as secondary messengers via Ca^{2+} spiking in the nuclear region of root hairs. PGPR produce IAA which, in turn, induces the production of nitric oxide (NO), which acts as a second messenger to trigger a complex signaling network leading to improved root growth and developmental processes (Molina-Favero et al. 2007).

10.6 Biopesticides

Biopesticides encompass a broad array of microbes and biochemicals derived from microorganisms that confer protection against pest damage (Gupta and Dikshit 2010). Microbes are formulated in solid carriers like talc, peat, lignite, clay etc., while liquid formulations are prepared in solvents such as water, oil, and organic solvents. Solid formulations have shorter shelf life, susceptibility to environmental conditions, high contamination and low field performance, while liquid formulations offer longer shelf life (up to 2 years), with high purity, carrier-free activity, ease in handling and application (Mazid et al. 2011c, d). They include nutrients, cell protectants, and inducers responsible for cell/spore/cyst formation leading to improved efficacy. The microbes are present in dormant cyst form which gives rise to active cells upon application in the field, and this helps increase its shelf life for more than one year. Liquid formulations showed their efficacy against insect and nematode pests (Rao et al. 2015). They possess high selectivity to target pests, safety to humans and nontarget organisms, amenability to individual applications, integrated pest management, and suitability for organic niche products in contrast to chemical pesticides that possess broad spectrum and affect nontarget organisms including predators, parasites, as well as humans. Biopesticides mainly include fungus used in weed control, bacteria controlling fungal and bacterial diseases, and viruses active against insect pests.

Liquid formulation of pesticides shows high efficacy for longer period of time. *Pseudomonas fluorescens* formulation in glycerol (10 mM) shows high efficacy of pest destruction for longer duration (6 months). Addition of glycerol increased the stability of the liquid formulations of *Bacillus thuringiensis* (*Bt*) used against *Helicoverpa armigera*. Liquid formulations of *Pochonia* (*Verticillium*) *lecanii* in glycerol, Tween 80, and arachnid oil effectively reduced mealy bug (*Maconellicoccus hirsutus*) infection in grapes. Seed treatment with liquid formulation reduced the disease incidence of *Fusarium* (wilt) in tomato and increased fruit yield. Bentonite oil-based liquid formulations (bentonite, corn oil, gum,

glycerin) control fungus in *Beauveria bassiana*. Spray application of the bacterial suspensions of *Agrobacterium radiobacter* and *Bacillus sphaericus* isolates cause significant reduction (24–41%) in root infection of potato caused by cyst nematodes, *Globodera pallida*. Emulsion (water in oil) of *M. anisopliae* showed efficacy against whiteflies, *Bemisia tabaci*, red spider mites and *Tetranychus cinnabarinus* in eggplants. Liquid formulations in vitro produced endospores of bacterial bioagent, *Pasteuria penetrans*, that suppressed the host nematode, *Belonolaimus longicaudatus*, more effectively (59–63%). Liquid biopesticides of *B. subtilis*, *Pseudomonas putida*, *Pseudomonas fluorescens*, *Paecilomyces lilacinus*, *Trichoderma viride* and *Trichoderma harzianum* with shelf life more than 12 months proved their efficacy in controlling nematode pests like root-knot nematodes (*Meloidogyne incognita*), reniform nematode (*Rotylenchulus reniformis*), and lesion nematodes (*Radopholus similis*). Liquid formulation of *B. subtilis* (1%) reduced root-knot nematode population to a significant extent.

The total world production of biopesticides is over 3000 tons/year. About 1400 biopesticide products are marketed worldwide, and the global market is expected to reach US$ 3.2 billion by 2014. The biopesticide market in India represents 2.89% of the overall pesticide market and is expected to reach more than US$ 1 billion (Shukla and Shukla 2012).

10.6.1 Categories of Biopesticides

Biopesticides have been broadly categorized as follows.

10.6.1.1 Microbial Pesticides

They consist of a naturally occurring or genetically modified microorganisms (e.g., a bacterium, fungus, virus, or protozoan). They are effective against kinds of pests, though each microbe is relatively specific for its target pest.

Bacteria Bacterial biopesticides are cheaper and the most widely used biopesticides. Bacteria belonging to the genus *Bacillus* are most widely used pesticides. The most commonly known microbial pesticides are bacterium *Bacillus thuringiensis*, or Bt. This bacterium produces protein crystals that are harmful to specific insect pest. Several strains of Bt have been developed and applied to plant foliage. The ingestion of the protein crystals by insects paralyzes their digestive tracts, killing them within 24–48 h. Different Bt strains produce crystals specific for each insect or small number of related insect species. It has been successfully used in controlling caterpillars/larvae moths, mosquitoes and black flies feeding on cabbage, potatoes and other crops. The commercial preparations of *B. thuringiensis* contain a mixture of spores, *cry* protein and an inert carrier. Bt is the first commercially used biopesticide throughout the world. Control of diamond-back moths, *Helicoverpa* and *Trichoplausia ni*, on cotton, pigeon pea and tomato is controlled by *Bacillus thuringiensis*. Till date, over hundred *B. thuringiensis*-based bioinsecticides have been developed and used against lepidopteran, dipteran, and

coleopteran larvae. The *cry* genes coding for the insecticidal crystal proteins have been successfully transferred into different crop plants. *B. thuringiensis* and *cry* proteins are efficient, safe, and sustainable alternatives to chemical pesticides for the control of insect pests. These proteins form the pores or ion channels in the membrane that lead to osmotic cell lysis. In addition, *cry* toxin monomers promote cell death in insect cells through a mechanism involving an adenylyl cyclase/PKA signaling pathway (Zhang et al. 2006).

Bacillus subtilis, a Gram-positive rod-shaped bacterium, produces endospore. It produces antimicrobial metabolites that target bacterial and fungal soil inhabitants including plant pathogens. It promotes growth of plants by production of phytohormones, sequestration of nutrients, stimulation of systemic induced resistance in plants and suppression of plant pathogen activities. Antimicrobial metabolites from *B. subtilis* have been employed in biocontrol and plant protection by exploiting their antibiosis activity on phytopathogens (Romero et al. 2007).

Fungi Fungi also control insect pests. Entomopathogenic fungi are regulators of insect populations and have potential as mycoinsecticide agents against diverse insect pests in agriculture. They invade their hosts by penetrating through the cuticle, gaining access to the hemolymph and producing toxins, and hence are used for the control of pests with piercing mouthparts such as aphids and whiteflies. *Trichoderma*, a fungicide has been found effective against soilborne diseases such as root rot and wilts in crops such as groundnut, black gram, green gram and chickpea (Nargund et al. 2007). Insect-pathogenic fungus *Metarhizium anisopliae* has been used against adult *Aedes aegypti* and *Aedes albopictus* mosquitoes. Fungal isolates of *Beauveria bassiana*, *Metarhizium anisopliae* and *Paecilomyces fumosoroseus* showed lethal effects on the eggs of the carmine spider mite, *Tetranychus cinnabarinus*. The ovicidal activity of the fungal species acts as biocontrol against spider mites such as *T. cinnabarinus*. Fungi *Beauveria bassiana* SG8702 and *Paecilomyces fumosoroseus* Pfr153 showed efficacy for control of *T. cinnabarinus* eggs. The potato psyllid has been controlled by *Bactericera cockerelli* (Sulc). *Aspergillus fumigatus*, *Alternaria tenuissima*, *Penicillium* spp. and *Fusarium* spp. produce harmful mycotoxins which also aid in pest control. Entomopathogenic fungi act as an alternate to insecticide and vital component of integrated pest management (Mazid et al. 2011b).

The combination of *B. bassiana* suspension and neem gave the highest *B. tabaci* egg and nymph mortalities with lowest LT50 value (Mazid et al. 2011b). The use of the insect-pathogenic fungus *Metarhizium anisopliae* was found effective against adult *Aedes aegypti* and *Aedes albopictus* mosquitoes. Fungal biocontrol agents such as isolates of *Beauveria bassiana*, *Metarhizium anisopliae*, and *Paecilomyces fumosoroseus* showed lethal effects on the eggs of the carmine spider mite, *Tetranychus cinnabarinus*. The fungal species showed the ovicidal activity and suggested the capacity as biocontrol agents against spider mites such as *T. cinnabarinus*. Isolates of entomopathogenic fungi, *Beauveria bassiana* SG8702 and *Paecilomyces fumosoroseus* Pfr153, showed effectivity against *T. cinnabarinus*

eggs. Several other entomopathogenic fungi (*Hypocreales*) have been used for the control of potato psyllid, *Bactericera cockerelli* (Sulc).

Viruses A family of viruses called baculoviruses infect their hosts through ingestion. They are characterized by rod-shaped enveloped virions and a circular dsDNA genome of 80–180 kbp. They control lepidopteran and sawfly forest pests. Virus particles invade the cells of the gut before colonizing the rest of the body. Infection reduces mobility and feeding and insects are killed in five to eight days. The virus kills insect pests by taking over the metabolic processes of the host insect for viral multiplication and transmission. This process involves both an active, replicating virus and the production of variety of enzymes and proteins that lead to enhanced infection and insect death (Hubbard et al. 2014).

Baculoviruses encode a number of proteins and enzymes that enhance their ability to specifically infect and replicate in insects. This increases the efficiency of spread and persistence of viruses within the host insect environment. All baculoviruses encode an occlusion body protein (polyhedrin or granulin) which forms the bulk of the proteinaceous crystal or occlusion bodies (OB) that occludes the virions in the later stages of virus replication. OB protein, polyhedrin, makes up to 30% of infected cell protein and is the powerful promoter element of the gene required for the development of baculoviruses expression system. The virion structure, novel polymerases are required for virus-specific DNA replication, gene transcription, and oral infectivity factors essential for infection of insect midgut cells. Two virion phenotypes, namely, occlusion-derived virions (ODV) and budded virions (BV), occur in baculovirus infections. ODV spread the infection to tissues orally throughout the host and infect midgut epithelial cells after ingestion and dissolution in the alkaline condition of the midgut. Upon ingestion of OB by a host insect larva, the polyhedrin protein is degraded in the alkaline environment of the insect midgut by proteases. The process releases the infectious occluded virions into the midgut lumen. The infection in the midgut epithelial cells of virions occurs via peritrophic matrix (PM), a chitin and glycoprotein layer which lines the midgut lumen. Baculoviruses also encode a family of metalloproteases associated with the OB or are incorporated into the envelope of the occluded virion referred as "viral enhancing factor" or "enhancins." Enhancins increase the oral infectivity of baculoviruses (2- to 15-fold).

Baculoviruses infecting Lepidoptera encode an enzyme referred to as ecdysteroid UDP-glucosyltransferase (EGT). EGT catalyzes the transfer of glucose from UDP glucose to ecdysone, thus inactivating the hormone and blocking the molting process from one larval instar to the next. It blocks the molting process and extends the life span of a baculovirus-infected larva. The development of recombinant baculoviruses with the EGT gene under the control of an inducible promoter allows production of more OB in insects. A gene encoding a protein tyrosine phosphatase has been implicated in the hyperactivity and wandering behavior of infected lepidopteran larvae. Chitinase genes are found in almost all baculoviruses infecting lepidopteran insects (Macedo et al. 2015). Chitinase is responsible for the

digestion of chitin which is the major component of insect exoskeleton. A virus-encoded protease, cathepsin, is expressed late in baculovirus infection. The enzyme protease aids in the breakdown of host cellular structure and eventually the integrity of the infected cadaver, thus maximizing baculovirus OB dispersion. Since CaV channels are not highly conserved in insects, this makes them attractive alternatives and represents a novel mode of action to conventional pesticides. Fusion protein technology, in which insecticidal peptides are linked to a plant lectin "carrier" protein, developed to allow proteins such as spider venom toxins to act as orally delivered biopesticides. ω-Hexatoxin-Hv1a (Hv1a) from the Australian funnel web spider *Hadronyche versuta* acts on CaV channels in the insect central nervous system (CNS), causing paralysis. Fusion of this insecticidal molecule to the carrier protein snowdrop lectin (*Galanthus nivalis* agglutinin, GNA) allows Hv1a to traverse the insect gut epithelium and access its sites of action, producing an orally active insecticidal protein. The Hv1a/GNA fusion protein has oral insecticidal activity against insects from a range of orders, including Lepidoptera, Coleoptera, Diptera and Hemiptera (Macedo et al. 2015).

The baculoviruses are classified into two genera, nucleopolyhedroviruses (NPVs) and granuloviruses (GVs) Erayya et al. (2013). Nuclear polyhedrosis viruses include *Helicoverpa armigera*, *Amsacta moorei*, *Agrotis ipsilon*, *A. segetum*, *Anadividia peponis*, *Trichoplusia orichalcea*, *Adisura atkinsoni*, *Plutella xylostella*, *Corcyra cephalonica*, *Mythimna separata* and *Phthorimaea operculella*.

The first viral insecticide Elcar™ that consists of a preparation of *Heliothis zea* is relatively broad range NPV baculovirus and infects many species belonging to genera *Helicoverpa* and *Heliothis*. HzSNPV is a product of choice for biocontrol of *Helicoverpa armigera*. Another baculovirus, HaSNPV, identical to HzSNPV was registered in China as a pesticide. HaNPV has relatively broad host spectrum and potentially used on a variety of crops infested with pests including *Spodoptera* and *Helicoverpa*. Another baculovirus, HaSNPV, has been used for large-scale biopesticide production. It has been extensively used on cotton fields. HzSNPV provides control of cotton bollworm and other pests attacking crops such a soybean, sorghum, maize, tomato, and beans. GV is the active component of a number of biopesticides used for protection of apple and pear orchards against the codling moth, *Cydia pomonella*. GV-based products are available in the trade names of Granusal™ in Germany, Carpovirusine™ in France, Madex™ and Granupom™ in Switzerland, and Virin-CyAP in Russia. *Autographa californica* and *Anagrapha falcifera* NPVs have a relatively broad host spectrum and potentially used on a variety of crops infested with pests belonging to a number of genera, including *Spodoptera* and *Helicoverpa* (Ranga Rao et al. 2015). Baculovirus *Anticarsia gemmatalis*, a nucleopolyhedrovirus (AgMNPV), is used to control the velvetbean caterpillar in soybean. Two commercial preparations of *Spodoptera* NPV are available in the USA and Europe. Two NPVs have a relatively broad host spectrum and used on a variety of crops infested with pests belonging to a number of genera, including *Spodoptera* and *Helicoverpa*. These include SPOD-X™ containing *Spodoptera exigua* NPV to control insects on vegetable crops and Spodopterin™

containing *Spodoptera littoralis* NPV which protect cotton, corn, and tomatoes (Mazid et al. 2011b).

10.6.1.2 Insects

Trichogramma are minute wasps which are exclusively egg parasites. They lay eggs in the eggs of various lepidopteran pests. After hatching, the *Trichogramma* larvae feed on and destroy the host egg. It is effective against lepidopteran pests like the sugarcane internode borer, pink bollworm, spotted bollworms in cotton, and stem borers in rice. They are also used against vegetable and fruit pests. It kills the pest in the egg stage, ensuring that the parasite is destroyed before preventing damage to the crop. Control of sugarcane borers and rots has been reported by *Trichogramma*. Ladybugs and praying mantis have been successful in combating scale insects or aphids which feed on plant sap. Control of mango hoppers and mealy bugs and coffee pod borer is reported by *Beauveria*. *Entomophthora ignobilis* (a fungus) is effective against green peach aphid of potato (*Myzus persicae*) (Macedo et al. 2015).

10.6.1.3 Plant-Based Pesticides

Pesticides obtained from plants mainly include (1) azadirachtin (*Azadirachta indica*), (2) rotenones obtained from the roots of *Derris elliptica* and *Lonchocarpus nicou*, (3) nicotine, (4) pyrethrum (*Chrysanthemum cinerariifolium*, *C. coccineum*, and *C. marshallii*), and (5) thurioside. Neem products have been effective in controlling a large number of insects which include 350 species of arthropods, 12 species of nematodes, 15 species of fungi, three viruses, two species of snails, and one crustacean species. Neem biopesticides are systemic in nature and provide long-term protection to plants against pests. Azadirachtin, a tetranortriterpenoid, is a major active ingredient isolated from neem and is known to disrupt the metamorphosis of insects. Two tetracyclic triterpenoids, meliantetyraolenone and odoratone, isolated from neem also exhibit insecticidal activity against *Anopheles stephensi*. Neem seed kernel extract (NSKE) has also been found most effective in reducing the larval population of *Helicoverpa armigera* in chickpea. Neem formulations also have a significant effect against eggs of peach fruit fly *Bactrocera zonata* (Saunders). Root extracts of *Tagetes* or *Asparagus* exhibit nematicidal properties, while *Chenopodium* and *Bougainvillea* act as antivirus agents (Isman 2000).

10.7 Biochemical Compounds

These are naturally occurring substances that control pests by nontoxic mechanisms. These include substances such as insect sex pheromones that interfere with mating, as well as plant extracts that attract insect pests to traps. Pheromones are chemicals emitted by living organisms used to send messages to individuals of the opposite sex of the same species. These include plant growth regulators or substances that repel or attract pests and interfere with growth or mating. These

have been found effective against rice cutworm, tobacco caterpillar, rice green leaf hopper and several species of aphids and mites. Mating disruption has been successful in controlling a number of insect pests (Mazid et al. 2011b).

Recombinant fusion proteins containing neuroactive peptides/proteins linked to a "carrier" protein can act as effective pest controls. The non-peptidic analog can be used in the development of novel insecticides overcoming the bioavailability of peptides penetrating the insect cuticle or gut mucosa. Hv1a/GNA (*Galanthus nivalis* agglutinin), containing an insect-specific spider venom Ca channel blocker (ω-hexatoxin-Hv1a) linked to snowdrop lectin (GNA) a "carrier," is an effective oral biopesticide toward various insect pests. Internalized Hv1a/GNA reach the brain within 1 h of exposure. It is unlikely to cause detrimental effects on honeybees, indicating that atracotoxins targeting Ca channels are potential alternatives to conventional pesticides. Pseudophomins A and B produced by *Pseudomonas fluorescens* strain BRG100 are found to be effective as herbicide and pesticide. Pseudophomin A acts as a bioherbicide, while pseudophomin B is an antifungal compound (Pedras et al. 2003).

10.8 Commercially Available Biopesticides

The success stories of application of biopesticides have been reported all over the world. Some of the commercially available biopesticides include BioNEEM, Azatin XL, Nema-Q and Biomite. BioNEEM is a broad-spectrum biocide isolated from kernel of neem seeds. The main component is *Azadirachtin*, a water-soluble emulsifiable concentrate that provides the most effective control of major pests of agricultural and plantation crops. It possesses broad specificity against red spiders and insect pests like helopeltis, aphids, jassids, thrips and caterpillars, etc. The active ingredient inhibits sensory receptors of mouth parts, resulting in disorientation of normal probing, feeding and intake of food by insects and mites. It has very strong repellent and deterrent effects which prevent the insects and mites from colonizing the treated plants.

The successful utilization of biopesticides and biocontrol agents in agriculture includes:

- Use of *Bacillus thuringiensis* for control of diamondback moth and *Helicoverpa* on cotton, pigeon pea, and tomato
- Use of *Beauveria* for control of mango hoppers, mealy bugs, and coffee pod borer
- Use of neem products for control of white fly on cotton
- Use of nuclear polyhedrosis virus (NPV) for control of *Helicoverpa* on gram
- Use of *Trichogramma* for control of sugarcane borers
- Use of *Trichoderma*-based products for control of rots and wilts in various crops (Kalra and Khanuja 2007)

10.9 Molecular Approaches

Several biotechnological and molecular approaches have been followed to enhance the capacity of microbes to achieve maximum pest control properties. These include the following.

10.9.1 Plant-Incorporated Protectants (PIPs)

The genetic modification of plants by expressing genes encoding insecticidal toxins, namely, δ-endotoxins, derived from the soil bacterium *Bacillus thuringiensis* (*Bt* plants). *Bt* endotoxins prove lethal to the alkaline environment of the insect gut. These proteins have provided protection against major pests of cotton, tobacco, tomato, potato, corn, maize and rice. More than 60 Cry proteins have been identified. Most *Bt* maize hybrids express the Cry1Ab, Cry1Ac, Cry9C and Cry3Bb1 protein targeted against the European corn borer (*Ostrinia nubilalis*) and corn rootworm complex (*Diabrotica* spp.), a major pest of maize in North America and Europe. Maize hybrids express the Cry3Bb1 protein, which is targeted against the corn rootworm complex (*Diabrotica* spp.) (Coleoptera), also a major pest of maize, especially in North America. Cotton expressing the Cry1Ac protein is targeted against the cotton bollworm (*Helicoverpa zea* Boddie) (Lepidoptera), which is a major pest of cotton; potato expressing the Cry3A or Cry3C is targeted against the Colorado potato beetle (*Leptinotarsa decemlineata* Say) (Coleoptera), which is a major pest of potato; and Cry4 proteins are targeted against some Diptera, such as certain flies (e.g., *Lycoriella castanescens* Lengersdorf) and mosquitoes (e.g., *Culex pipiens* L.). The expression of these toxins confers protection against insect crop destruction. Potato expressing the Cry3A or Cry3C is targeted against the Colorado potato beetle (*Leptinotarsa decemlineata* Say) (Coleoptera), which is a major pest of potato; and Cry4 proteins are targeted against some Diptera, such as certain flies (*Lycoriella castanescens*) and mosquitoes (e.g., *Culex pipiens* L.) (Saxena et al. 2010).

10.9.2 Other Proteins and Peptides

Macrocidins and *P. macrostoma* enter weed tissues via locations adjacent to root hairs, colonizing intercellularly the vascular trachea, thereby interfering with tissue functionality. *P. macrostoma* also colonizes resistant plants and only in the outer layers of the root. There are two major groups of macrocidins: macrocidin A (Graupner et al. 2003) and macrocidin Z (Bailey et al. 2011). *P. macrostoma* could provide a valuable alternative to control weeds with resistance to carotenoid biosynthesis targeting synthetic herbicides.

The antimicrobial peptides are synthesized as two distinct systems: (1) - non-ribosomal peptide synthesis, e.g., cyclic lipopeptides, iturin, and fengycin families, and (2) ribosomal peptide synthesis, which is further subdivided into

(a) posttranslationally modified, e.g., subtilisin; (b) non-modified, e.g., thurincins; and (c) large heat-labile proteins. Peptide synthetases constitute the non-ribosomal biosynthetic machinery that produces a diverse array of lipopeptides (LPs), such as iturin, fengycin and surfactin families. The LPs are amphiphilic and have cell wall and membrane surface active properties, facilitating the formation of pores in membranes of phytopathogenic bacteria and fungi. Loss of cell membrane integrity causes a disruption in active transport (i.e., nutrient and ion transport) leading to cell death. Surfactins have antibacterial activity, while iturins and fengycins have antifungal properties. Optimum biocontrol activity and colonization of the rhizosphere by *B. subtilis* require coordinated production and synergy of complementary LPs. Surfactin is a signal molecule in the quorum-sensing (QS) system in *B. subtilis*. Surfactin may be involved in initiating biofilm development by subpopulations of the colony and detection of microbial community diversity in the rhizosphere. QS molecules elicit regulation of genes required for microbial survival and act as microbial interspecies communication signals. Subtilisin exhibits broad-spectrum activity toward Gram-positive bacteria by disrupting membrane function.

10.10 Conclusions

Biopesticides and biofertilizers form an important component of integrated pest management (IPM). Biofertilizers increase soil fertility and sustainability by mobilizing macro- and micronutrients or by converting insoluble form to soluble forms available to plants. They stimulate root growth and produce phytohormones that change the root physiology to increase nutrient and water uptake. The advantages such as easy biodegradability and less pesticide residues resulting in less pollution make them a sustainable, environmentally safe pest control agent (Copping and Menn 2000). More research needs to be carried out to overcome the limitations such as narrow target range, specific mode of action, shorter shelf life and limited field persistence and achieve maximum benefit from microbes. Molecular techniques can help in development of modified insecticides which possess enhanced activity against a wide range of pathogens and diseases.

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Approaches to Agro-industrial Solid Waste Disposal and Bioenergy Generation

11

Aneet Kaur

Abstract

Increasing concern about the environment, food and feed shortages, and hike in the price of petroleum has stimulated interest in the new ways of producing more bioenergy. The interest is rapidly increasing toward converting agricultural and industrial wastes to commercially valuable products. Waste disposal and pollution are inextricably linked. Unwanted residues that are usually perceived to be of negative value are described as waste. The production of citrus juice on an industrial level leads to a considerable quantity of solid and liquid residue (8–20 million tons year^{-1}), which is considered as waste. Citrus processing residues possess no economic value. They are rich in soluble sugars, cellulose, hemicellulose, pectin, and essential oils that could form the basis of several industrial processes. Possible applications of these waste residues include fertilizer, cattle feed, charcoal, adsorption of chemical compounds, bioethanol production, and extraction of essential oils and pectin.

The majority of waste disposal situations involve pollution of various kinds. Thus, the solid wastes and its disposal is one of the serious problems in developing countries, which require eco-friendly treatment options. The bioethanol made from citrus waste biomass can offer immediate and sustained greenhouse gas advantages and also solve the problem of its disposal. The study proposes alternatives for the minimization and recovery of solid and liquid residues generated in the production of citrus processing with a view of industrial plants for its reuse and value addition, thus saving environment from its hazards.

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Bioenergy • Bioethanol • Waste disposal • Citrus peel waste

11.1 Introduction

11.1.1 Agro-industrial Waste Generation and Disposal

11.1.1.1 Solid Waste Generation

Rapid population growth and socioeconomic development in the country have increased the generation of waste. There is an estimated increase of 1–1.33% in solid waste generation during the last few decades in India (Beukering 1999). This enormous increase in solid waste generation will have significant impacts in terms of the land required for disposing this waste as well as on methane emissions. The increase in solid waste generation would impose serious threat to the environment. It is evident from the fact that the cumulative requirement of land for disposal of agro-industrial solid in India would amount to around 1400 km by 2047 (Singhal and Pandey 2001). Solid waste generation, urbanization, and increase in population are highly interlinked and affect the process of land acquisition for disposal of waste. Improper disposal of waste practices in the past has created several hazards as well as aesthetically unpleasant sites. According to the Environmental Protection Agency (EPA), in a global level, it is estimated that in a 1990 survey, in cities approximately 1.3 billion metric tons of municipal solid waste was generated, averaging about the average person dumps almost 4.5 pounds of waste into landfills every single day (Beede and Bloom 1995). In terms of composition, the difference between the wastes of high- and low-income countries is considerable (Cointreau et al. 1984). Two problems are foremost in India and in the other developing countries:

- Absence of adequate dumping sites
- Absence of appropriate primary treatment of agro-industrial waste

In addition, the hazardous content in the waste is quite high since the regulatory and enforcement system to control such waste disposal is usually nonexistent or not functional. This is a particular problem with waste from hospitals located within the urban area, often found mixed with other solid municipal wastes which get dumped in open fields resulting in landfills (Fig. 11.1). With the population skyrocketing explosion across the globe, landfill dumps and solid waste will only become more of a public concern as time passes by. Despite the arguments over landfills in general, there are no legislatives available which help to combat the problem, and this actually contributes to the environmental problem of landfills.

Differences in waste management systems thus require distinct approaches to solve it. The nature and contents of waste in the developing countries are highly organic and susceptible to rapid decomposition or decay. Studies have shown that

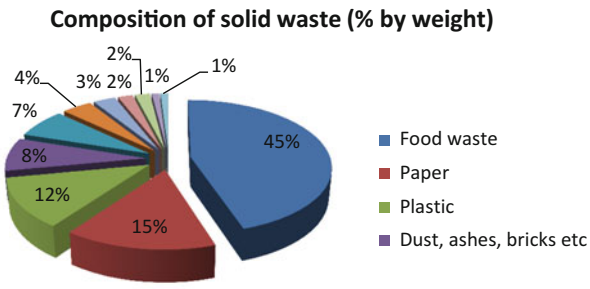

Fig. 11.1 Typical composition of solid waste in Indian cities (Source: Status of solid waste generation, collection, treatment, and disposal in metropolitan cities (Beukering et al. 1999)

expensive collection trucks and compactors developed and used in urban countries are difficult to operate as it requires skilled operators, are difficult to maintain, and are unsuitable for densely populated narrow lane areas (Cointreau and de Kadt 1991).

11.1.2 Global Bioenergy Demand

11.1.2.1 World Scenario

Increasing world energy demands and environmental concerns due to the fossil fuel consumptions motivate considerable efforts toward the development of sustainable and renewable energy resources, such as biofuels. There is a rapid increase in interest toward converting agricultural wastes to commercially valuable products. Biofuels made from waste biomass offer greenhouse gas effect and sustainable environment. The strategic and economic matters associated to the oil economy have promoted new interest for the so-called biofuels, which include bioethanol, biodiesel, and biogas.

Recent hike in oil prices has led to increased interest in biofuels. With the lower price and more advanced state of development, they are drawing the greatest attention globally. It is expected that in the future, technological advances will increase the competitiveness of second-generation biofuels. Currently, many governments worldwide are looking to biofuels as a way of reducing reliance on oil imports and reducing greenhouse gas emissions. The Biofuels Initiative goals of the US Department of Energy include making cellulosic ethanol costs comparable with gasoline and replacing 30% of the current levels of petrol consumption with biofuels by the next decade.

The high price of petroleum and other factors, such as environment degradation and global warming have raised the demand for bioenergy. The most commercially spread biofuel is ethanol. This is the main alternative to fossil fuel. There are many increasing potential benefits on our dependence on renewable sources of energy, such as biofuels. They cause lesser pollution in the environment, thus being potentially more environmentally friendly. The bioenergy generation by taking advantage of biomass conversion or organic waste utilization is one of the future industries. The source plants absorb carbon dioxide from the air as they are

growing, and consequently, the carbon dioxide that is released when biofuels are burned does not represent a net addition of that greenhouse gas to the atmosphere (Cherubini 2010). The main advantage of second generation biofuels are that they offer the best way to reduce greenhouse gas emissions and alternate to the pollutant fossil fuels (Searcy and Flynn 2008; Fleming et al. 2006).

11.1.2.2 Indian Scenario

In 2005, India ranked seventh in the world in terms of energy demand, accounting for 3.4% of the total energy utilized. As the population in the country is growing on a fast pace, the demand is also expected to grow exponentially in the near future. India is also initiating the use of ethanol as an automotive fuel and to produce a cost-effective method of ethanol production. Under the approved National Biofuel Policy, India aims to rise the blending of biofuel with petrol and diesel by 20% by the year 2017.

11.2 Approaches for Production of Bioenergy

11.2.1 Conversion of Solid Waste

Utilization of available solid wastes from existing industries to produce bioenergy is the gaining interest globally. Production of value-added products from agro-industrial and food processing wastes is now a focusing area, as it reduces environmental pollution in addition to energy generation. Low-cost crop and forest residues, wood process wastes, and the organic fraction of municipal solid wastes can all be used as lignocellulosic feedstocks for the generation of bioenergy (Sims et al. 2010). In the recent years, different new substrates and new processes have been employed for the production of ethanol.

Substrates for ethanol production ranging from sugar cane molasses, corn meal, cassava wastewater, and potato flour to banana biomass fruit peels and bread wastes, brewer's spent grain, etc. were utilized by direct fermentations as well as by using pretreatments and enzymatic hydrolysis using various enzymes from microbial sources like cellulases, pectinases, and polygalacturonases that have been used for the transformations.

There is a considerable industrial interest in the enzymatic transformation of flavonoids to hydrolysis products that offer a pathway to bioenergy generation. Hydrolysis of flavonoids by specific enzymatic action liberates important sugars from the citrus wastes. Ethanol is considered a valuable biofuel and is a renewable energy resource. From the application and industrial point of view, bioethanol production from citrus waste has been very beneficial to the society and has a lot of economic potential (Grohmann et al. 1994).

Many countries have established the setup for converting waste and production of bioethanol. In India we have a large amount of solid residue available, but lack of technology and resources is restricting their use, and the waste is posing serious problems for the environmental communities. Conventional crops, such as corn and

sugarcane are unable to meet the global demand of bioethanol production due to their primary value of food and feed. There are various other sources from which bioethanol could be produced, and various researches have worked and explored different sources. Many studies are currently conducted on the development and optimization of biofuel production from various other biomasses, such as wood and agricultural/industrial and municipal wastes. Various new methods and techniques are being developed to convert the waste material by microorganisms to valuable products such as biogas, ethanol, citric acid, chemicals, various enzymes, volatile flavoring compounds, fatty acids, pectins, essential oils, antibacterial agents, and microbial biomass (Dhillon et al. 2004).

11.3 Production of Bioethanol from Citrus Industry Waste

World citrus production has been significantly increasing since the 1980s and is about 120 million tons per year. As a result, citrus processing industries, especially in developed countries, have expanded rapidly (Khadir and Khan 2011).

India is ranked sixth in citrus fruit production throughout the world. The estimated annual production of citrus waste is approximately 15 million tons (Marin et al. 2007). In India, more than 1.7 million metric tons of citrus fruits are produced annually. The Punjab state produces about 90% of the country's orange and grapefruit juice, and the citrus industry generates about 5 million tons a year of pith and peel. India is also one of the potential producers of bioethanol.

The industrially important citrus crops include oranges, lemons, grapefruit, and mandarins. These industries processed the citrus crops for juice extraction, where few other industries processed citrus including their peels, segment membrane, and seeds that end up as wastes (Wilkins et al. 2007a). These solid residues are referred to as citrus processing wastes (CPWs) with an estimated worldwide production of 15 million tons per year (Marin et al. 2007). Citrus peel wastes are considered as an important feedstock for ethanol production since two decades ago (Grohmann and Baldwin 1992). These solid remains are generally a very good source of carbohydrates. The cell wall polysaccharides and other functionally important bioactive contribute to various important industrial processes. Citrus peel waste constitutes various polysaccharides, bioactive compounds, and phenolic compounds which can be hydrolyzed to sugars, and these sugars are fermented into ethanol. Limonene, as well as pectin, can also be recovered from the citrus processing waste (Pourbafrani et al. 2010). These carbohydrate polymers and polyphenols make it an ideal feedstock for bioenergy generation through biological conversion to biofuels, such as ethanol and biogas (Wilkins et al. 2007b). Various pretreatment methods of lignocellulosic waste can yield high ethanol and also biogas production (Zhou et al. 2007).

The global concerns are to identify technically feasible and environmentally acceptable options for converting these solid residues into usable forms of energy. Out of many potential options of waste management, each option addresses the

environmental problems and can be used to generate significant amounts of usable bioenergy.

Collectively the various practices of waste management remain insufficient and carry many disadvantages. Out of most of the practices of the solid waste disposal, a large amount is incinerated in disposal yards and dumped into the ocean bottom, which leads to eutrophication and death of marine biodiversity posing severe consequences.

Recently, parts of the dried CPWs are being used for low-protein cattle feed production. These are called as "citrus pulp pellets (CPP)" or biopellets, and the rest of the debris are disposed in landfills, resulting in severe environmental problems. Further, the operating cost would also require for landfilling activities in addition to existing waste disposal costs (Tripodo et al. 2004).

The problem with aerobic digestion of citrus wastes is the presence of citrus oil, inhibit its digestion creates the concern for additional research to ascertain the digestibility of peel press liquor and other liquid citrus wastes. Solid citrus wastes can also be reduced through mechanical pressing and subsequent drying to a low moisture level to sustain combustion, but this has apparently not been tried commercially. In fact, such dried and shredded citrus wastes have similar characteristics to cane bagasse, which is commonly used as fuel for energy generation by combustion. The anaerobic digestion of these waste materials consists of various technical processes not attempted so far on a commercial level for such agro-industrial wastes. By combining the energy produced from combustion of solid wastes with the energy from anaerobic digestion could produce large amount of steam sufficient for all of the facility's process steam and electrical requirements. The resultant would be able to displace 100% of current fossil fuel consumption or oil-fired boiler-based systems.

Cattle feed and by-product recovery from agro-industrial waste were also tried from several processes but found to be uneconomical in many countries as liquid effluent would also need to be properly treated before they are discharged to minimize environmental pollution. Although this option has been the focus of much attention, the options described below are considered more attractive for a country like India.

Kinnow (*C. reticulata*) is the most important commercial crop in India. It occupies about 39% of the area under citrus cultivation. The chief centers of production are Nagpur, Assam, and Punjab. Areas considered to be good for production of kinnow Hoshiarpur, Muktsar and Abohar districts. The growth of agricultural-based industries worldwide has generated huge quantities of fruit wastes (25–40% of the total fruits processed). Production of bioethanol using yeast *Saccharomyces cerevisiae* through fermentations has also been well established and extensively exploited. Suitable conditions with chosen microorganism are used for ethanol production for rapid fermentation and ethanol production. Simultaneous saccharification and fermentation (SiSF) process by *Saccharomyces cerevisiae* is the most tested process which increases the hydrolysis rate, simplifies the operational process, and also decreases the processing time, thereby improving process economics. These options represent the maximum potential generation of

usable energy from citrus wastes while simultaneously alleviating current waste disposal problems.

11.4 Conclusions

The appropriateness of different bioenergy production systems in economic, environmental, and social terms will depend to a large extent on national and local circumstances. To plan a bioenergy strategy, analysis of different options and their broad impacts should be carried out to achieve the policy objectives. Dramatic improvements in policy and technology are needed to meet global demand for both food and biofuel feedstocks. Citrus waste material being potentially valuable for production of bioethanol drags the area of interest these days. All of these constituents of citrus waste have been extensively studied by various researchers for the production of ethanol, but one of the constituents which is flavonoids has yet to be fully exploited. The flavonoid naringin which is responsible for the bitterness of citrus fruit juices can be used for sugar production (rhamnose) with the help of enzyme naringinase. This will add up in ensuring the replacement of non-eco-friendly fuels with the renewable green fuel that is bioethanol and help save the environment. Biofuel could replace petroleum fuels, such as natural gas, diesel, jet fuel, and gasoline. This replacement reduces dependency on oil and gas and also helps the environment through reduction of greenhouse gas emissions.

The economics of ethanol production by fermentation can be significantly influenced by the availability and cost of the raw materials, which accounts for more than half of the production costs. To achieve a lower production cost, the supply of cheap raw material is thus a necessity. The rationale of the chapter was to identify feasibilities and environmentally acceptable options for converting agro-industrial waste or dumped wastes in India into usable sources of bioenergy. Many of the options generate significant amounts of usable energy. Many potential options have already been identified by many researchers. Each option addresses the environmental as well as economical aspects. Production of value-added products from agro-industrial and food processing wastes is now a focusing area, as it reduces pollution in the environment in addition to energy generation.

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Role of Genetically Modified Microorganisms in Heavy Metal Bioremediation

12

Saurabh Gupta and Daljeet Singh

Abstract

Heavy metals are natural constituents of the earth's crust. There is a significant alteration in the geochemical cycles and biological balance of these heavy metals due to various anthropogenic activities. These anthropogenic activities result in the release of bioavailable forms of various heavy metals such as mercury, lead, cadmium, nickel, copper, zinc, etc. into soil and aquatic environments. Prolonged exposures to these heavy elements lead to harmful health implications on different domains of terrestrial and aquatic life. Due to several limitations associated with physical and chemical methods for remediation of contaminated sites, bioremediation has been explored these days as an alternate technology for treatment of heavy metal pollution in soil and water. Various microorganisms such as bacteria and fungi along with plants play a vital role in biotransformation of these heavy metals into nontoxic forms, through processes such as bioremediation and phytoremediation, respectively. Recent progress in genetics has provided the driving force toward the use of engineering improved microbes and enzymes for bioremediation. Keeping these future remediation tolls in mind, present review investigated the abilities of wild microorganisms and plants in terms of tolerance and biotransformation of heavy metals along with their genetically engineered counterparts to explore these immense and valuable biological resources for bioremediation.

Keywords

Heavy metals • Toxicity • Bioremediation • GMOs • Phytoremediation • Genetically modified plants

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12.1 Introduction

The earth's crust is a major reservoir of electron-rich elements known as metals. These elements share their characteristic properties such as hardness, opacity, and shininess along with good electrical and thermal conductivity. Many of these metals are essential for different forms of life as trace elements. Some metals and their compounds play a vital role in various physiological pathways. At the other end, exposure to high concentrations of these metals results in toxicity and other health issues. Thus, these elements are kept in three different categories from their physiological roles played in different living beings: (i) nontoxic and essential metals and their compounds (e.g., calcium and magnesium), (ii) essential for normal functioning, but are harmful at high concentrations (e.g., iron, manganese, zinc, copper, cobalt, nickel, molybdenum, and selenium), and (iii) highly toxic (e.g., mercury, lead, and cadmium) (Valls and Lorenzo 2002).

Heavy metals have a peculiar property of high atomic density (<4000 kg m^{-3}), which is about five times more than water (Garbarino et al. 2005). These heavy metals and metalloids are categorized into essential and nonessential elements on the basis of their requirements in the biological systems (Graz et al. 2011). In an ecosystem, heavy metals and their compounds are generally more stable and persistent as compared to organic contaminants such as pesticides, phenols, and petroleum by-products. Persistence and nonbiodegradability is an inherited property harbored by these heavy metals due to association of nuclear fission process for degradation of any element. However, these metals can be only transformed from one form to another (Lasat 2002). Cadmium, chromium, cobalt, copper, lead, nickel, mercury, and zinc are the metals of most immediate concern according to the World Health Organization (WHO 2008). These bioavailable forms of heavy metals enter into the environment through different anthropogenic activities such as mining of rocks, smelting process, power stations, synthesis and use of pesticides and fertilizers containing metal ions, irresponsible disposal of wastes by various industries, colored material, batteries, erosion of rocks, combustion by-products, traffics, etc. (Vadkertiova and Slavikova 2006). Due to accumulation of bioavailable forms of these heavy metals in the soils which leads to their entry into biological systems, microbial activity in soil, plants, and human health is affected adversely. Heavy metals have a strong tendency to bind with different biomolecules which leads to accumulation of these metals in biological systems and, in due course, their entry into the food web via different mechanisms (Giller et al. 1998). Thus, pollution caused by these heavy metals leads to a severe danger to both the ecosystem and humans. Removal of these contaminants by physical and chemical methods is an expensive cleanup methodology. These metals and their compounds are difficult to take away from the environment, and unlike many other pollutants, these cannot be degraded chemically or biologically. Toxic effects of heavy metals include:

Tubular proteinuria
Lung disease
Cadmium pneumonitis
Inhibition of hemoglobin synthesis

Dysfunctions in the kidneys, joints, reproductive systems, and cardiovascular system
Acute and chronic damage to the central nervous system (CNS) and peripheral nervous system
Vomiting
Diarrhea
Bloody urine
Icterus (yellow mucus membrane)
Anemia
Gingivitis
Stomatitis
Abdominal cramps
Dyspnea
Muscular weakness
System intervention on cellular enzymatic systems (Ferner 2001; Lenntech 2004; Young 2005; Saberi et al. 2010)

Heavy metals also exhibit toxic effects on plants such as inhibiting seed germination, root and stem elongation, and leaf expansion, propagation effects on the repair process of vascular plants, visible injuries and physiological disorders, chlorosis, growth inhibition, and browning of root tips (Mohanpuria et al. 2007; Zhou et al. 2007; Guo et al. 2008).

12.2 Occurrence and Health Implications of Heavy Metals

12.2.1 Mercury

Mercury is one of the most toxic elements present on this planet (Nies 1999). Different anthropogenic activities such as metal mining, chlor-alkali, power plants, electronic industries, and refining and electroplating industries result in the discharge of mercury and its compounds into the atmosphere and surface water. Finally these metal compounds find their natural sinks, i.e., soil and water bodies including groundwater from where these enter into the food chain (Sinha and Khare 2012). The anthropogenic activities contribute about 2190 tons of mercury in the environment (Li et al. 2009). Besides these human activities, many natural processes, such as volcanic eruptions, geothermal activities, soil erosion, hydrological cycle, wild fires, and the re-emission of the deposited mercury contribute to the global mercury load through the elemental form of mercury. Approximately 54% of total global mercury emission from all anthropogenic activities is contributed by Asian countries in the year 2000 (Pacyna et al. 2006). Indiscriminate release of mercury and its compounds is projected to reach the level of 2390–4860 Mg by the year 2050 (Streets et al. 2009). Mercury shows toxic effects by binding with sulfhydryl groups present in various enzymes and proteins, thereby resulting in altered properties of these biomolecules and finally negative impact on various cell

functions. In particular, mercury binds to cysteine amino acids of proteins and nitrogen atom of nucleic acids causing extreme toxicity and physiological disorders (Wagner-Dobler et al. 2000). Mercury is also readily absorbed through nerve endings and is rapidly transported inside the axon of the nerves leading to neurological disorders (Brodkin et al. 2007; Holmes et al. 2009). Mercury accumulation in the human body results in different disorders such as allergy, coronary heart disease, damage to the nervous system, paresthesia, and numbness in the fingers, which are common symptoms of Minamata disease (Salonen et al. 1995; Weiss et al. 2002; UNEP 2003).

12.2.2 Cadmium

Cadmium is a heavy metal mostly present in ores with zinc, lead, and copper. Cadmium compounds find their wide applications as stabilizers in PVC products, color pigment, and several alloys. These days cadmium has been exploited in rechargeable nickel-cadmium batteries. Cadmium has been declared as recalcitrant and carcinogenic due to its prolonged persistence in living beings with adverse effects, respectively (IARC 1993). Cadmium and its compounds are discharged into the environment by various means such as mining and metallurgy of cadmium, cadmium electroplating, ceramic industrial wastewater discharge, mine tailing, and effluents from textile, leather, tannery, electroplating and galvanizing industries, as well as cadmium batteries. Cadmium is a nonessential element and highly toxic to organisms even at very low dosages. The WHO "safe" level for human ingestion of Cd has been estimated to 7 μg cadmium/week/kg body weight (WHO 1992). Cadmium is easily taken up by the plants and transferred through food chain to cause adverse effects on human health (Kumar et al. 2012). Exposure to cadmium and its compounds results in cell damage due to their strong affinity toward glutathione and sulfhydryl groups of proteins. Along with this, it also has the tendency to displace zinc and iron ions from proteins (Cunningham and Lundie 1993). In Japan, itai-itai disease occurred due to high concentration of cadmium causing skeleton deformation and spontaneous fractures. Moreover, cadmium metal also leads to other detrimental effects in humans such as brain damage, reproductive failures, nervous system failures, spongy bone disease, kidney disorders, lung disorders, autoimmune diseases, and destruction of red blood cells along with cancers and tumor formation (Titus and Pfister 1984; Koplan 1999; Johansson 2002; Satarug and Moore 2004; Zaki and Farag 2010). Chronic exposure to cadmium also leads to liver damage (Santra et al. 2000). It also affects apoptosis, differentiation, and proliferation and increases the chances of oncogene activation. Cadmium being highly stable also binds to essential respiratory enzymes causing oxidative stress. Furthermore cadmium is also related to generation of reactive oxygen species (ROS) (Zeng et al. 2010).

12.2.3 Lead

Lead (Pb) is one of the most hazardous heavy metal present on earth. Lead is primarily released in the environment through various human activities such as mining and smelting, combustion of lead-containing fossil fuels, use of sewage sludge as land application, use and disposal of lead-containing battery and other products (Adriano 2001; Cho et al. 2004). These activities result in significant input of Pb which finally leads to accumulation in high concentrations of Pb in soils (Huang et al. 2006b). In nature, lead is mostly present in its elemental form and oxidized state. However, Pb^{2+} is the most reactive and common form of lead present. On a worldwide scale, elevated levels of lead represented the greatest risk to human health. WHO has recommended 10 μg/L lead as safe permissible level in the drinking water (Watt et al. 2000). Besides the mere presence of lead into the soil, its toxicity and bioavailability are affected by soil pH, redox potential, and predominant lead species. Lead is found in soil mainly as carbonate-bound, Fe/Mn oxide-bound, organic, and residual phases (Halim et al. 2005). Ultimately, soluble form of lead and its presence in exchangeable phases pose more threat to environment, ecosystem, and human beings as compared to immobilized Pb present in other phases (Chen et al. 2003). Lead toxicity is usually associated with its impact on the nervous system in both adults and children. Prolonged exposure to high levels of lead causes damage to the brain and kidneys along with anemia (Flora 2002). Being mutagenic and teratogenic, lead causes harmful effects on biological systems, viz., neurodegenerative impairment, reproductive damage, and cancer (Fowler 1998; Tong et al. 2000; Watt et al. 2000; Lam et al. 2007).

12.2.4 Arsenic

Arsenic (As) is a toxic metalloid with an atomic weight of 74. Arsenic (As) is abundantly found in rocks, soil, water, air, and sediments. It has been exploited as an ingredient of many commonly used materials, such as wood preservatives, pigments, insecticides, herbicides, rodenticides, fungicides, and animal feed additives (Mandal and Suzuki 2002). Hence, arsenic is released into the environment in significant proportion during cycling of these materials. Both natural and anthropogenic activities play a considerable role in the recycling of arsenic (Eisler 2004). Arsenic exists in several oxidation states in the ecosystem such as arsenate [As(V)], arsenite [As(III)], elemental [As(0)], and arsenide [As(-III)] (Selvi et al. 2014). Arsenate and arsenite are the two most toxic and bioavailable forms of arsenic present in the environment, while arsenite [As(III)] is 25–60 times more toxic than arsenate [As(V)] (Munawar et al. 2012). Major release of arsenic into land comes from commercial wastes (40%) followed by coal ash (22%), mining industry (16%), and atmospheric fallout from the steel industry (13%) (Eisler 2004). Most of arsenic and its compounds have been used in the manufacturing of products with agricultural applications. More than 80% of arsenic compounds are used to manufacture insecticides, herbicides, fungicides, algaecides, and sheep

dips, along with the medicines used for eradication of tapeworms in sheep and cattle (NAS 1977). Besides this, the manufacture of ceramic and glass, electronics, pigments and antifouling agents, cosmetics, fireworks, and Cu-based alloys uses arsenic trioxide (As_2O_3) extensively (Leonard 1991). Inhalation is the principal route of arsenic entry in occupational settings, while ingestion of contaminated drinking water is the predominant source of arsenic contamination (Tchounwou et al. 2003). Acute exposure of humans with high levels of inorganic arsenic can be fatal, while acute exposure to lower levels leads to vomiting, decrease in red and white blood cells, abnormal heartbeat, and damage to blood vessels. Arsenic poisoning also leads to development of skin lesions (Smedley et al. 2002). It is also a neurotoxin, damaging peripheral and central nervous systems (Mazumder et al. 1999). Arsenic induces chromosomal aberrations at cellular level which leads to inhibition of lymphoblast proliferation (Liu et al. 2000).

12.3 Bioremediation

Bioremediation is a generalized term used to represent all the actions and activities of any biological system that take place to improve the environment by transforming/degrading contaminants to restore its original status. A number of biological tools have been reported with their possible role in bioremediation. Primarily, microorganisms and their metabolic processes lead to degradation or transformation of recalcitrant environmental contaminants into harmless or less toxic forms (Garbisu and Alkorta 2003). Bioremediation has been explored over current physico-chemical methods of remediation due to its potentially low cost and environmentally agreeable scenario which do not lead to secondary pollution (EPA 2008; Huang et al. 2006a). Bioremediation technology finds its wide application both at the site of contamination (*in situ*) and under controlled condition where contaminants are physically removed from the original site (Sharma 2012). Bioremediation process is aided by various techniques, such as bioventing, bioleaching, land farming, bioreactor, composting, bio-augmentation, rhizofiltration and biostimulation (Chowdhury et al. 2012). Hence, diverse microbial metabolic ability with these bioremediation aids has been exploited for degradation/removal/transformation of potential environmental pollutants as an economic and safe alternative compared to other physico-chemical methodologies. Presence of highly diverse and specialized microbial communities along with physico-chemical properties of contaminated site limits the removal of contaminants. Slow rate of degradation and transformations of these potential pollutants leads to accumulation of these pollutants and their metabolites in the environment, which is hazardous for both biotic and abiotic environment. This is particularly valid for heavy metal pollution as these contaminants are only transformed by microbial activity and remain present at the site all the time. Hence, heavy metal contamination is one of the most significant environmental issues, since metals are highly toxic to biota, decrease metabolic activity, diversity, and also affect the qualitative & quantitative structure of microbial communities. More than 50% of the researchers preferred the use of environment-friendly approaches, including microbial remediation and

phytoremediation for the treatment of contaminated areas (Hussein et al. 2001; EPA 2007). Low-cost in situ bioremediation technologies, such as monitored natural attenuation have been explored in developed nations at a very large scale. In contrary, developing economies still rely upon the use of more expensive *ex situ* technology. Many different strategies have been evolved by microorganisms as well as plants for remediation of heavy metal-contaminated sites (Gan et al. 2009). These biological systems use different mechanisms such as biosorption, bioaccumulation, biotransformation, and biomineralization for their survival in heavy metal-polluted habitats which are exploited for bioremediation either ex situ or in situ (Gadd 2000; Lim et al. 2003; Malik 2004).

12.4 Genetically Modified Microorganisms

Any biological entity whose genetic material has been modified using recombinant DNA technology is termed as genetically modified organism. The term GMO is very close to the technical legal term, "living modified organism" defined in the Cartagena Protocol on Biosafety. It regulates international trade in living GMOs specifically, "any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology." Although no sexual reproduction has been reported in bacteria, still genetic exchange between bacteria takes place naturally through transformation, transduction, and conjugation. Besides these natural means, recombinant bacteria are also generated through genetic engineering techniques exploiting the natural mean for transfer of genetic material, i.e., transformation. Although the use of genetically engineered microorganisms (GEM) has received great deal of attention in bioremediation, it is largely limited to the laboratory trials only. Use of genetically engineered microorganisms is principally associated with regulatory risk assessment concerns and uncertainty of practical impact and delivery of these organisms under field conditions. Use of genetically modified microorganisms is fruitful only when it not only survives and grows in the contaminated site but also expresses the desirable genes for the significant remediation. Hence, these characteristics of the organism are largely affected by growth rate, inoculum size, environmental conditions, spatial distribution, and the presence of competing microorganisms. The spatial distribution of GMOs introduced into contaminated site leads to their interactions with native microflora and other components of the ecosystem which in turn affect the bioremediation. In general, a bacterium that has been recently isolated from a natural environment is more likely to survive when released back into that same environment. The prerequisite for development of GEMs for bioremediation of contaminated sites involves minimally four principal approaches. These include:

1. Modification of enzyme specificity and affinity
2. Construction and regulation of specific pathways
3. Development of bioprocess for remediation and its monitoring and control
4. Use and applications of biosensors for chemical sensing, toxicity reduction, and end point analysis.

For an instance, recombinant DNA technology has been exploited to produce genetically improved strains for biosorption of heavy metals. Many of these strategies equip the bacterial cell wall with metal ion-binding polypeptides which results in anchoring/binding of pollutants with the recombinant strains. Valls et al. (2000) reported a fusion protein (β-domain of IgA protease) for improved biosorption along transfer of plasmids containing the necessary genetic material between recombinant and indigenous strains for better results. Besides the use of microorganisms, genetically engineered plants are also at the forefront for the bioremediation of various heavy metal-contaminated sites due to its eco-friendliness and reduced health hazards as compared to physico-chemical-based strategies (Singh 2011). A number of microbial genes have been harnessed in the genetically modified plants for accumulation of inorganic contaminants to a confined region (Maestri and Marmiroli 2011). Expression of genes encoding the chelators, such as metallothioneins and phytochelatins results in improved uptake, transport, and accumulation of various heavy metals by the plants. Similarly, expression of some bacterial genes in the plants leads to volatilization of mercury and other heavy metals by converting these elements into their potential volatile organic compounds such as methylmercury and dimethylmercury besides elemental mercury. Many reports have been published in the recent past in which plants enriched with various microbial catabolic genes and specific transporters resulted in enhanced transport, accumulation, and transformation into non-available forms hence bioremediation (Gullner 2001; Doty 2007). Bittsanszkya et al. (2005) reported that the expression of mercuric reductase and γ-glutamylcysteine synthetase genes is followed by an increase in resistance to Hg and Cd and Cu with high accumulation of these metals (Bittsanszkya et al. 2005).

12.5 Genetically Modified Microorganisms and Heavy Metals

12.5.1 Mercury

Mercury is one of the most toxic heavy metals in the environment. Bioremediation of mercury using bacteria is generally associated with the expression of bacterial *mer* genes (Jackson 1982). Mercury-resistant bacteria harbor the mer operon in their genome. The mer operon includes certain functional genes along with promoter, regulator, and operator. The most common functional genes are merA and merB, which code for mercuric ion reductase and organomercurial lyase, respectively. The lyase is responsible for reducing highly toxic organomercurial compounds such as methylmercury and phenylmercuric acetate into almost non-toxic volatile elemental mercury with the help of the enzyme reductase (Dash and Das 2012). Mercuric reductase enzyme results in the conversion of bioavailable form (Hg^{2+}) of mercury into a non-bioavailable form (Hg^{0}) of mercury. Elemental mercury being volatile is then volatilized from the cell. Some of the bacterial strains harbor an organomercurial lyase whose activity leads to cleave bond of many organomercurial compounds between carbon and mercury. A wide diversity has

been reported in the genes coding for *mer* operons. An additional *mer*B has been reported in some bacteria that confer broad-spectrum resistance to mercuric compounds (Barkay et al. 1992, 2003). Most *mer* operons contain a regulatory gene, *mer R*, which is transcribed separately and divergently from the structural *mer* genes. MerR, the metalloregulatory protein, binds the promoter-operator region, where it both positively and negatively regulates the expression of the divergently transcribed structural genes and also negatively regulates its own expression. MerR protein activates transcription of the operon in the presence of inducing concentrations of Hg^{2+}. It represses transcription of the structural genes from the *mer* operon (*merTPCFAD*) in the absence of Hg^{2+} and activates transcription in the presence of Hg^{2+}. However, in Gram-positive bacteria, the *merR* genes of pI258 from *Staphylococcus aureus* and RC 607 from *Bacillus* sp. are transcribed in the same direction as the structural genes (Laddaga et al. 1987; Wang et al. 1989). *Deinococcus radiodurans*, the mostly studied radiation-resistant organism for treatment of mixed radioactive wastes, has been genetically modified using cloning and expression of merA gene from *Escherichia coli* BL308 to remediate the sites containing ionic mercury (Brim et al. 2000). A transconjugant strain *Cupriavidus metallidurans* MSR33 has been developed using heavy metal-resistant *Cupriavidus metallidurans* strain CH34 with two large plasmids, pMOL28 and pMOL30. Individually, each plasmid contained a merRTPADE operon with a narrow-spectrum mercury resistance resulting in development of a broad-spectrum mercury resistance after transconjugation with a strong ability to remove mercury from polluted water (Mergeay et al. 2003).

12.5.2 Arsenic

Arsenic is highly toxic when it is present in oxidized forms. Reduction of As(V) to As(III) is not a potential remediation pathway as As(III) is more toxic than As(V). Hence, bioremediation of arsenic is greatly associated with conversion of these bioavailable soluble forms into the volatile arsenic compounds such as mono-, di-, and trimethylarsine. Although a number of microorganisms have been reported with a property to volatilize arsenic, indigenous microflora has been reported to volatilize 2.2–4.5% of arsenic in 30 days under soil system (Liu et al. 2011). Development of genetically modified microorganisms with ability to volatilizations of arsenic compounds is an upcoming research field as microbial biomethylation has emerged as major pathway for the removal of contaminated sites (Bentley and Chasteen 2002). Volatilization of arsenic compounds proceeds through reduction of As(V) to As(III) followed by a series of methylation reactions of As(III) with trimethylarsine (TMA) as a final product (Turpeinen et al. 2002; Bentley and Chasteen 2002). Besides this, a significant increase in formation of volatilized arsenic forms has been reported in transgenic bacteria as compared to wild type. In the last decade, efforts have been made for cloning and expression of arsenite S-adenosyl methionine methyltransferase gene *(arsM)* isolated from *Rhodopseudomonas palustris* in *Escherichia coli* to methylate inorganic arsenic

to volatile trimethylarsine (Xu and Rosen 1997; Qin et al. 2006; Yuan et al. 2008). Cloning of *arsM* gene *Sphingomonas desiccabilis* and *Bacillus idriensis* leads to tenfold increase in the release of methylated arsenic gas compared to wild strains in aqueous system. Besides this, *Thermus thermophilus* HB8 has been reported to have TTHB128 and TTHB127 genes associated with production of arsenite oxidase capable of oxidizing toxic forms of arsenic to nontoxic form. More than 80% of arsenite has been oxidized by various transgenic microorganisms when these genes were cloned into broad-host-range vector pBBR1MCS-5 followed by their transfer in potential strains (Yang 2010). Plasmid pSinA of *Sinorhizobium* sp. and *M14* (*Alphaproteobacteria*) is the first described, natural, self-transferable plasmid harboring a complete set of genes for oxidation of arsenite. Removal of this plasmid from cells of the host strain caused the loss of resistance to arsenic (Drewniak et al. 2013).

12.5.3 Lead and Cadmium

Bioremediation of lead (Pb) is associated with active uptake and accumulation of Pb in various microorganisms with a potential to convert it into non-available forms. Both chromosomal and extrachromosomal genetic material have been reported to harbor genes whose expression facilitates uptake and accumulation of lead within the bacterial cell. Microorganisms such as *Pseudomonas aeruginosa* strain 4EA and *Salmonella choleraesuis* strain 4A along with *Proteus penneri* strain GM10 possess genes encoding bacterial metallothioneins (*smt A* and *smtAB*) responsible for lead resistance on plasmids and genomic DNA, respectively (Naik and Dubey 2013).

Phytochelatins (PCs) are naturally occurring peptides consisting of the repeating γ-Glu-Cys dipeptide unit terminated by a Gly residue with high affinity to bind heavy metals such as cadmium (Cd), Hg, As, and Pb, especially cadmium through thiolate complexes (Maitani et al. 1996; Mejare and Bulow 2001). More than seven times increase in uptake and accumulation of Cd has been reported after cloning and expression of PC synthase gene in *Escherichia coli* from *Schizosaccharomyces pombe* (SpPCS). Further increase of approximately tenfold and twofold in PC production and cadmium accumulation has been achieved by co-expression of a variant glutamylcysteine synthetase desensitized to feedback inhibition (GshI), respectively, which leads to increase in the supply of PC precursor glutathione (Kang et al. 2007). Cloning and expression of plant PC genes in *E. coli* resulted in substantial increase in intracellular cadmium content as compared to control strain (Sauge-Merle et al. 2003). Sriprang et al. (2003) reported an enhanced accumulation of Cd^{2+} by a *Mesorhizobium huakuii*, transformed with a gene from *Arabidopsis thaliana* coding for PCs. Besides the use of these phytochelatin genes, a manganese transport gene (*mntA*) and a metal-sequestering protein (metallothionein [MT]) gene were cloned in *Escherichia coli* resulting in development of strain JM109 with a potential to accumulate cadmium (Cd) in an aqueous phase (Kim et al. 2005). Along with these nonspecific genes exploited for cadmium

accumulation, Deng et al. (2007) demonstrated accumulation of Cd^{2+} specifically from multicomponent metal-polluted site due to presence of specific cadmium transport system and metallothionein (MT) protein in genetically engineered bacteria. Expression of RsaA-6His fusion protein in recombinant *Caulobacter crescentus* strain JS4022/p723-6H enhanced the removal efficiency (94.3–99.9%) of the Cd(II) as compared to control strain (11.4–37.0%) (Patel et al. 2010).

12.6 Heavy Metal Bioremediation Using Genetically Engineered Plants

12.6.1 Phytoremediation

The term phytoremediation ("phyto" meaning plant and the Latin suffix "remedium" meaning to clean or restore) is a process in which a number of plants play an important role in combating the pollution caused by various recalcitrant compounds through various mechanisms. These days phytoremediation may be carried out by naturally occurring plants or genetically engineered plants to clean contaminated environments (Flathman and Lanza 1998). Phytoremediation finds its numerous advantages over the other remediation techniques such as low remedial costs, habitat restoration, and in situ cleanup of contamination than ex situ avoiding creation of secondary pollutant sites (Zynda 2001). Phytoremediation involves a number of different mechanisms such as phytoextraction, phytodegradation, phytostimulation, phytostabilization, rhizofiltration, and phytovolatilization dependent upon the nature of the pollutant toward cleanup of the contaminated sites as mentioned in Table 12.1 (Raskin and Ensley 2002).

Nowadays, a number of plant families such as Asteraceae, Brassicaceae, Caryophyllaceae, Cyperaceae, Cunoniaceae, Fabaceae, Flacourtiaceae, Lamiaceae, Poaceae, Violaceae, and Euphobiaceae have been reported with a potential to accumulate significantly high concentration of heavy metals. However, remediation of heavy metal-contaminated sites was initially reintroduced and developed by Utsunamyia in 1980 (Utsunamyia 1980) and Chaney in 1983 (Chaney 1983). Baker et al. (1991) conducted first field trial on phytoremediation of cadmium by phytoextraction. Genetic manipulation of potential plant species using recombinant DNA technology resulted in successful alteration of the biological functions of plants through modification of primary and secondary metabolism. Further these transgenic plants facilitate better understanding and improving phytoremediation properties by adding new phenotypic and genotypic characters to plants (Davison 2005). Metal hyperaccumulating plants and microbes represent an important reservoir of unique genes with unique abilities to tolerate, accumulate, and detoxify metals and metalloids (Danika and Norman 2005).

Table 12.1 Different techniques and mechanisms used in phytoremediation

Technique involved	Type of contaminant treated	Mechanism
Phytoextraction	Metals	Hyperaccumulator plants uptake metals and accumulate it in confined space
Phytodegradation	Organic compounds	Production of certain enzymes leading to breakdown of contaminants
Phytostimulation	Organic compounds	Plants release certain microbial growth-promoting substances in soil resulting in enhanced microbial activity with significant degradation of contaminants
Phytostabilization	Generally metals	Converts bioavailable forms of contaminants into non-available forms and hence restricts their entry into the biological system
Rhizofiltration	Metals	Plant roots absorb, concentrate, and precipitate metals
Phytovolatilization	Metals	Plants have the tendency to uptake heavy metals such as mercury from soil and volatilize it from the foliage region

12.6.2 Genetically Modified Plants and Heavy Metals

Plants, such as *Arabidopsis thaliana*, *Brassica juncea*, *Populus angustifolia*, *Nicotiana tabacum*, or *Silene cucubalus* have been successfully genetically engineered with different bacterial genes thereby resulting in enhanced heavy metal accumulation and transformation as compared with corresponding wild plants. Glutathione synthetase is the major enzyme involved in uptake of heavy metals in plants followed by their sequestration with heavy metal-binding peptides called phytochelatins (Zhu et al. 1999). Hence, *Brassica juncea* was genetically engineered with *Escherichia coli gshII* gene encoding glutathione synthetase (GS) to overcome the initial rate-limiting factors for glutathione production. A significant increase in tolerance and accumulation (up to threefold) toward exposure to cadmium has been reported after transfer and expression of genes encoding for *gshI* (gamma-glutamylcysteine synthetase) and *gshII* in *Brassica juncea* (Ow 1996). Besides these *E. coli* enzymes, an increase in concentrations of phytochelatins and total non-protein thiols has also been reported leading to enhanced biomass production as compared to wild-type seedlings. A significant increase in tolerance and conversion of methylmercury into volatile and less toxic elemental mercury has been reported in *Arabidopsis thaliana* transformed with two bacterial genes *merA* and *merB* encoding for mercuric reductase and organomercurial lyase, respectively (Bizily et al. 2000; Eapen and D'Souza 2005). Transformation and overexpression of phytochelatin synthase (*TaPCS1*) gene in *Nicotiana* resulted in significant increase in tolerance of this plant toward different heavy metals especially lead (Gisbert et al. 2003). Cloning and expression of bacterial genes encoding for arsenate reductase (arsC) and gamma-glutamylcysteine synthetase (gamma-ECS) in genetically engineered *Arabidopsis thaliana* plants resulted in substantially high tolerance to arsenic along with significant increase in arsenic

accumulation in transgenic plants than wild plants (Dhankher et al. 2002; Mello-Farias and Chaves 2008). Similarly successful transfer and expression of merA9 and merA18 genes in eastern cottonwood (*Populus deltoides*) resulted in fourfold increase in elemental mercury accumulation relative to wild plants grown *in situ* on a Georgia Piedmont soil contaminated with Hg(II) (Che et al. 2003).

12.7 Conclusions and Future Perspectives

Although traditional physical, chemical, and biological methods have been widely used for remediation of heavy metal-contaminated sites, use and application of genetically modified organisms marked their importance and role significantly for removal of these pollutants. Field applications of these genetically altered organisms are still scarce due to certain inherited issues associated with use of biological system such as their reach to the contaminants, activity, competition, and mostly widespread contaminated sites. Hence, further improvements in genetically modified microorganisms in terms of their survival, completion with indigenous population, and chemotaxis toward the pollutants along with structural genes associated with bioremediation of contaminants should also be considered while developing GMOs for environment cleanup.

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Agriculture Biotechnology

13

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Abstract

Agricultural biotechnology is the area of biotechnology involving applications to agriculture. Agricultural biotechnology has been practiced for a long time, as people have sought to improve agriculturally important organisms by selection and breeding. In the twentieth century, breeding became more sophisticated, as the traits that breeders select for include increased yield, disease and pest resistance, drought resistance and enhanced flavor. Traits are passed from one generation to the next through genes, which are made of DNA. Based on an understanding of DNA, scientists have developed solutions to increase agricultural productivity. Starting from the ability to identify genes that may confer advantages on certain crops and the ability to work with such characteristics very precisely, biotechnology enhances breeders' ability to make improvements in crops and livestock.

Keywords

Agriculture • Biotechnology • Resistance • Disease • Crops • Farmers

13.1 Introduction

Over the recent years, biotechnology applied to agriculture has been considered a realistic alternative to improving effectiveness in agricultural production. There is no qualm that the judicious use of biotechnological tool tilting to agricultural production will create positive impact in developing countries. Agricultural biotechnology has been adopted for a long time, as people have sought to perk up

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agriculturally important organisms by selection and breeding (Ania 2003). An example of conventional technology is the improvement of disease-resistant wheat varieties by crossbreeding of different wheat types. The consequences of the invention of DNA-based molecular techniques and their application to agriculture have been pervasive, both within the agriculture sector and outside it. Increased food production and profits were probably the primary hoped-for results by scientists who pioneered agricultural biotechnology, while widespread public cynicism and even vociferous opposition probably were not anticipated. Researchers have always modified plants to improve growth rates and yields, create varieties resistant to pests and diseases and infuse special nutritional or handling characteristics. Such modifications have been achieved by crossbreeding plants and animals with desirable traits, through hybridization and other methods. With the help of recombinant DNA technology, scientists uses genetically modifying plants by selecting specific genes that bear desirable traits (e.g., resistance to a pest or disease) from one organism, and infusing them into another, and producing plants that can be economically important for food, fiber, pharmaceutical, or industrial uses (Tadlock 2011). Farmers have rapidly adopted genetically engineered (GE) varieties of soybean, cotton, and corn crops since their commercialization in the mid-1990s. In the USA, the genetic engineering varieties have augmented from 3.6 million acres to 165 million acres from the last decade (Hohn and Vasquez 2011). Globally, 29 countries planted GE crops on approximately 366 million acres in 2010. Genetically engineered soybean, cotton and corn production in the USA have increased at a fast rate, and they are also continuing to expand rapidly in other countries. The overall population of the developing countries is 4.6 billion and is growing at a rate of 1.9% per year. Hence, there should be a upliftment in the agriculture which would not only increase the productivity of major crops but reduces the use of chemical fertilizers and pesticides into soil and replacing them with biologically based products (Allison et al. 1998). A safe and sufficient food supply, grown in an environmentally responsible manner, is essential for humanity. The day from when these technologies have been put forward, there has been crop improvement using biotechnological application and have been used safely, with benefits. Out of many factors involved, agricultural biotechnology is the only one factor that influences the health and welfare of farmers and other citizens in the developing world. As biotechnology continues to evolve, factual and open public discourse is vital to define the role it should play in society (Chawanje 1992).

13.2 Use of Biotechnological Techniques in the Field of Agriculture

Various biotechnological approaches are available that specifically target to improve the agricultural output. These mainly include:

- Genetic engineering incorporates fragments of DNA into chromosomes of cells and then uses tissue culture to regenerate the cells into a whole organism with a different genetic composition from the original cells. This is also known as rDNA technology; it produces transgenic microorganism. Generally, all crops which are grown using transferred DNA (often called GM crops or GMOs) till date have been developed to help farmers to increase crop productivity by reducing crop damage from pest, weeds, diseases, or insects.
- The cells, anthers, pollen grains, or other tissues are manipulated by tissue culture technique; so they endure for extended periods under laboratory conditions or become whole, living, growing organisms. Few of the economically important crops which are produced through tissue culture are citrus, pineapples, avocados, mangoes, bananas, coffee, and papaya.
- Embryo rescue is a technique which keeps embryos containing transferred genes into tissue culture to absolute their development into whole organisms. Embryo rescue is often used to smooth the progress of "wide crossing" by producing whole plants from embryos that are the result of crossing two plants that would not normally produce offspring (Thompson 2008).
- In case of somatic hybridization, the cell walls of cells from different organisms are hereby removed, and further it induces the direct mixing of DNA from the treated cells, which are then regenerated into whole organisms all the way through tissue culture (Smith et al. 2001).
- Selective crossbreeding: In conventional plant breeding, newfangled varieties are developed either by selecting plants with enviable characteristics or by combining traits from two closely related plants through selective breeding. These appearances may, for example, be resistant to a particular pest or disease or tolerance to climatic conditions. Pollen-containing genes having the preferred trait is transferred from plants of one crop variety to the flowers of another variety with other fortunate traits. Eventually, in the course of vigilant selection of offspring, the desired trait will appear in a fresh variety of plants. Customary plant breeding has produced abundant flourishing new varieties of crops over the centuries. There have also been many less than unbeaten crosses made. In conventional breeding, crosses are often made in a relatively unrestrained manner. The breeder chooses the parents to cross, but at the genetic level, the results are erratic. The parental DNA recombines arbitrarily, and desirable traits like pest resistance may be bundled with undesirable traits, such as low-grade yield or poor quality. The parent plants must be strongly related to produce offspring (Burne Cristy 2006).Traditional propagation programs are prolonged, often taking decades to produce new feasible crop varieties, and labor intensive. Enormous effort is required in order to separate undesirable from desirable traits, and this is not always thriftily practical. Throughout this process, many potential benefits are lost alongside, as plants that fail to demonstrate the introduced characteristics are discarded. It takes about 12–15 years for a long-established plant breeding to produce a new crop variety.
- Molecular breeding and marker-assisted selection (MAS): Marker-aided genetic analysis studies showed DNA sequences to identify genes, QTLs (quantitative

trait loci), and other molecular markers and to associate them with organism functions, i.e., gene identification. Conventional breeding involves selection of individual plants based on perceptible or quantifiable traits. Scientists can use molecular-assisted markers, after examining the DNA of an organism in order to select plants that possess a pleasing gene, even in the deficiency of a discernible trait. The mainstream of MAS techniques is relies on the use of the polymerase chain reaction (PCR) and strengthening of selected genes or parts of genes and involves correlated technologies such as SSRs SNPs, AFLP, ISTR, RFLP and RAPD. Further techniques, such as diversity array technology (DArT) based on DNA microarray technology, are related to genetic mapping and diversity analysis. These molecular breeding technologies facilitate the identification, mapping and evaluation of plants which carries useful traits in a breeding population. Thus, breeding is more accurate and resourceful. The International Institute of Tropical Agriculture has used molecular markers to achieve cowpea resistant to bruchid (a beetle), disease-resistant white yam, and cassava resistant to cassava mosaic disease, in the midst of others (Ribaut et al. 2010). An additional use of molecular markers is to recognize undesirable genes that can be eliminated in future generations.

- The process of identification and inheritance tracing of previously identified DNA fragments through a series of generations is termed as marker-aided selection. Genomics implies the analysis of whole genomes of species along with other biological data about the species so as to have an idea as to what DNA confers what trait in the organisms. In the same way, proteomics works for an analysis of the proteins in a tissue to know the gene expression in that tissue and also to understand the specific function of proteins encoded by particular genes. Both of these, together with metabolomics (metabolites) and phenomics (phenotypes), are considered as the subcategories of bioinformatics (Coombs 1992; Zaid et al. 1999). Functional genomics may also be referred sometimes as molecular phenotyping. The sole aim is to implement high-throughput methodologies that includes proteomics, transcriptomics, and metabolomics, which can help in acquiring a more clear perception of the crop and its metabolic pathways that interrelates its traits with their respective genes, thereby enhancing the level of understanding of crop biology and also the scopes to target interventions in breeding. The total number of nations growing such crops reached up to 29 in 2010, four more than in 2008. Approximately 60% of the total transgenic crop area was in the USA, about 20% in Argentina; Canada, Brazil and China each had about 6%, whereas the rest of the world had less than 2% of the total area in 2005 (James 2004).

13.3 Classical Breeding with Induced Mutation

Mutations mean a manifestation in the genetic organization of a plant. Sometimes mutations occur naturally and result in the development of new as well as beneficial traits. In 1940, mutagenesis was introduced by plant breeders which means

mutations in a faster rate. Radiation or chemicals changes the plant's DNA which is the basic molecular unit of all organisms' genetic material. The prime goal is to bring a change in the sequence of the base pairs of DNA that provides a set of biochemical instructions for the development of plants. The resultant plants may contain new and desirable characteristics via this modification of their genetic structure. During this entire process, plant breeders must grow and thus accordingly evaluate each plant from each seed that is produced.

13.4 Agricultural Biotechnology Research and Commercial Technologies

13.4.1 Input Traits

The impact of plant biotechnology has been mainly on crops of high economic value to the developed world. In addition to that, there is little evidence from scientific studies that reveals about potential risks of genetic engineering. Organisms modified via transgenesis can offer a wide range of benefits compared to those that has emerged from innovations from conventional agricultural biotechnology. A few examples of benefits that has resulted from application of currently available methods of genetic engineering to agricultural biotechnology are elucidated. For example, in the year 1998, the five main transgenic crops were (in decreasing order of area) soybean, corn/maize, cotton, canola/rapeseed and potato. The transgenic soybean alone had an acreage of over 50%; wheat, sunflower and rice were also important. Herbicide resistance (71%) and insect resistance (28%) were the most common transgenic traits. These are almost the same as those of conventional plant breeding, apart from one or two exceptions. In generalized way, the goal falls into two major categories: augmenting crop performance in the field (so-called input traits) and developing new products with increased value (output traits).

So far, the transgenic crops that are most commercially developed features input traits, but in the next few years to come, the commercial release of such more crops with output traits is highly anticipated (Dunwell 2000).

13.4.1.1 Pest and Disease Resistance

In developing countries, the rate of agricultural crops are decreased due to harmful pest and disease caused by them. Genes responsible for resistance to pest and disease also have the potential to reduce crop loss as well as to eliminate the use of agrochemical pesticide may be subjected for genetic transformation.

13.4.1.2 Virus Resistance

Eighty percent of crops are destroyed by various plant viruses. DNA obtained from plant viruses are genetically engineered into crops in order to provide the crops a natural shield against viral disease. The state of "immunity" is passed on to the next generations of plants. Such modified plants from over 20 different plant varieties,

which are also resistant to more than 30 different viral diseases, have now been developed by using this modern technology (Yuan et al. 2011). A virus named Sharka is a disease that has come forward first in 1915 in Bulgarian plums. During World War II, Sharka was discovered in Hungary and quickly spread to Austria and Germany. The symptoms produced on exposure of the crops by this disease are leaf discoloration and rings or spots on fruit. Besides these symptoms also include premature fruit drop, deformed fruit, and discoloration of the skin and flesh. As a result of the infection, the fruit becomes unsuitable for direct consumption or industrial processing (dried, jams, or brandied). Measures have been developed to produce Sharka-resistant stone fruit trees using modern biotechnology tools that have given fruitful results in the USA, Austria and France. Another disease, i.e., tomato yellow leaf curl virus (TYLCV) first came into notice in European tomatoes and is spread by whiteflies (Christou et al. 1988). Symptoms of this disease include severe stunting, marked reduction in leaf size, flower abscission and significant yield reduction. In some particular regions, it has led to 100% yield losses which has ultimately forced netting use in greenhouses in many countries. Italian researchers have also conducted the process of transformation in tomato plants by incorporating a gene of the TYLCV.

13.4.1.3 Insect Pest Resistance

Majority of the crop produced by means of genetic engineering have been appreciated by farmers that currently a third of the corn and about three-quarters of the soybean and cotton which are grown in the USA are those of the varieties that are produced through genetic engineering. Twelve transgenic crops (corn, tomato, soybean, cotton, potato, rapeseed (canola), squash, beets, papaya, rice, flax and chicory) have been permitted for production on commercial basis in the USA. (Bur 2003). The most commonly grown are *Bt* corn and cotton and glyphosate-resistant soybeans. The widely accepted approach used to enhance plant resistance to insect pests is the *Bt* strategy. With an idea of increasing cotton yields, the Indian government approved the introduction of transgenic cotton variants with the Bt insect-resistant trait in 2002, mainly focusing on the withstanding capacity of the crop against pest such as the bollworm as well as lowering the pesticide requirements (Halpin 2005). The gene encoding the protein that is toxic to many insect pest is naturally present in the soil bacterium *Bacillus thuringiensis*, which is inserted into plants. The toxin is then produced inside the plants enabling themselves to be resistant to insect attack. Different varieties of *Bt* toxin with least difference in mode of action specific to different species variety of insects have been identified. The *Bt* protein is particularly effective against lepidopteran or caterpillar insects as well as the European corn borer (ECB), which is a major insect pest of field corn in the USA. The ECB takes up the Bt protein when it begins to feed on the plant. The toxin binds itself to the membrane of the gut, which leads to the death of the ECB larvae. Many different types of *Bt* corn have been recorded. The outcome of corn produced through *Bt* technology results in higher productivity and lowers the use of pesticide (Cooley et al. 1995).

13.4.1.4 Herbicide Resistance

The idea of developing transgenic plants having resistance to herbicide seems to be an unlikely goal. 70% of genetically modified crops have been cultivated globally (Kumar et al. 2006). The alteration allows the use of basic weed management strategies that are attractive to farmers. Herbicide resistance genes occur naturally in lower organism like bacteria and can be incorporated into plants. An example of such herbicide is Roundup™, which is broad spectrum in nature and has low toxicity to mammals and is also rapidly recyclable. Herbicide-resistant varieties of soybeans, cotton, corn, canola, and rice can now be found commercially. Herbicide-resistant wheat and sugar beet are likely to be introduced soon (Ives et al. 2011).

13.4.2 Output Traits

Herbicide tolerance and *Bt* genes both make a direct divergence in farming, but newer types of transgenic crops are foreseen with human health or environmental applications. Many transgenic varieties are being developed by different companies which produce transgenic varieties without the antinutritive or allergenic factors that some foods, such as peanuts, soy and wheat, naturally contain as well as other kinds of plants designed to improve health. Specific targets include plants that are developed so as to contain increased more quantity of nutritionally advantageous components (including lysine, methionine, zinc, iron, and vitamin A) or condensed undesirables, for example, trans fats. Other applications are aimed at plants that remediate heavy metals from polluted soils and plants with a more level of sugars to help in high productivity of feedstock for ethanol (Arawaka et al. 1998; Cockburn 2004; Jefferson-Moore and Traxler 2005). Out of these, only a handful of these varieties have been subjected commercialized, and none of these products has yet achieved "significant commercial acreage" (Williams et al. 2004).

13.4.2.1 Foods with Improved Nutritional Value

13.4.2.1.1 Enhanced Vitamin Content

Genetic engineering could also be used to produce crops that have elevated amounts of vitamins in order to improve their nutritional quality. The thickness of micronutrients, such as vitamin A and the minerals iron and zinc, can be augmented through genetic approaches. Genetically altered "golden rice," contains three transplanted genes that permit plants to manufacture beta-carotene, a complex that is converted to vitamin A. Vitamin A deficiency is the world's foremost cause of blindness—affects as many as 250 million children. It imparts a light yellow color to the grain; the beta-carotene is transformed to vitamin A after ingestion which gives nutrition to the person ingesting it (Herdt 1995; Toenniessen 2003; Lusk and Rozanb 2005).

13.4.2.1.2 Healthier Oils

Biotechnology is being used to adjust the content of many oil crops. A broad range of oil crops has been genetically customized, either to increase the amount of oil or to modify the types of oils they produce by these crop plants: oils having different degrees of "saturation" have dissimilar properties. Genetically improved soybeans have lower saturated fats but have high oleic acid and are more stable during frying without further processing. Certain oils, such as soybean and canola oils, have been developed to contain less saturated fat inside the human body.

13.4.2.1.3 Plants Producing Novel Products

Researchers are using biotechnology to expand edible vaccines in plants. These vaccines are genetically integrated into food plants, and they do not require any kind of refrigeration, sterilization equipment, or needles. This innovative technology will be in particular useful for delivering economically safe and highly effectual vaccines throughout the world. Researchers are mounting a vaccine against hepatitis in bananas and vaccines against *E. coli* and cholera in potatoes. In the USA, scientists have used genetic adaptation to develop potatoes that contain a vaccine for human papilloma virus (HPV). HPV, which is transmitted sexually, is the major cause of cervical cancer. Mainly in developing countries, a plant-based vaccine would be chiefly useful where customary vaccines are both complex and expensive to administer. Oilseed crops could supply farmers with value-added industrial crops that reinstate petrochemical-sourced industrial materials—e.g., valuable oils, such as gamma-linolenic acid.

13.4.2.1.4 Drought-Resistant Crops

Biotechnology has helped to increase crop yield by introducing such qualities as disease resistance and increased drought tolerance to the crops. At this time, researchers can identify and select genes for disease resistance from different species and transfer them to imperative crops. Though plant breeding tribulations are sometimes mentioned as an explanation for biotechnology, none is more commonly cited than drought. Commercial companies are as apprehensive as the public sector to build up more drought-tolerant cultivars and make out that the "potential benefits of combining genomic tools with conventional breeding have been a source of prevalent interest and resulted in copious efforts to achieve the desired synergy" (Chapman et al. 2000; Morgan 2000; Ishitania et al. 2004; Campos et al. 2004). Two extensive approaches are being used. In the first, one attempts to optimize phenotypic qualities, including deep roots, vigorous root systems, stomata control, osmoregulation and leaf epicuticular wax and in the second, to optimize reply to drought by manipulating manufacture rates of plant growth enzymes (Morgan 1999). Labors have been made to categorize molecular markers allied with drought tolerance and hundreds of genes induced by drought have been recognized (Lopez et al. 2002). Scientists from the Australian Centre for Plant Functional Genomics (ACPFG) and Department of Primary Industries at La Trobe University in Victoria are mounting frost-resistant cereal crop varieties using genes taken from the Antarctic hair grass. The final goal of this research is to breed

plants that can bear temperatures two degrees lower than at present available varieties, which would help make them less vulnerable to frost damage.

13.5 Conclusion and Future Agriculture Demands

Agricultural biotechnology has also been hailed as a key stratagem in raising world food supplies. The enhance will most commonly come from developing countries. Biotechnology clearly holds promise as a solution to some developing country production problem and to solve them in an environmentally friendly manner. Crop improvement can only be complemented by innovative crop management. The relevance of biotechnology is most liable to diminish yield variability but not to increase maximum yield. Different policies on intellectual property right, market concentration, and agricultural research are expected to take a place on the worldwide market then they have now on industrialized country. Besides, public sector agricultural research in many developing countries is rigorously underfunded and human capital development may not be satisfactory for the successful improvement of agricultural biotechnology. Agricultural modernization is nowadays progressively carried out by the private sector and public research projects are conducted in a different policy and market environment. The green revolution was motivated by public institutions for local markets and was operated through subsidies and protected markets. Agricultural novelty today is to a large amount driven by the private sector, exercising a much more inflexible IP protection. Agricultural markets are also, with some exceptions, to a much lesser degree under government control and less influenced by subsidies. Additionally this research stands a much greater chance of success if it is not performed but in partnership with scientist of developing countries and with real indulgent of the constraints (Lesser 2005).

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Recent Advances in Phytoremediation Technology

14

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Abstract

The rapid increase in the environmental contaminants due to various anthropogenic activities has become a serious issue worldwide. New and efficient measures are explored to remove or contain the threat from the increasing levels of environmental pollution. Plant-based soil and water remediation (phytoremediation) is one such method which can prove to be a sustainable and promising treatment to remediate environmental problems. Phytoremediation exploits the abilities of green plants to uptake, stabilize, or metabolize the pollutants. Moreover, it is a cost-effective and environmentally safe approach as compared to conventional methods to solve the problems of soil and water pollution. Phytoremediation technique has been successfully applied to treat various contaminated sites and pollutants such as heavy metals, dyes, fly ash, hydrocarbons etc. and furthermore, research is underway for exploring new ways to improve the phytoremediation process.

Keywords

Environmental • Phytoremediation • Pollutants • Heavy metals

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14.1 Introduction

Rapid industrialization with increasing urbanization has enhanced the levels of contaminants in the environment and has become a serious global concern. Over the past few decades, there has been a considerable increase in the pollutants due to anthropogenic activities. Therefore, it is necessary to find new and efficient remediation methods to remove, reduce, or stabilize toxic substances introduced into the environment. Pollutants can be both organic and inorganic chemicals/compounds which include heavy metals as the major constituent of inorganic contaminants, xenobiotic compounds, hazardous wastes, explosives and petroleum products (Nikolić and Stevović 2015). These harmful compounds are toxic by nature and can enter the food chain causing mutagenicity and carcinogenicity in animals and human (Afzal et al. 2014). Therefore, their removal from soil and aquatic ecosystem is one of the important issues in the field of environmental sciences and engineering.

Traditional methods of soil remediation, such as liming, washing, leaching, turning and deep plowing, are usually energy-consuming and require expensive machinery that often causes secondary pollution. Conventional physical and chemical methods for the cleanup of soil and water polluted with organic compounds are often costly and environmentally destructive. Plant-based soil and water remediation (phytoremediation) is a sustainable and promising treatment that uses plants to remediate environmental problems. Moreover, phytoremediation is considered as a cost-effective and environmentally safe approach to conventional methods to solve the problems of soil and water pollution. Application of phytoremediation in cities is an attractive initiative to meet the prospects of the community (Table 14.1). Covering a contaminated site with vegetation creates open green spaces which have shown to reduce stress levels in people, improving their mental health, particularly in urban communities. These open green spaces have been recognized as physically, mentally and socially good for our communities, making them healthier (Nikolić and Stevović 2015).

Based on economic implications, phytoremediation may be focused on three major goals: (1) plant-based extraction of metals with commercial values, i.e., Ni; (2) risk minimization (phytostabilization); and (3) sustainable soil management in which phytoremediation steadily enhances soil fertility to boost up crop growth with increased economic value (Mahar et al. 2016).

14.2 Definition of Phytoremediation

Phytoremediation is defined as the use of plants to remove, metabolize, degrade and immobilize transferor detoxifying contaminants such as metals, hydrocarbons, dyes, and toxic substances from the soil, water, or air through their natural metabolic pathways and functions. Phytoremediation exploits the abilities of green plants to uptake pollutants. It is a promising new technology which is low cost, long term, environmentally, and aesthetically friendly.

Table 14.1 Advantages and disadvantages of phytoremediation

S. no.	Advantages	Disadvantages
1.	Low investment cost and minimal equipment requirement (constitutes substantial savings)	Incomplete removal of pollutants with long-term low performance
2.	Lower labor costs and reduced cost in operations	Limited applicability to different types of wastes, especially with high-level toxicity wastes
3.	Can be applied in situ, i.e., on-site removal of contaminants, whether in the soil, water, or groundwater	Mainly applicable to the upper layer of the soil and mine tailings
4.	Aesthetically pleasing and widely accepted to the public community	Proper disposal of plant matter is required with proper risk assessment
5.	Nondestructive, nonintrusive, highly biologically active, therefore have very low environmental impact on soil and water	Possibility of introduction and spreading of undesirable invasive species of plants
6.	Reduces erosion of soils, especially thinner inorganic soils	Effectiveness of the phytoremediation process is affected by seasonal factors
7.	Reduces leaching of particulate matter and spreading of toxicants	Plant deaths may occur in highly toxic sites which could increase the cost of the process
8.	Contaminants can be recovered from the plant tissues and offer opportunity for commercialization	Risk of bioaccumulation of pollutants in the food chain
9.	Very effective at sites where low amount/toxic contaminants are present	Good cultivation practices and maintenance are required to avoid accidents
10.	Can be used for phytoremediation of soils that are nonproductive for agriculture	Better understanding of the behavior and physiological changes of plants in response to different types of wastes is needed

Filippis (2015)

Over the past few decades, it has been recognized as a sustainable means of detoxifying contaminated soil and water sources. Historically, phytoremediation was first identified as a natural process and proved more than 300 years ago, and the first plant species discovered to treat sewage waste were *Thlaspi caerulescens* and *Viola calaminaria* (Nikolić and Stevović 2015). Phytoremediation can be applied to detoxify areas with trivial pollution of metal, nutrients, organic matter, or contaminants. It is emerging as an efficient technique with widespread application of different plant species in detoxification of various types of wastes (Table 14.2). With the discovery of new plants species having phytoremediation potential and better understanding of their metabolic pathways, phytoremediation could be the solution to the challenges of the twenty-first century.

Table 14.2 Examples of plant species with phytoremediation potential

S. no.	Plant species	Pollutants	References
1.	*Cernuella virgata*	Zn, Cu, Mn, Fe	Nikolić and Stevovićc (2015)
2.	*Chromolaena odorata* (L.)	Hg2+, radionuclide pollutants	Nikolić and Stevovićc (2015)
3.	*Helianthus annuus* (L.)	Zn, Pb, Cd, Ni and Cu, As, radionuclides, especially U, xenobiotic	Nikolić and Stevovićc (2015)
4.	*Brassica juncea*	Pb, Au	Mahar et al. (2016)
5.	*Eleocharis acicularis*	Cu, Zn, Cd, As	Mahar et al. (2016)
6.	*Nasturtium officinale*	Acid Blue 92 dye	Khandare and Govindwar (2015)
7.	*Spartina maritima*	Ni, Zn	Curado et al. (2014)
8.	*Momordica charantia*	Disperse red 17, Disperse brown 1 dyes	Tahir et al. (2015)
9.	*Jatropha curcas*	POPs, heavy metals	Tripathi et al. (2016)
10.	*Ricinus communis*	Cd, DDT	Tripathi et al. (2016)
11.	Alfalfa (*Medicago sativa* L.)	TPH	Ndimele (2010)
12.	Indian grass (*Sorghastrum nutans*)	TPH	Ndimele (2010)
13.	*Sesuvium portulacastrum (L.)* L.	Reactive green 19A–HE4BD dye	Lokhande et al. (2015)
14.	Sunflower	Endosulfan	Mitton et al. (2016)
15.	*Cynara cardunculus* L.	Sewage sludge	Pandey et al. (2016)

Abbreviations: POPs persistent organic pollutants, *DDT* dichlorodiphenyltrichloroethane, *TPH* total petroleum hydrocarbons

14.3 Phytoremediation Techniques

Phytoremediation can be classified into different categories/techniques on the basis of their mechanism (Fig. 14.1) such as (1) phytoextraction, (2) phytostabilization, (3) phytodegradation (phytotransformation), (4) phytovolatilization, and (5) rhizofiltration.

14.3.1 Phytoextraction

It is mainly used for accumulation of metals and involves plant root uptake of metals and their translocation through the xylem to the shoots and leaves, which are

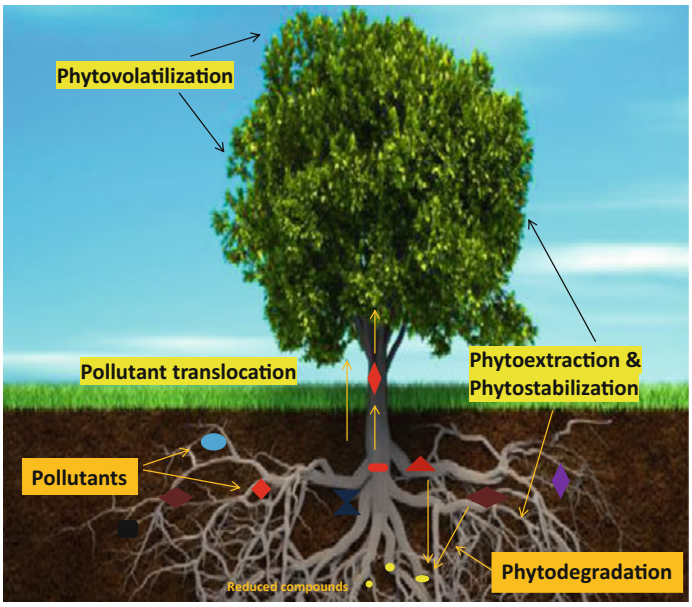

Fig. 14.1 Types of phytoremediation

then harvested and subsequently removed from the site (Nikolić and Stevović 2015). This technology is an advanced form of phytoremediation in which high-biomass crops are grown in the contaminated areas and harvested to recover heavy metals. Thus, it can be commercialized by mineral industries to produce metals using bioharvesting. It is, therefore, also called as phytomining or biomining. This technique has several advantages as compared to conventional methods, i.e., it is cost-effective and reduces the erosion of soil as well. On the other hand, there are several factors that limit the potential of phytoextraction such as metal bioavailability within the rhizosphere, which depends on soil pH and clay content and cellular tolerance to toxic metals. Sunflower (*Helianthus annuus*) has been one of the most exploited species for phytoextraction, because of its fast growth, high biomass, and high potential in the remediation of certain heavy metals and other toxic compounds (Nikolić and Stevović 2015).

14.3.2 Phytostabilization

This approach mainly uses plant roots to immobilize and restrict contaminants in soil and groundwater through adsorption on roots or precipitation within the root zone (rhizosphere). The main role of phytostabilization is confiscation of contaminants in the rhizosphere, but not in the plant parts. This process prevents the pollutants from migrating to the groundwater, hence reducing their bioavailability into the food chain. Thus, the future adverse effects of pollutants in the

surroundings can be controlled by restricting them from entering the groundwater or spreading in the air. This approach is useful at those contaminated sites where there is no available method for detoxification. It is a simple and cost-effective technique for stabilizing and reducing bioavailability of contaminants in the environment. However, the major disadvantage of this method is that contaminants are not removed from the soil and require a regular monitoring. Phytostabilization is a suitable technique to remediate Cd, Cu, As, Zn and Cr. In recent studies, perennial ryegrass (*Lolium perenne* L.) was observed as potential plant for the phytostabilization of mine-polluted soil and municipal solid waste compost and, to a lesser extent, sewage sludge (Nikolić and Stevović 2015).

14.3.3 Phytodegradation (Phytotransformation)

Phytodegradation includes plant metabolic system in association with microorganisms to detoxify heavy metals and organic pollutants such as herbicides, insecticides, chlorinated solvents and inorganic contaminants. It is also known as phytotransformation. In this approach, the pollutants are absorbed by plants and further broken down into insoluble and inert materials, which are either released as exudates or stored in lignin tissues. Pollutants can also be metabolized in the plant tissues with the help of enzyme-catalyzed metabolic process within plant root and/or shoot cells. The metabolic enzymes, e.g., dehydrogenases, oxygenases and reductases, transform pollutants into nutrients that can be utilized by the plants in their own metabolism. Contaminants thus are transformed, biochemically bounded to plant tissues, and become biologically less harmful to the environment (Nikolić and Stevović 2015).

14.3.4 Phytovolatilization

Phytovolatilization involves the use of green plants to absorb pollutants from contaminated sites, transform them into volatile compounds, and transpire them out through their aerial organs. It was observed in a study at USDA Agricultural Research Service that some plants are able to transform Se in the form of dimethylselenide and dimethyldiselenide in high-selenium media which can be transcribed out of the plants (Ahmadpour et al. 2015). Phytovolatilization has been used for contaminants like mercury (Hg); inorganic chemicals that have volatile forms, such as selenium (Se) and arsenic (As); as well as volatile organic compounds (VOCs), e.g., trichloroethene. The advantage of phytovolatilization is that toxic pollutant may be transformed into less toxic substance and then released into the atmosphere. However, the substance released into the atmosphere is likely to be recycled by precipitation and then redeposited into aquatic ecosystem, repeating the production of the toxic form of the substance (Nikolić and Stevović 2015).

14.3.5 Rhizofiltration

Rhizofiltration is the combination of phytoextraction and phytostabilization. This process involves absorption, concentration, and precipitation of contaminants in wastewater, surface water, or polluted groundwater by plant roots. The absorbed and concentrated pollutants are precipitated as carbonates and phosphates inside plant roots. Recently, it has been shown that hydroponically grown terrestrial plants, such as mustard and sunflower, effectively remove Cu, Cd, Cr, Ni, Pb, Zn and Fe from aqueous solutions. Besides metals, it was also observed that rhizofiltration can be used to remove organic compounds such as tetrachloroethane, trichloroethylene (TCE), metolachlor, atrazine, nitrotoluenesanilines, dioxins and various petroleum hydrocarbons. Terrestrial plants are more advantageous for rhizofiltration process due to their fibrous and much longer root systems. However, the plants need to be disposed regularly and harvested periodically which limits the scope of this rhizofiltration process (Nikolić and Stevović 2015).

14.4 Plant-Assisted Microbial Degradation

This phenomenon involves degradation of contaminants by microorganisms like bacteria and fungi which are present within the plant root zone or rhizosphere. The phytochemicals released by the plant roots, such as sugars, amino acids, enzymes and other compounds, are taken up by these microorganisms to enhance their metabolism and biological activity. In this way, plants accelerate biodegradation of contaminants by helping the soil microbes to flourish in their surroundings. These rhizosphere microorganisms can act directly on organic and inorganic pollutants through volatilization, transformation and rhizodegradation. For example, toxic metals like mercury (Hg) and selenium (Se) are phytovolatilized by microbial interactions with the plant. Furthermore, several bacterial species contain plasmids that have resistance genes to heavy metals and metalloids, e.g., Ag^{+}, Cd^{2+}, Hg^{2+}, Ni^{2+} etc. which can be genetically altered to enhance the removal of these toxic metals. In addition, soil microbes prevent phytopathogens from infecting plants by increasing biomass production, which in turn make the plants much more efficacious in phytoremediation (Nikolić and Stevović 2015).

14.5 Endophytic Bacteria

Endophytic bacteria are generally found in all plant species and inhabit different plant tissues without conferring pathogenicity to the host. Some of these bacteria may have beneficial effects to their host plant by several mechanisms which are similar to rhizosphere bacteria. These endophytic bacteria produce several enzymes that help in degradation of various organic compounds present in the rhizosphere and endosphere of plants growing in polluted environments. Endophytes may have several advantages as compared to rhizosphere bacteria in regard to the degradation

of xenobiotic compounds. They may degrade contaminants in both rhizosphere and endosphere as they are efficient colonizers of both the environments. Thus, they are likely to detoxify the plant environment more efficiently in such manner. Endophytes, in many cases, are efficient plant growth promoters and induce tolerance to abiotic stress in their host plants. Another significant advantage of endophytes is that the toxic compounds are degraded within the plant tissues, thus eliminating any toxic effects on herbivorous fauna residing on or near contaminated sites (Afzal et al. 2014). Several endophytes like *Enterobacter* sp. and *Pseudomonas putida* that have been isolated from common plants such as poplar (*Populus deltoides*) are able to degrade volatile organic compounds such as trichloroethylene (TCE).

14.6 Application of Phytoremediation

14.6.1 Role of Phytoremediation in Heavy Metal Removal

Heavy metals are considered as one of the most significant environmental pollutants that can have serious effects on soil and water quality, plant and animal growth, as well as human health. The generic term of "heavy metals" refers to elements that have metallic properties such as high specific gravity, density, conductivity, stability as cations, and an atomic number greater than 20 (Oosten and Albino 2014). For example, arsenic (As), cobalt (Co), chromium (Cr), silver (Ag), cadmium (Cd), copper (Cu), molybdenum (Mo), nickel (Ni), iron (Fe), mercury (Hg), manganese (Mn), lead (Pb) and zinc (Zn) are considered as heavy metals, also called as PTE (potential toxic elements).

Heavy metals enter the environment through two different ways that are from natural and anthropogenic sources. Natural sources constitute a little of the contribution, while anthropogenic activities such as mining, smelters, foundries, coal-fired thermal power plants, metal plating, tanneries and battery and paper industry are the major sources of the heavy metal contamination. Heavy metal contamination is a serious issue of global concern as these metals are persistent in the environment, unlike other organic material. Accumulation of these heavy metals in living organisms is of particular concern as these metals are carcinogenic and can bioaccumulate to higher toxic concentrations. Metal ions cause toxicity through the following mechanisms: (1) generation of reactive oxygen species (ROS) that reacts with antioxidants and cause oxidative stress; (2) direct interaction with proteins through affinity to different functional groups such as thionyl, histidyl, and carboxyl groups; and (3) displacement of essential cations in specific binding (Oosten and Albino 2014).

Therefore, there is an urgent need for the removal/stabilization of these heavy metals from the contaminated environment. Conventional methods are generally used but they have their own drawbacks. Phytoremediation can be used as an important alternative to the conventional strategies as it is cheap and eco-friendly. Most of the plant species have the ability to absorb/immobilize

metals. There are certain woody or herbaceous plants that can accumulate and tolerate higher levels of heavy metals in their tissues and are known as hyperaccumulator (Ahmadpour et al. 2015). These naturally occurring hyperaccumulator plant species are suitable candidates for phytoremediation. A few examples of the hyperaccumulator plant species are *Aeolanthus biformifolius* (Cu), *Berkheya coddii* (Ni), *Euphorbia cheiradenia* (Pb), *Hordeum* spp. (Hg), *Pteris cretica* (As), etc. (Mahar et al. 2016).

14.6.2 Phytoremediation of Textile Dyes and Effluents

Application of dyes in a variety of industrial activities such as pulp and paper processing, paint and pigment manufacturing, leather tanning, and textile dyeing results in dumping of dye containing effluents into water pools and surrounding industrial areas, which subsequently affects ground and surface water resources and soil properties as well. Dyes are complex aromatic compounds that are used for coloration of fabrics, leathers, papers etc. Natural dyes are extracted from plant or animal sources, while dyes that are produced by chemical synthesis are called as synthetic dyes. During the process of dyeing, large proportions of these dyes remain unbound or unfixed, and therefore end up as effluents in sewage water or natural environment. It has been estimated that 10–15% of these dyes are released as effluents into the environment during textile dyeing, whereas unreactive dye losses of up to 50% have been reported. Dye-based effluents and/or wastewater usually have higher concentrations of suspended solids, while the presence of dyes in water bodies along with posing turbidity problems also causes an increase in BOD and COD levels. Moreover, chromophoric groups of dyes strongly absorb sunlight, thereby inhibiting the photosynthetic activity of phytoplanktons including aquatic plants and algal species by preventing light penetration (Tahir et al. 2015). Thus, apart from destroying natural quality of water bodies, these dyes also threaten aquatic biota such as flora and fauna by disturbing the ecological balance and posing serious environmental concerns and hence need to be treated or removed prior to their disposal or dispersal into water bodies or surrounding environment. Conventional strategies are not that much promising; instead, recent studies have shown that a great deal of plant species are able to metabolize, absorb, or detoxify various dyestuff and colorants. Plant species like *Brassica juncea*, *Rheum rhabarbarum*, *Tagetes patula*, *Thymus vulgaris*, *Rosmarinus officinalis*, *Phragmites australis*, *Rumex acetosa*, *Typha angustifolia*, *Hydrilla verticillata*, *Nasturtium officinale*, *Petunia grandiflora* Juss., *Glandularia pulchella*, *Armoracia rusticana* (horseradish), etc. have proved to be potential degrader of synthetic dyes. *Typhonium flagelliforme* was found to have significant dye degradation competency (67%) while *Chara vulgaris* displayed 95% decolorization of Congo red, whereas biotransformation rates of approximately 60% and 40% have been achieved in the case of *Momordica charantia* against Disperse Red 17 and Disperse Brown 1 dyes. A few other examples are *Z. angustifolia*, *Brassica juncea*, *Blumea malcolmii*

Hook.f., *T. patula* and *Hydrocotyle vulgaris* which have shown potential for the removal of textile effluents (Tahir et al. 2015).

14.6.3 Removal of Fly Ash

Coal-based thermal power plants produce an enormous amount of coal fly ash (600 million tonnes/year) and are a major constituent of the air and land pollution. India is one of the largest producers of fly ash (FA) with much of its energy requirement (approx. 80%) achieved by coal-based thermal power plants (TPP). The disposal of FA causes significant health and environmental hazards, and thus its disposal and utilization have become a worldwide concern. A total of only 30% of the global FA production is being utilized annually which is far less than required. Moreover, mobilization of its harmful constituents and airborne particulate matter and high amounts of toxic/heavy metals possess a great risk to the ecosystem (Ghosh et al. 2014).

Additionally, measures such as landfill cannot be considered as an eco-friendly alternative due to its high concentration of heavy metal content and the presence of radionuclides. Therefore, phytoremediation can be used as a practical, economical, and eco-friendly alternative for revegetation and reclamation of FA dump sites. A recent study has shown vetiver (*Vetiveria zizanioides*) grass system to be capable of remediating FA by stabilizing the metals in the root. Vetiver system is tolerant to extreme environment and capable of protecting the upper layer of soil. Besides phytostabilization of the heavy metals, vetiver also plays a role in reduction of genotoxicity caused by fly ash (Ghosh et al. 2014).

14.6.4 Phytodegradation of Oil Hydrocarbons

Oil spills are one of the main sources of aquatic contamination as a result of petroleum production, transportation, refining, and accidents. They are the major cause of disrupting the aquatic ecosystems throughout the world. Petroleum is highly toxic and can prove to be lethal to living organisms. The heavy fraction of the crude oil mostly contains naphthene–aromatics and poly-aromatic compounds which are carcinogenic, and if exposed for longer periods, these compounds may lead to tumors and failure of nervous system. (Abha and Singh 2012). It is estimated that nearly 8 million metric tonnes of crude oil are released in the aquatic environment every year. For example, the oil spill of North Slope into Prince William Sound, Alaska, from the Exxon Valdez, 1989, led to the death of thousands of seabirds and marine fauna and many long-term environmental effects. Another devastating spill occurred due to the explosion of Transocean Deepwater Horizon rig in 2010, killing 11 people and threatening coastal Louisiana, Gulf of Mexico, and surrounding ecosystems (Ndimele 2010). Conventional strategies for cleaning oil spills are various physicochemical methods such as booming and skimming, manual removal (wiping), water flushing, sediment relocation, and tiling. Apart

from that, a range of bacteria, microscopic fungi, and yeasts are well-known degraders of oil hydrocarbons and are considered ecologically progressive approaches as compared to physicochemical treatments.

However, recent studies have observed that several species of plants have the ability to grow in the oil-contaminated sites and actually extract the contaminants from the area. A few examples are western wheatgrass (*Agropyron smithii*), prairie buffalo grass (*Buchlo dactyloides* var. prairie), soybean (*Glycine max*), etc. which have demonstrated potential to phytoremediate oil hydrocarbons. Grasses are considered to be superior candidates for phytoremediation as they have an extensive, fibrous root systems which can penetrate deep (3 m) in the soil. Water hyacinth (*Eichornia crassipes*) would also prove to be a promising agent for phytoremediation because it has fibrous root system like prairie grass system while it floats on the surface of the water. As most of the oil spills occur in the water bodies, an aquatic floating plant like water hyacinth is, therefore, of extreme importance. A recent study has shown that soybean (*Glycine max*) and sunflower (*Helianthus annuus*) could degrade motor oil from the soil-contaminated site (Ndimele 2010).

14.6.5 Phytoremediation of Landfills

The most common method of waste management practiced globally is disposal of waste to landfills with up to 95% of generated refuse dumped in landfill. Landfilling offers an easy and relatively inexpensive means of waste disposal; however, if not managed suitably, it can cause serious contamination of the surroundings due to release of various contaminants such as liquids, gases, or dust particles (Kim and Owens 2010). The major drawback concerning with landfill sites includes soil and groundwater contamination and gas emissions, which as a result have adverse effects on the human population as well as the plants surrounding the area. On the basis of waste disposed, landfill sites can be broadly categorized into three groups: (1) municipal waste, (2) industrial waste, or (3) a combination of municipal and industrial wastes. Municipal waste mostly contains a large range of organic waste, while industrial waste is composed of unknown components and may include certain toxic materials and heavy metal (Kim and Owens 2010). These components are leached subsequently from the landfill sites and can disrupt the surrounding ecosystem. Landfill gas is mainly composed of methane, carbon dioxide, carbon monoxide, oxygen, nitrogen, hydrogen and hydrogen sulfide in different proportions. The main components are methane and carbon dioxide, comprising between 40 and 60% of the total gas emissions from landfill and are considered harmful to the environment. Methane and carbon dioxide are well-established as greenhouse gases and contribute to global warming.

Recent studies have aimed at developing an alternative to the conventional engineering-based remediation methods, and phytoremediation has proved to be a promising technique as it has the advantages of being cost-effective, environmentally friendly, and less disruptive to the soil, thus maintaining the ecosystem. There

are two different approaches that can be used for phytoremediation of landfill sites: (1) contaminant extraction/degradation and (2) stabilization. In the first approach, plants grown in heavy metal-contaminated sites can accumulate heavy metals into different plant tissues through their uptake mechanism which can further be harvested and removed from the site. The second approach involves the stabilization and prevention of runoff and dust blow to surrounding areas with the help of plant vegetation. Further, plants can prevent inorganic contaminants from leaching to groundwater by stabilizing them around the root zone. Trees growing on landfill caps can also promote favorable environments for methane-oxidizing bacteria, thus reducing its emissions from landfills.

In particular, communities in residential areas would be more attractive toward phytoremediation technologies due to the environmentally friendly nature of such systems.

14.6.6 Removal of Radioactive Waste

Radiation exists all around us, and the radioactive substances emitting these radiations are beneficial to humans and society in certain ways. For example, radiations are utilized in scientific, medical, agricultural, industrial and energy generation programs. Therefore it is inevitable that these diverse activities lead to generation of radioactive waste. Radioactive waste can be solid, liquid, or gas from various group of operations and activities (nuclear power and uranium mining) and accidents (spills and reactor meltdown), but irrespective of its origin, radioactivity poses a great risk to humans, the environment, and the ecosystems. However, at present, there are no established permanent repositories and storage facilities for the most dangerous high-level nuclear wastes from nuclear power plants. The radiation leak that occurred due to the nuclear accident at Chernobyl, Ukraine, alone is believed to have increased the risk of cancer to humans by 0.1% (Filippis 2015). Current practices for removal of radioactive-contaminated soils are mostly focused on "excavation and dump" or "encapsulation," neither of which removes the contaminant from the soil. Immobilization and extraction by physicochemical techniques are expensive and can only be applied for small areas, where rapid, complete decontamination is required and the contaminant is moved safely elsewhere (Filippis 2015).Therefore, the low-technology in situ approach of phytoremediation is attractive, as it offers partial site restoration, partial decontamination, preservation of ecosystem, and physical structure of soils and is low cost. Moreover, it offers the possibility of biorecovery of useful radioactive nuclides from the plants. Phytoremediation has become a fast growing field of research and development for application to radionuclide waste. In recent studies, three different grass species, namely, *Paspalum notatum*, *Sorghum halepense* and *Panicum virgatum*, have been used to accumulate 137Cs (100+8 Bq g^{-1}) and 90Sr (112 +7 Bq g^{-1}) in soil contaminated with these radionuclides. Other examples are *Amaranthus retroflexus*, *Brassica juncea and Phaseolus acutifolius* which have shown potential in removal of radioactive nuclides (Singh et al. 2008).

Phytoremediation, although still an emerging technology for radioactive contaminated sites, has the potential for greater industrial acceptance due to its low cost and environment-friendly approach.

14.7 Genetic Engineering to Improve Phytoremediation

Genetic engineering is the manipulation of the genetic makeup of an organism using biotechnological tools. It is one of the important techniques that can be employed to improve the phytoremediation potential of plant species to a greater extent. Biotechnological approaches (genetic engineering) can be used to insert one or more effective accumulator genes from taller plants into other smaller plants, and thus the final biomass can be increased. For example, genes responsible for metal-scavenging properties of hyperaccumulating plants, such as *T. caerulescens*, can be inserted into high-biomass generating species, such as Indian mustard (*Brassica juncea*) or maize (*Zea mays*) to enhance their phytoremediation potential for commercial utilization. Recent studies have shown successful utilization of genetic engineering to manipulate metal uptake and stress resistance properties in various species. One example is the enhanced metal resistance in tobacco (*Nicotiana tabacum*), achieved by expressing the mammalian metallothionein metal-binding proteins. Currently, transgenic plant species are being produced using genetic engineering and are employed in phytoremediation of soil contaminated with methyl mercury (a neurotoxic agent). Transgenic tobacco and *Arabidopsis* are a few examples which express bacterial genes *merB* and *merA* and have the potential to remove mercury from the soil. The *merB* gene present in these transgenic plants carry out protonolysis of the carbon–mercury bond and thus liberate Hg^{2+}, a reduced mobile mercury species, and the *merA* gene converts Hg^{2+} to Hg^{0} (elemental Hg) which is a less toxic, volatile element and is liberated into the atmosphere (Malik et al. 2015). Genetic engineering can also be applied to the bacteria assisting plants in phytoremediation. In these bacteria, genes may be inserted for biodegradative enzymes, biotic and abiotic stress, metal uptake regulators and risk mitigation which, in turn, enhance phytoremediation. One such example is expression of phytochelatin synthase (PCS) genes from *Schizosaccharomyces pombe* into *Pseudomonas putida* KT2440 which resulted in recombinant strain KT2440-*spPCS* with increased resistance to Hg, Cd and Ag and a 3–5-fold increase in Cd accumulation, hence increasing the efficiency of phytoremediation (Yong et al. 2013).

These studies reveal that genetic engineering has a tremendous potential in increasing phytoremediation opportunities for different plant species.

14.8 Challenges for Phytoremediation

There are several challenges that need to be overcome to increase the use of phytoremediation for waste treatment. A few are listed below:

- Longer period (several years) required for remediation process.
- Phytoextraction efficiency of most of the hyperaccumulator plants is usually limited due to their low-biomass production and restricted growth rate.
- Climatic factors affect the accumulation capacity of some plants and in some cases, pests and disease can hinder the phytoremediation process.
- Invasiveness of some of the hyperaccumulator plant species which affect the indigenous flora diversity.
- Accumulation of pollutants into food chain if not handled carefully (Mahar et al. 2016).

14.9 Conclusion and Future Prospects

Environmental contamination is a serious global concern, and therefore efficient and economical remediation alternatives are necessary. Phytoremediation is an attractive approach with good community acceptance. It is an eco-friendly and solar-driven technology which is cost-effective and offers opportunity for its commercialization as well. At present, phytoremediation technology is in its early stages, and there are many technical problems that are needed to be addressed for its development. However, advancements in genetic engineering and development of innovative agronomic practices would greatly help this technique to be more effective and relatively simple. Besides that, there is an urgent need to discover and explore new hyperaccumulator plant species for their potential and mechanism of phytoremediation. A thorough research is required for the optimization of processes, understanding the dynamics of plant–pollutant interactions, plant–microbe interactions and means of proper disposal of waste with minimum damage to the environment. The role of various additives and other factors that influence the phytoremediation process is still needed to be explored. Moreover, the use of applied molecular techniques and development of transgenic plant with enhanced phytoremediation activity are gaining widespread acceptance; therefore, genetic engineering is expected to play an important role in boosting the applicability of phytoremediation technologies. Studies concerning these strategies would be very helpful in the development of easier and cost-effective tools for phytoremediation.

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Microbial Flora and Biodegradation of Pesticides: Trends, Scope, and Relevance

15

Ridhima Arya, Raman Kumar, Navnit Kumar Mishra, and Anil Kumar Sharma

Abstract

Pesticides, although proving as a fast remedy in pest control, are polluting the environment in a number of ways acting as havoc to mankind and environment. The presence of pesticides above tolerance level has raised concerns about their removal from soil and environment through novel ways like microbial bioremediation. The present book chapter highlights about the microorganisms and their degradation pathways used in removal of a number of pesticides like carbendazim, chlorpyrifos, endosulfan, and sulfosulfuron. There are a number of living and nonliving factors such as pH, temperature of soil, and availability of degrading microbes. Research has been done on isolation of pesticide-degrading microbes, which could act as an efficient and novel bioremediation agents in the future like *Brevibacillus borstelensis* and *Streptomyces albogriseolus* that have the ability to remove carbendazim and sulfosulfuron.

Keywords

Bioremediation • Carbendazim • Degradation • Pesticides • Sulfosulfuron

15.1 Introduction

A pesticide is a chemical compound, such as lindane, parathion, thymol, and heptachlor or even a biological agent like virus or bacteria as per defined by the Environmental Protection Agency, USA. Pests are living organisms which damage the crops, humankinds, or other animals. These may include insects, fungi, mice, other animals, unwanted weeds, and even microorganisms such as bacteria and

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viruses. The vast increase in use of pesticides, herbicides, and insecticides in agriculture as well as increased industrialization has led to ecological contamination around the world. Pesticides and their degradative products in the top layer of soil have become a serious threat not only to humans and animals but to the soil microbes especially the nitrifying and ammonifying ones. It is considered that the rate with which pesticides are being used at present would malign the environment rendering it unfit for human health. Most of the applied pesticides approximately 20–70% and their breakdown products that are percolated to the soil cause many undesired effects to the environment (Arya et al. 2015).

For controlling the huge range of growing pests, great arrays of pesticides have been used in food production technologies (Osteen and Livingstion 2007; Ghaly and Dave 2012; Ahemad and Khan 2012a, b). In a survey of food commodities, 51% pesticide contamination were detected; however, 20% was found to be pesticides above the maximum tolerance level. The presence of pesticides above tolerance level has raised an alarm for human health concern (Selvaraj et al. 2014). A number of pesticides like aldrin, chlordane, ethyl mercury chloride, methomyl, carbofuran, benzyl hexachloride, 2,4-T, endosulfan, and many more have been banned by the Government of India. While others like DDT, lindane, methyl parathion, and diazinon have been restricted in use, use of pesticides has raised an alarm as these have shown adverse health effects on even nontarget organisms including man. Breakdown of pesticides occurs in soil and water and could be caused by plants, microbes, other chemicals in environment, and UV radiations as well. But the most important type of degradation occurs by microorganisms especially fungi and bacteria. In literature, previous studies have shown that the microbes use pesticides as supply medium and energy source and obtain essential elements from them.

Nowadays, herbicides/weedicide like fenoxaprop-p-ethyl, clodinafop, and sulfosulfuron are efficiently used in weed crop for weed control (Chhonkar and Malik 2002). The use of these herbicides/weedicides especially sulfosulfuron at more than recommended doses or imperfect calibration and wrong methods of application have raised a concern about the health hazards for animals and humans because of the residues left in soil and crops after the pesticide application. The use of benzimidazole fungicides started way back in 1960s and had increased thereon. These fungicides are found to be efficient at low doses and act by inhibiting cell division and thus play a crucial role in modern agriculture (Maltby et al. 2009).

The most commonly used systemic fungicides include thiabendazole, benomyl, thiophanate-methyl, fuberidazol, and carbendazim (Delp 1987). The excessive use of these fungicides has sharply reduced the resistance of healthy crop plants against pathogen attack (Medina et al. 2007; Garcia et al. 2001). Using of pesticides symbolizes the agricultural development where they are used to control pests and vectors, but the environmental and health hazards caused by them have raised a concern on the excessive use of these harmful chemicals. Increase in environmental contamination is the result of excessive use of pesticides in agriculture which causes the long-term harmful effects to human health (Bhanti and Taneja 2007). Contamination of foods associated to pesticides' used in agriculture has increased a

major concern of human health such as nausea, headaches, reproductive problem, cancer, and endocrine disorder (Berrada et al. 2010). In countries like India, Colombia, Argentina, Zimbabwe, Mexico, and Kenya, floriculture has increased nowadays because of the optimization of greenhouse conditions (Illing 1997; Ribeiro et al. 2012). In the greenhouse production of medicinal herbs and vegetables, application of carbendazim increased nowadays making it compulsory to discover the ways to remediate carbendazim from the contaminated soil and environment. Likewise, sulfonylurea herbicides are very much persistent in environment and are used more often. As compared to conventional herbicides, the herbicides from sulfonylurea group have showed higher potency even at low concentration (Brown 1990). Moreover, it has been observed that even at lower concentration of herbicides as low as 1% causes the damage to sensitive plants (Beyer et al. 1987).

15.2 Classification of Pesticides

Pesticides is a class of agrochemicals including a large group of chemical compounds classified into various subclasses of herbicides, fungicides, insecticides, rodenticides, garden chemicals, etc. based on their target.

They can be classified as botanical, synthetic, and inorganic pesticides. These are also divided on the basis of their mode of action, targeted pest species, and their chemical composition.

Pesticide classification has been done according to the type of pest as follows:

- Algicides are used to control the algae.
- Avicides are used to control the birds.
- Bactericides are used to control the bacteria.
- Fungicides are used to control the fungi.
- Herbicides are used to control the weeds.
- Insecticides are used to control the insects.
- Molluscicides are used to control the slugs and snails.
- Nematicides are used to control the nematodes.
- Rodenticides are used to control the rodents.

Pesticide classification on basis of mode of action:

- Systemic or noncontact
- Nonsystemic or contact

Systemic pesticides are those pesticides such as 2, 4-D and glyphosate, which are absorbed through the plant tissues efficiently and reach to the vascular system of plant showing its consequence. The nonsystemic or contact ones are those pesticides which target the pest like paraquat when they come in contact without entering in plant tissue.

Pesticide classification on basis of chemical composition:

- Organochlorines
- Organophosphates
- Carbamates
- Pyrethroids

Organochlorine pesticides contain five or more chlorine atoms in their structure. In agriculture they were the first synthetic pesticides. Examples include DDT, endosulfan, Lindane, and aldrin.

Organophosphates consist of a phosphate group in their chemical moieties. Examples include parathion, glyphosate, and malathion.

Carbamates are carbamic acid derivatives. Examples include carbofuran and aminocarb.

Pyrethroids are the chemical molecule, which is an analogue of pyrethrins, secondary metabolites of flower chrysanthemum. Examples include cypermethrin and deltamethrin.

15.3 Mode of Formulations of Pesticides

Pesticides are marketed as various formulations. Pesticide formulation is composed of an active ingredient and an inert ingredient. The active chemical moiety in a pesticide controls the target pest. Most of the pesticides also consist of chemically inert ingredients, which mainly reduce their toxicity for human handling making them more effective; usually, they are diluted in water, a petroleum-based solvent, or other diluents. Formulations are further of two types: liquid and dry.

15.3.1 Liquid Formulations

These formulations are in liquid form and further of following types:

1. *Emulsifiable concentrates (EC)*

These formulations consist of one or more organic solvents, an active liquid ingredient, and a chemical agent which allows the formulation to be emulsified with water. Usually, 25–75% of the active ingredient is present in one gallon of EC.

2. *Solutions (S)*

An active counterpart of some pesticides is easily solubilized either in water or organic solvent. After forming a solution, they do not easily sediment or cannot be separated. Further, they are of the following three types:

(a) *Ready to Use (RTU)*: These agrochemical solutions hold the recommended amount of solvent. Therefore, there is no need to dilute these solutions before application. These formulations contain small amounts of active ingredient usually.
(b) *Concentrate Solutions (C or LC)*: These agrochemicals should be further diluted with either organic or inorganic solvent application. Occasionally, the solvent is liquid; more often the solvent could be a petroleum-based solvent or refined oil.
(c) *Ultra-Low Volume (ULV)*: These concentrate solutions could have 100% of the active ingredient. These can be used by diluting with a small quantity.

3. *Flowables (F)*

Flowables are the suspension, which contains active ingredients in a liquid solvent with inert ingredients. Mostly, the suspensions are prepared with water.

4. *Aerosols (A)*

Aerosol formulation consists of few active ingredients in a liquid solvent. However, the amount of the active ingredient is very low.

5. *Formulations for Smoke or Fog Generators*

These types of formulations are made to be disintegrated into aerosol using a machine which uses a rapidly moving disk or heated surface.

6. *Invert Emulsions*

Invert emulsion is a mixture of pesticide in 40% water added in oil. In this emulsifier pesticide is dispersed in oil/water suspension, which forms large droplets that hinder their slide.

15.3.2 Dry Formulations

These formulations are in dry powder forms and further of following types:

1. *Dusts (D)*

The dust formulations consist of dry inert carrier such as clay, talc, and volcanic ash mixed with lesser amount of active ingredients (0.5–10%). They are often applied dry and easily dispersed on the target or nontarget sites.

2. *Baits (B)*

This formulation consists of an active ingredient added in food. The bait attracts the pests, and when they consume it, they die due to the presence of pesticide in it. Normally, the amount of active ingredients in the bat formulations is less than 5%.

3. *Granules (G)*

Granular formulations are similar in some extent to dust formulations; moreover, this formulations contain larger and heavier granular particles. These granular particles consist of absorptive material like walnut shells, corncobs, or clay. These formulations are made up of 1–15% of active ingredient.

4. *Pellets (P or PS)*

Most of the pellet formulations are similar to granular formulations. Moreover, in the pellet formulation, the active particles are of alike shape and weight.

5. *Wettable Powders (WP or W)*

Wettable powders are similar in some extent to dust formulations. Prior to application they must be mixed with water. This formulation contains 5–95% of active ingredient. As they do not dissolve in water, therefore, constant mixing is applied to maintain the suspension.

6. *Soluble Powders (SP or WSP)*

These formulations are alike of wettable powders. They form the true solution when dissolved in water. The formulation consists of 15–95% of active ingredients.

15.4 Effect of Pesticides on Environment, Man, and Other Living Organisms

Residue pesticide levels for 253 different pesticides in 100 samples of 13 different dried vegetables were tested in Seoul, Korea, and residual pesticides were found in exceeded MRLs in 2 samples out of 11 agricultural products tested and 1 dried pepper leaf sample (Seo et al. 2013). In Iran, pesticide use has increased in previous years as insecticides being used are 33% followed by herbicides 30%, fungicides 20%, acaricides 6.2%, rodenticides 3.8%, and nematicides 1.5% (Sara et al. 2013). China is the global leader in the use of pesticides since the 1990s (Wang 1999) with the use of chemical pesticides found to be threefold greater than in developed countries (Zhang 2001; Yu 2006). Application of pesticides worldwide has guaranteed production potential, but their heavy use, persistence, and transfer in cross-ecosystems and trophic food webs have caused major environmental contamination (Pimental 2007;

Ackerman 2007). Pesticide application has led to changes in soil nutrient levels and alterations to soil microbial activity, diversity, as well as genetic structure (Girvan et al. 2004; Ros et al. 2006). A major impact of herbicides is on aquatic environment which enters by spray drift, runoff, and leaching to field drains which then passes into the food chain (Davies et al. 2003). For the sustainable agricultural fertility and productivity, soil health with special reference to biological features maintaining the functions of both natural and managed ecosystems is very much required (Enriqueta-Arias et al. 2005).

The most widely used active ingredient in the benzimidazole carbamate fungicides has been carbendazim or methyl-2-benzimidazole carbamate (MBC), which has both protective and curative activities against fungal pathogens. The fungicidal property of carbendazim has been embattled by disruption of microtubule formation and stopping mitotic cell division (Foster et al. 1987). The residues of carbendazim have been detected from orange (Shen et al. 2009) and sandy soil (Yarden et al. 1985). A study carried out on degradation and dissipation of pesticides such as carbendazim, difenoconazole, and azoxystrobin in the pomegranate fruit has shown the residues of carbendazim and difenoconazole in outer rind of the pomegranate. All these pesticides, viz., azoxystrobin, difenoconazole, and carbendazim, have been found to follow the first-order kinetics for their dissipation (Utture et al. 2011). About 208 litchi soil samples of Guangdong area of China have been investigated by the authors for the detection of nine pesticides, viz., cyhalothrin, mancozeb, cypermethrin, metalaxyl, dichlorvos, dipterex, deltamethrin carbendazim, and dimethoate. Cypermethrin, mancozeb, metalaxyl, and cyhalothrin along with carbendazim ranges from 3.4 to 59.1% (Yao et al. 2010).

Residues of pesticides have been determined in tomato samples in Bogota, Columbia. Among pesticides carbendazim, acephate, dimethomorph, and pyrimethanil are among the frequently detected ones (Arias et al. 2014). Study carried out by Hernandez et al. (2012) on soil samples and surface waters used for cultivation of rice crop from different sites of Usosaldaña, Colombia, revealed the occurrence of fungicides like azoxystrobin, carbendazim, epoxiconazole, propiconazole, and herbicides like atrazine, diuron, and insecticides such as thiacloprid. Residues of ten pesticides have been detected in paddy rice, eight in rice bran, and seven in brown rice. These residues are obtained after industrial processing of paddy rice from the 14 pesticides including carbendazim evaluated for their persistence in the cropping and processing of rice crop in the season 2009–2010 in Uruguay (Pareja et al. 2012). In 204 samples of 19 different vegetables, 215 pesticide residues have been monitored, and most commonly detected pesticides are organophosphorous followed by pyrethroids, triazoles, and carbamates.

Carbendazim (methyl-1*H*-benzimidazol-2-yl-carbamate) or MBC has mutagenic and teratogenic effects in animals even at low concentration and can harm the liver and endocrine system (Zuelke and Perreault 1995; Moffit et al. 2007; Rajeswary et al. 2007; Yu et al. 2009). After an oral exposure to carbendazim, it gets well absorbed (80–85%) and subsequently metabolized into many compounds within the organism, and main metabolites include 5-hydroxy-2-benzimidazole

carbamate and 5, 6-hydroxy-2-benzimidazole carbamate-N-oxides. These metabolites are poorly catabolized in humans and animals and thus retained in tissues, such as gonads, liver, skin, adrenals, adipose, and other organs as reported by WHO in 1990. Carbendazim at higher doses reduces sperm production in male rats and fetal viability in female rats. Alteration in morphology of sperm, weight of testis and epididymis, sperm motility, and post-implantation losses were also observed (Gray et al. 1990). Carbendazim is investigated to be behind the benomyl-induced toxicity of testes as well as inhibition of the microtubular assembly of the testes in rats. Within an hour of carbendazim administration, sloughing of the seminiferous tubules starts and reaches severity in 2 h (Lim and Miller 1997). A number of abnormalities in sexual differentiation and reproduction are found to occur because of endocrine disruptor chemicals. In a study on human, ovarian granulose-like cells possessing high levels of aromatase activity is carried out to demonstrate the effect of benomyl, the only known benzimidazole fungicide, and a microtubule-interfering agent, which is found to induce aromatase activity. This activity is presumed to be mediated by its metabolite, carbendazim (Morinaga et al. 2004).

Benomyl, isothiocyanates, captan, iprodione, and carbendazim have been found to inhibit the respiratory and fermentative metabolism in yeast (Chiba et al. 1987). Quinlan et al. (1980) studied the effect of carbendazim on the cell cycle and nuclear division in yeast *Saccharomyces cerevisiae* and found it to result in the accumulation of large doublets of cells, spindle and cytoplasmic microtubule disappearance, alteration in morphology of spindle polar bodies, increase in nuclear size, and showing inhibition of microtubule polymerization. Carbendazim causes loss of mitotic chromosomes at high frequency, disruption of the mitotic spindle structure, and function along with nondisjunction of chromosomes in *Saccharomyces cerevisiae* (Wood 1982).

The effects of carbendazim and chloramphenicol on the soil bacterial/fungal ratios and on soil enzyme activities both singly and together were studied, and it was found that carbendazim had an inhibitory effect on the bacterial/fungal ratios. The inhibitory effect of chloramphenicol on neutral phosphatase was found to be increased in the presence of carbendazim (Yan et al. 2011a, b). Effects of carbendazim, 2, 4-D, and atrazine were studied on rhizospheric soil of groundnut crop, and it was found that the total counts of bacteria, fungi, and actinomycetes were lower in treated soil than in the untreated soil along with reduction in number of *Rhizobium*, *Azospirillum*, and phosphate-solubilizing bacteria. Also there was reduction in soil enzyme activities (Mohiuddin and Mohammed 2014).

A study was conducted on Canadian prairies for checking dissipation behavior of some herbicides like tribenuron-methyl, metsulfuron-methyl, rimsulfuron, thifensulfuron-methyl, ethametsulfuron-methyl, sulfosulfuron, and nicosulfuron from cropland to surrounding aqueous systems. Of these, three most persistent pesticides, viz., metsulfuron-methyl, sulfosulfuron, and ethametsulfuron-methyl, are among the majority of detected pesticides in swamp sediments (Degenhardt et al. 2010). A study was conducted on the activity, adsorption, mobility, and field persistence of sulfosulfuron in a silty clay loam and sandy loam soil. There was an

increase in activity of sulfosulfuron observed with the increase in sulfosulfuron concentration, which is slightly greater in sandy loam soil than silty clay loam soil (Eleftherohorinos et al. 2004). A sensitive and very fast analytical method was developed for simultaneous detection of 16 sulfonylurea herbicides including sulfosulfuron in surface water (Yan et al. 2011a, b). Residues of the sulfosulfuron and their harmful effects were detected in crops including sunflower, canola, bean, soybean, lens, sorghum, pea, sugar beet, corn, barley, and sorghum (Hadizadeh 2010).

A survey during the crop season of 2005–2006 was conducted on 286 farmers belonging to different districts of Punjab regarding bio-efficacy of herbicides used by the Punjab farmers for the control of *Phalaris minor* in wheat, and it was disclosed that 38.5% farmers use sulfosulfuron and 36.0% used clodinafop in agriculture. However, 13.6% farmers used unrecommended herbicides. It was discovered that more than 27% farmers use unrecommended herbicides or unapproved brands of recommended herbicides, and more than 19% of the farmers were found to use under- or overdoses of herbicide (Walia and Brar 2006).

Herbicide residue analysis for isoproturon, clodinafop-propargyl, fenoxaprop-p-ethyl, and sulfosulfuron in samples of postharvest soil, grain, and straw of wheat was analyzed by HPLC in a field experiment carried out at Gwalior, M.P., India, and higher values for isoproturon and clodinafop were detected (Arora et al. 2013).

15.5 Degradation of Pesticides in Soil, Water, or Environment by Abiotic and Biotic Factors

The pesticides undergo a complex series of interdependent reactions following their release in environment collectively called chemodynamics of pesticides. Abiotic factors like pH, salinity, temperature, moisture, precipitation, light intensity and topography, and inherent physicochemical properties affect the chemodynamic processes of pesticides. Major fate of pesticides is in the form of transportation, retention, degradation, and biota uptake. Degradation is the important path of environmental removal of pesticides, which entails the chemical degradation, photodegradation, and microbial degradation.

Pesticide degradation is the breakdown or chemical transformation of pesticide molecules into simpler forms that are less toxic as compared to the parent molecule. Sometimes, the molecules converted still remain toxic like that of the case with DDT. The DDT is converted to DDD, which is also toxic and acts as a pesticide. Chemical transformation of pesticides normally occurs in soil due to various interactions with soil components. These reactions are of oxidation, reduction, and hydrolysis type.

Photodegradation of pesticides occurs in the presence of sunlight as a result of rupturing of chemical bonds. Photocatalytic degradation of various pesticides like carbendazim, chlorpyrifos, simazine, and acetochlor has been investigated to form different degradation compounds resulting from the loss of the chloro, hydroxyl, and alkyl groups along with cleavage of the amide, ester, amino-alkyl, and alkyloxy

bonds finally leading to deamination and opening of the ring (Kiss and Virag 2009). Effective phototransformation of carbendazim has also been studied (Abdou et al. 1985; Panades et al. 2000).

Microbial degradation is the breakdown or transformation of pesticides by microorganisms present in soil, water, or air. Rate of degradation depends on the nature and amount of pesticide present in soil, microbial population in soil, and the abiotic factors of soil like temperature, pH, salinity, moisture, aeration, and organic matter. Pesticides are acted upon by bacteria, fungi, and other microbes which probably use them as a substrate for carbon or energy source. Some examples include bacterial genera like *Pseudomonas*, *Clostridium*, *Bacillus*, *Thiobacillus*, *Achromobacter*, etc. and fungal genera like *Trichoderma*, *Penicillium*, *Aspergillus*, *Rhizopus*, and *Fusarium*, etc. which are playing an important role in the degradation of the toxic chemicals or pesticides in soil (Kaufman 1987). A number of isolates capable of carrying out some form of degradation of carbofuran have been isolated from soils, and several bacterial taxa were recorded for the same including *Pseudomonas* sp. (Parekh et al. 1995), *Flavobacterium* (Chapalamadugu and Chaudhry 1991), *Achromobacter* (Chaudhry and Ali 1988), *Arthrobacter* sp. (Ramanand et al. 1988), and *Sphingomonas* sp. (Feng et al. 1997). Microorganisms serve as important agents to detoxify these harmful chemicals which affect human and animal health, helpful soil microbes, and crop production (Kale et al. 1989). A newly classified strain *Brevibacillus laterosporus* has been observed to use as biological control agent in crop field against bacterial brown strip of rice caused by *Acidovorax avenae* subsp. avenae (Li et al. 2015).

Carbendazim is known to be degraded up to 99.1 and 87.1% by a bacterial strain, a member of *Pseudomonas* sp., isolated from soil in mineral salt medium containing 10 ug/ml and 1 ug/ml, respectively (Fang et al. 2010). Carbendazim removal efficiency was found to increase effectively by combining carbendazim-degrading bacteria *Bacillus subtilis*, *Paracoccus* sp., *Flavobacterium*, and *Pseudomonas* sp. with *Sedum alfredii* and Cd (Xiao et al. 2012a, b). Carbendazim degradation along with effects of environmental factors by strain *Bacillus pumilus* (NY97–1) has been detected by HPLC. Detected organic nitrogenous sources were found to have higher role in degradation of carbendazim than the inorganic nitrogenous sources which were showing negative impact (Zhang et al. 2009). *Azospirillum brasilense* and *Rhodococcus erythropolis* discovered were found capable of using carbendazim as a lone nitrogen or carbon source for growth (Lin et al. 2011). *Streptomyces* sp. M7 was isolated from organochlorine pesticide contaminated sediment and was capable of degrading lindane up to a concentration of 300 ug/ml showing increase in the growth as the pesticide concentration increased from 100 to 300 ug/ml. There is increased degradation activity when the strain is used in a consortia containing *Streptomyces* sp. A2-A5-M7-A11 (Fuentes et al. 2010). A novel carbendazim-degrading actinobacterium *Rhodococcus jialingiae* sp. nov. djl-6-2 was isolated from the sludge of a wastewater (containing carbendazim) treatment facility present in Jiangsu province, China (Wang et al. 2010). Wheat soil has been used for the isolation of a proficient carbendazim-degrading bacterium *Brevibacillus borstelensis*, which was found to degrade carbendazim effectively in

48 h and degradation products, 2-aminobenzimidazole and 2-hydroxybenzimidazole, were detected (Arya and Sharma 2014a, b). Bacterium identified as *Streptomyces albogriseolus* after biochemical and morphological analysis was found to degrade MBC in a time-dependent manner from the initial concentration of 29.12 μg/ml–2.86 μg/ml and 0.63 μg/ml in 24 h and 48 h, respectively. LCMS/MS analysis showed the presence of metabolite, 2-aminobenzimidazole, after 10 h of growth which eventually disappeared after 24 h of growth (Arya and Sharma 2014a, b). When both the above isolated strains were grown together, they were found to be more efficient in the removal of carbendazim, with nearly zero in 10–12 h of growth. LCMS/MS studies further confirmed the presence of various metabolites (Arya and Sharma 2016).

The degradation of various organophosphorous and carbamate pesticides including carbendazim in the tropical freshwater was studied and found that degradation rate was increased as the pesticide reached to sediment after leaching out from water in the post-monsoon water. The effect of pH and organic matter on the rate of degradation was also observed (Bhushan et al. 1997). Carbendazim transformation induced by hydroxyl radicals generated by the UV photolysis of H_2O_2 in dilute aqueous solution has also been investigated previously (Mazeilier et al. 2002). Adsorption of carbendazim was found to be inversely proportional to pH range in soil in a study carried out on determining the effect of pH (3–7) on adsorption of carbendazim in three mineral agricultural soils, namely, Hypereutric Camisol, Haplic Luvisol, and Hyperdystric Arenosol (Paszko 2012). The capacity of carbendazim for adsorption in peat, montmorillonite, and soil is dependent on the organic matter, nitrogen, and clay content, as well as on the cation exchange capacity (Cancela et al. 2006).

A study was carried out on the adsorption and biotransformation of the two pesticides, carbendazim and iprodione, singly and together, and it was observed that carbendazim leads to reduction in adsorption of iprodione by 70%. Carbendazim had negative effect on transformation of iprodione and reduced it by 26%, while iprodione had a very little effect on transformation of carbendazim (Leistra and Matser 2004). The effect of environmental factors on the degradation capability of a microbial consortium for degradation of fungicide, carbendazim, herbicide, and 2, 4-D was studied for 2 months in a continuous column reactor. The study has been investigated for different flow rates and consistent ability of the consortium for 6 months (Nagase et al. 2006).

The photodegradation of carbendazim was found to be enhanced with increase in pH and increase in dissolved O_2 concentration (Panades et al. 2000). The extraction of pesticides like dimethoate, malathion, methyl parathion, carbaryl, carbofuran, and carbendazim was evaluated by HPLC followed by their persistence and degradation studies. High Ca content, moderate moisture, and higher pH enhanced degradation and the presence of organic matter leading to increase in persistence of the pesticides (Thapar et al. 1995).

The effect of physical parameters like soil moisture, cadmium, and the microbes on the degradation profile of carbendazim in the paddy soil has been studied earlier under lab conditions (Xiao et al. 2012a, b). Half-life of carbendazim was found to

be 12.6–13.8 times more in sterilized soils than in nonsterilized soils. It was found to decrease to 46.2–74% if soil moisture gets increased by 40–80% (Xiao et al. 2012a, b). Half-life of carbendazim was found to decrease by 32.1–52.4% in the presence of low levels of cadmium, while it decreased to nearly 34% in the presence of carbendazim-degrading strains along with cadmium (Xiao et al. 2012a, b). The absorption, desorption, and mobility of a pesticide is influenced by the coexistence of the other pesticides like carbendazim, imidacloprid, and atrazine in the soil (Jin et al. 2013).

The half-life of sulfosulfuron was detected to be 28 days in high pH soil and 11 days in low pH soil. After 120 days, 14% and 5% of the sulfosulfuron remained in high and low pH soils, respectively (Brar et al. 2006a, b). Investigations were done on the effects of pH on the hydrolysis pattern of some sulfonylurea herbicides in soil and aqueous solutions. Also functional relationships between pH versus hydrolysis rate constants, temperature, and the presence of minerals were analyzed (Sarmah and Sabadie 2002). The stability of sulfosulfuron was studied in a controlled environment of pH, temperature, solvent, and surface, as well as in alkaline and acidic conditions (Saha and Kulshrestha 2002). Sulfosulfuron was found to have a half-life of 93 days in unsterilized soil and 120 days in sterilized soil (Brar et al. 2006a, b). The photocatalytic degradation of five sulfonylurea herbicides, viz., chlorosulfuron, nicosulfuron, flazosulfuron, triasulfuron, and sulfosulfuron, was studied, and their degradation followed first-order kinetics and none of the pesticides were detected after 120 min. of illumination except chlorosulfuron (Fenoll et al. 2012).

An experiment done across Canadian Prairies has shown the long persistence of sulfonylurea herbicides in artificially created farm dugouts. These herbicides were found to be resistant to hydrolysis showing their slower microbial degradation (Cessana et al. 2006). The dissipation of sulfosulfuron in water along with its bioaccumulation in fish has been investigated. The dissipation rate followed first-order kinetics, and the metabolites, ethyl sulfone, aminopyrimidine, desmethyl sulfosulfuron, sulphonamide, guanidine, and rearranged amine were detected in water and fish samples by LCMS/MS analysis (Ramesh et al. 2007).

15.6 Pathways for Degradation of Pesticides

Reaction of dissolved oxygen in the environment with pesticides is called oxidation. Oxidation process can be accomplished by singlet oxygen, ozone, hydrogen, peroxide, and other hydroxyl radicals. Hydroxyl radicals are considered the primary agents that bring about chemical oxidation of pesticides in water or atmosphere. For example, DDT shows both reduction as well as oxidation reactions in the soil with the help of *Enterobacter aerogenes* under UV light in the presence or absence of iron catalyst to form DDE and DDD as well as dichlorobenzophenone. Carbendazim transformation by UV/H_2O_2 is a second-order reaction, and it was observed that hydroxyl radicals get quenched with the generation of carbonate radicals (Mazellier et al. 2003).

When a pesticide undergoes reduction in its oxidation state, the chemical reaction that persists is called reduction of pesticides. The reducing agents in the environment are usually H+ve. As an example malathion performs reduction in acidic/aquatic environment that continues by the replacement of any ethyl group with H^+ resulting in the construction of two functional isomeric molecules of malathion monoacid.

Acephate is degraded to methamidophos detected in HPLC and LCMS/MS studies by aerobic bacteria belonging to genus *Pseudomonas*, and no further degradation indicates the capability of bacteria to breakdown at initial steps only (Pinjari et al. 2012). A strain *Bacillus subtilis* was isolated by Xiao et al. (2015) capable of degrading beta-cypermethrin efficiently along with some other pesticides like deltamethrin, beta-cyfluthrin, and cypermethrin. Seven metabolites were detected in beta-cypermethrin degradation pathway.

Pseudomonas sp. RPT 52 discovered by Gupta et al. (2016) was capable of degrading imidacloprid, Coragen, and endosulfan in a time range of 40 h. Degradation kinetics studies showed first-order kinetics for imidacloprid and endosulfan, while zero-order kinetics for Coragen. Rotary drum and windrow composting of vegetable waste resulted in removal of pesticides, endosulfan, and aldrin by degradation into metabolites chlorendic acid and chloroendic anhydride by epoxidation reaction and oxygenation of carbon bridge of aldrin and the presence of endosulfan sulfate and dehydration reaction resulting in dieldrin and hydroxychlorodene formation (Ali et al. 2016).

The degradation of various organophosphorous and carbamate pesticides including carbendazim in the tropical freshwater was also studied and found that degradation rate was increased as the pesticide reached to sediment after leaching out from water in the post-monsoon water. The effect of pH and organic matter on the rate of degradation was also observed (Bhushan et al. 1997).

Photolysis of carbendazim along with degradation products, viz., 2-aminobenzimidazole and two unidentified compounds, were reported in a study carried out on phototransformation by UV photolysis, and kinetics of photodecomposition was studied using HPLC-diode array (Boudina et al. 2011). Carbendazim is hydrolyzed to 2-aminobenzimidazole and then changed to benzimidazole, 2-hydroxy benzimidazole, by a novel actinobacterial strain *R. jialingiae* djl-6-2 (Zhichun et al. 2010). Rajeswari and Kanmani (2009) proposed the mechanism of carbendazim degradation and deduced its pathway using TiO_2-based photocatalysis and ozonation process.

Arya and Sharma (2016) suggested the degradation of carbendazim by the isolated strains *Brevibacillus borstelensis* and *Streptomyces albogriseolus* together reduced carbendazim to benzimidazole and 2-hydroxybenzimidazole in 12 h of growth. Carbendazim could first be converted to 2-aminobenzimidazole as in the case of *Brevibacillus borstelensis*, which is very rapidly converted to benzimidazole or 2-hydroxybenzimidazole. 2-amino benzimidazole could also have acted as an intermediate. 2-hydoxybenzimidazole and benzimidazole could be converted very rapidly to catechol and then even to CO_2 after ring cleavage (Fig. 15.1).

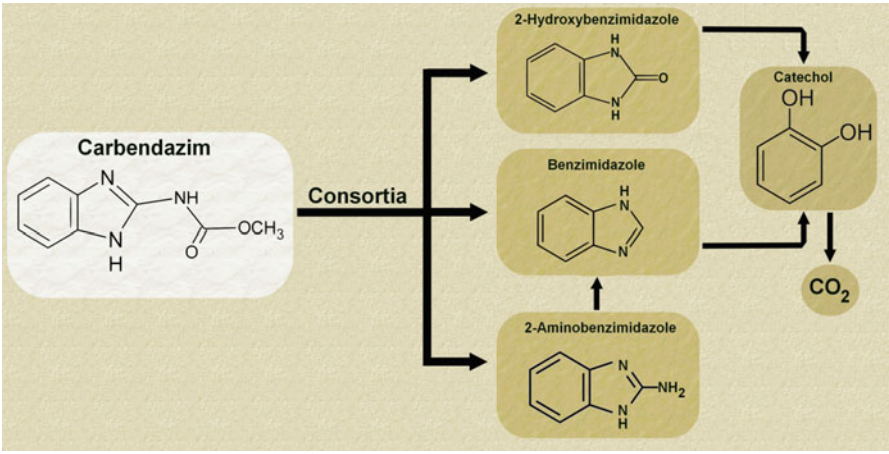

Fig. 15.1 Proposed pathway for degradation of carbendazim (Arya and Sharma 2016)

Sulfosulfuron [1-(2-ehtylsulfonylimidazo [1,2-a]pyridine-3-ylsufonyl)-3-(4,6-dimethoxypyramidin-2yl) urea] degrades in alkaline conditions to the metabolite, 1-(2-ethylsulfonylimidazo [1,2-a]pyridine)-3-(4,6-dimethoxypyramidin-2-yl) amine. However, in acidic conditions, the metabolites formed are 1-(2-ethylsulfonyl imidazo [1, 2-a] pyridine)-3-sulfonamide and 4, 6-dimethoxy-2-aminopyramidine. Metabolites formed by photodegradation are similar to acidic hydrolysis because of the cleavage of sulfonylurea bridge, while in alkaline conditions, contraction of bridge was found (Saha et al. 2003).

Ramesh et al. (2007) investigated the presence of metabolites, ethyl sulfone, aminopyrimidine, desmethyl sulfosulfuron, sulphonamide, guanidine, and a rearranged amine in water and fish samples by LCMS/MS analysis. A fungus *Trichoderma* was isolated from contaminated soil of wheat rhizosphere by Yadav and Choudhury (2014), which was able to degrade sulfosulfuron up to concentration of 2 g/l. In LCMS analysis, the authors observed presence of metabolites, 2-amino-4,6-dimethoxypyrimidine and 2-ethylsulfonyl imidazo{1,2-a} pyridine-3-sulfonamide-2-ethylsulfonyl imidazo{1,2-a} pyridine-3-sulfonamide, N-(4,6-dimethoxypyrimidin-2-yl)urea, N-(4,6-dimethoxypyrimidin-2-yl)-N-″-hydroxyurea (IV) and N, N″-bis(4,6-dimethoxypyrimidin-2-yl)urea. Carbendazim-degrading bacterial strains *Brevibacillus borstelensis* and *Streptomyces albogriseolus* isolated by Arya and Sharma (2016) has also been found to degrade sulfosulfuron to 2-aminopyrimidine and a rearranged amine in their growth individually as well as together with same effectiveness (unpublished work). Novel bacteria identified as *Pseudomonas* sp. has been isolated from carbendazim-contaminated soil which was found to decrease the degradation half-life of MBC to 3.06 days from 14.15 days. HPLC studies revealed the presence of 2 aminobenzimidazole, 2-hydoxybenzimidazole, and benzimidazole.

15.7 Genetic Studies

Bacteria, identified as *Brevibacillus borstelensis* AG1 on the basis of phenotypic, biochemical, and molecular characteristics (using 16S rRNA gene sequencing technique), were isolated from Marcha (fermentable local wine in Northeast India). This bacterium produces a bacteriocin-like inhibitory substance which has been tested against six food-borne/spoilage-causing pathogens, viz., *Listeria monocytogenes* MTCC 839, *Clostridium perfringens* MTCC 450, *Bacillus subtilis* MTCC 121, *Staphylococcus aureus*, *Lactobacillus plantarum*, and *Leuconostoc mesenteroides* MTCC 107 (Sharma et al. 2013).

In previous studies, it has been discovered that major pathways for degradation of aromatic compounds is to bring about by a number of enzymes converting to some of the intermediates normally leading to catechol and finally finding an entry to tricarboxylic acid cycle (Chaudhry and Chapalamadugu 1991; Clarke 1982; Commandeur and Parsons 1990; Fewson 1988; Reineke 1984; Reineke and Knackmuss 1988). The catabolic genes present on plasmid NAH7 codes for enzyme degrading naphthalene via an intermediate salicylic acid which are present on nah and sal operons. Toluene-degrading genes todF and todJ were discovered encoded by tod operon (Horn et al. 1991). Catechol-degrading *cat* genes and protocatechuate-degrading *pca* genes have been identified in different species showing varied patterns (Doten et al. 1987; Hughes et al. 1988; Ornston et al. 1990).

Bacterial species like *Pseudomonas putida*, *P. cepacia*, and *P. aeruginosa* are suggested to have a family of intradiol dioxygenases enzymes with subgroups of catechol dioxygenases, protocatechuate dioxygenases, and chlorocatechol dioxygenases (Aldrich et al. 1987; Ornston et al. 1990). *P. mendocina* KR1 contains toluene-4-monooxygenases enzyme complex which converts toluene to p-cresol (Yen et al. 1991). The main enzymes for transformation reactions of halogenated aliphatic compounds were hydrolytic dehalogenases normally classified in two categories, viz., haloalkane dehalogenases and 2-haloacid dehalogenases which were detected in *Pseudomonas* sp. and some other organisms as well (Schneider and Frank 1991).

15.8 Conclusions and Future Perspectives

Overall, it has been seen that the microbial flora has great impact on biodegradation of pesticides. Several scientific studies have demonstrated their potential to break-down the hazardous chemical moieties of pesticides. There are several health issues associated with the application of pesticides like chlorpyrifos, endosulfan, carbendazim, sulfosulfuron, etc. These moieties have been identified in a number of samples collected from different fields near to their application. This review emphasized the use of natural microbial flora involved in the remediation of these harmful pesticides and elucidated the mechanistic view of their degradation.

Microorganisms are involved in breakdown of a number of pesticides like carbendazim, chlorpyrifos, endosulfan, sulfosulfuron, etc. through their metabolic activities. The breakdown of pesticides in the soil depends on a number of living and nonliving factors like pH, temperature of soil, and availability of degrading microbial flora. Scientific studies have proven the pesticide-degrading capacity of *Brevibacillus borstelensis*, *Streptomyces albogriseolus*, and other microorganisms, which could easily break down the hazardous chemical moieties of pesticide.

These bioremediation of pesticides enables them to be an excellent natural biota for further investigating their microbial and molecular evolution. Moreover, the biochemical pathways attributed to these degradations should be clearly understood. The resolution of these metabolic pathways requires metabolite analysis of pesticide degradation. Most of these bioremediation carried out through the microbial enzymes by their catalytic activities. The enzymes itself is of biotechnological interest for growing their recombinant model to produce large-scale inoculums. This is of utmost importance to get acquatinted with their sequence, structure, and function associated with those genes involved in breakdown. Upstream coding sequences are of much relevance as the significant variance in operon sequences is of concern. Furthermore, these breakdowns of hazardous chemical moieties via nanoparticle formation should be evaluated, which further enhances the catalytic breakdown rate.

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Biosensors: A Tool for Environmental Monitoring and Analysis

16

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Abstract

Biosensor is an analytical tool that consists an immobilized biological component to react with analyte; subsequently, the produced biological signal is converted to a readable signal with the help of a transducer. Biosensors are of great importance because of their several advantages over the conventional techniques in the field of analysis. Biosensors are researched and applied in several diverse areas, such as health, medicine, defense, agriculture and food safety, industry and environmental monitoring, etc. Present chapter provides an overview of application of biosensors in the field of environmental analysis and monitoring. Strategies developed involving different biocomponents, bioassay principle, transducers, and their application for different groups of analytes; for example, pesticides, BOD, heavy metals, and other categories of environmental pollutants have been discoursed. Future trends and commercial aspects of environmental biosensor have also been discussed.

Keywords

Environmental biosensors • BOD • Heavy metal • Pesticide • Toxicity

16.1 Introduction

A biosensor is defined by the International Union of Pure and Applied Chemistry (IUPAC) as a self-contained integrated device that is capable of providing specific quantitative or semiquantitative analytical information using a biological recognition element, which is retained in direct spatial contact with a transduction element (Thevenot et al. 2001).

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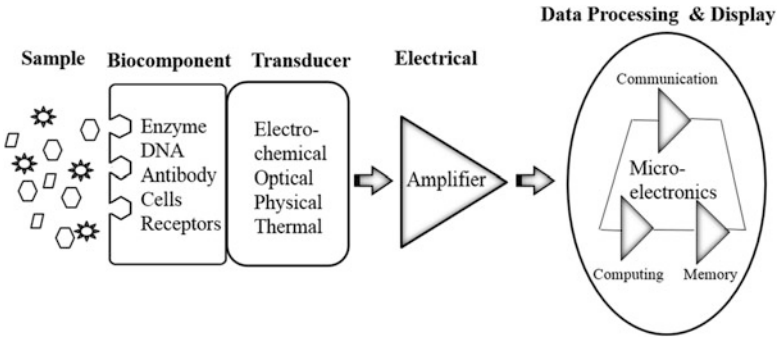

Fig. 16.1 Basic working principle of biosensors

Biosensor is an analytical tool that consists of an immobilized biological component (e.g., enzyme, DNA, cell organelle, whole cells or tissue, etc.) to interact with an analyte, in close proximity to a transducer with a readout device to convert the complicated biological interaction in an easily readable signal. (Fig. 16.1). Hence, biosensors exploit a biological element to make use of its natural specificity and sensitivity.

Biosensors constitute three essential components:

1. *Biological component*: Biocomponent interacts with the analyte present in the sample thereby shaping the basis of analysis. Therefore, selection of a biocomponent to develop biosensor for a particular analyte is vital for its success. Biocomponent can be a biological material or biological derivative.
2. *Transducer*: Biological signal produced from the interaction between biocomponent and analyte is converted to an electrochemical, optical, or some physic-chemical signal depending on the transducer. Selection of a transducer largely depends on the type of interaction and the biological signal produced, e.g., whether there is a change in pH after interaction, mass difference, activation and/or inactivation of enzyme etc. Combination of a transducer with the kind of interaction outlines bioassay principle of the particular biosensor.
3. *Readout Device*: Finally, the signal processed and displayed by the readout device with the associated microelectronics e.g., plate reader, electrochemical analyzer or fiber-optic spectrofluorometer.

The key benefits of biosensors over the conventional techniques (Rogers and Gerlac, 1999) are as follows:

- Rapid and continuous measurement
- High specificity and sensitivity
- Very less usage of reagents required for calibration
- Portability

Biosensors are usually named either after the biocomponent used (e.g., enzyme biosensors, whole cell biosensors or DNA biosensors, etc.), transducers (e.g., optical biosensors, electrochemical biosensors, etc.), or on the basis of analytes (e.g., pesticide biosensor, heavy metal biosensors, etc.).

A fast, sensitive, easy to handle, and cost-effective technique has always been a requisite; increasing number of potentially harmful pollutants in the environment amplify this requirement for extensive monitoring programs. Additionally, the increasing social concern, legislative actions for environmental pollution control over the last few years, forced the scientific community toward the development of biosensors for environmental applications.

16.2 Biocomponents and Bioassay Principles

The selection of biocomponents has its own importance in the development of a biosensor as it has to interact with the analyte. Specificity and sensitivity of a biosensor directly depend on its biocomponent. The selection of biocomponents largely depends on the application of biosensor; at one time specificity is required, other times it may not (e.g., toxicity), and one time a qualitative measurement is required, other time the requirement may be of a quantitative measurement. The following are the popular biocomponents that have been used for developing environmental biosensors.

16.2.1 Whole Cells

Whole cells are generally used as a biocomponent in developing biosensor for monitoring general toxicity or specific toxicity triggered by one or more pollutants. Whole cells are also used in the creation of biosensor for general quality parameters like BOD.

In addition to that, the cells are genetically engineered to respond against a particular pollutant. Various recombinant whole cell biosensors have been developed that use a whole cell carrying a genetically engineered reporter gene, which express only upon exposure to the pollutant/analyte. The response can be measured qualitatively or quantitatively depending on the set of promoter and reporter. Promoters, which are activated by toxic or hazardous chemicals, can be fused to reporter genes and inserted in a cell to monitor the presence of those chemicals. Variety of whole cell biosensors have been developed for different pollutants by quantifying light, fluorescence, color, or electric current. Bacterial cells have mostly been of choice and used because of their rapid growth, ease of genetic adjustments, and relatively fast response; however, eukaryotic cells have also been used occasionally (Yagi 2007).

16.2.2 Enzymes

Enzymes are one of the most exploited biocomponents for developing biosensors. Bioassay principle is usually based on its activity or inhibition; the analyte is either the substrate or inhibitor of enzyme activity. Thus activity of enzyme is correlated with the concentration of analyte and is measured amperometrically, potentiometrically, or optically (Cosnieret al. 1994; Liu et al. 1993; Nader et al. 1990). Enzyme biosensors are prepared by attaching an enzyme to the surface of electrode by different immobilization methods. Such systems typically involve the catalysis of redox reactions where either the substrate or the product is electrically charged. In addition to enzyme activity, enzyme inhibition has also been used as bioassay principle for development of biosensor for the analytes that have inhibitory effect for a particular enzyme (Verma et al. 2010 2011).

16.2.3 Antibody

Antibodies are produced in response to stimuli by foreign substance (antigen). Antibodies have a natural specificity for a particular site of that antigen and specifically bind to that site. If an antigen has multiple sites, antibodies elicited for a particular site are called monoclonal antibodies, while collection of antibodies elicited for different sites is called polyclonal antibodies. Both types of antibodies have been used to build electrochemical immunosensors. Antibody-based biosensors are known as immunosensors. Biosensors based on antibodies have seen a great development in analytical determination of organic micro-pollutants (Mallat et al. 2001).

16.2.4 Receptor

A receptor is a structure on the surface of a cell (or inside a cell) that selectively receives and binds a specific substance. Receptors can be adsorbed on the working electrode surface using several methods: capture behind a membrane, a polymeric matrix, or bilayer lipid membranes (Badihi-Mossberg et al. 2007). This type of affinity biosensor makes use of specific interactions between a biological receptor and an analyte (Borisov and Wolfbeis 2008).

16.2.5 Bacteriophage

A bacteriophage usually named as a phage is an intracellular parasite that infects specific bacterial species. Specificity of phages in the host selection is used for the constructing of a sensitive biosensor. In a lytic cycle, the linkage of phage-specific identification and the release of the inner enzymatic cell markers after the lysis of

the cell provide a powerful tool as a highly specific detection method of a given bacterial strain (Neufeld et al. 2003).

16.2.6 Aptamers

Aptamers are nucleic acid (DNA or RNA) that selectively bind to low molecular weight organic or inorganic substrate or to relatively big macromolecules. The affinity constant of aptamers toward their specific targets is reported in the micromolar to picomolar ranges, comparable to the binding constant of antibody and antigen affinity interaction. Nucleic acids, as a biological polymer for the storage and propagation of genetic information, retain extraordinary structural and functional characteristics (Hayat and Marty 2014). Aptamers being nucleic acid strand can be very easily immobilized on the transducer electrode surface so chip-based, portable, and miniaturized electrochemical systems can be designed accordingly.

16.2.7 Liposome

A liposome is an artificial spherical vesicle composed of a phospholipid bilayer surrounding an aqueous cavity, originally developed to study cell membranes. Liposomes offer a great potential to be used in environmental monitoring with its ability to bear different molecules in its cavity. Variety of molecules can be attached with liposome with different techniques, e.g., encapsulation, electrostatic interaction with the polar head or partitioning within the lipid, etc. For the purpose of monitoring an analyte, liposome enclosing a marker can be tagged with antibodies, haptens, or DNA (Ahn-Yoon et al. 2003).

16.3 Transducers

As mentioned above and as per definition of biosensor, biological component has to be in close proximity to a transducer so that result of the biological interaction between analyte and the biocomponent is converted to a quantifiable signal (Fig. 16.2). Selection of transducer in the development of a particular biosensor depends on the nature and consequence of biological interaction between the analyte and biocomponent.

16.4 Biosensors for Environmental Applications

As results of technological development of human being, a wide range of man-made chemicals and by-products from industrial processes has been still released in the environment. Some of these substances, such as pesticides, heavy metals, or PCBs, are well-recognized contaminants known to affect the quality of

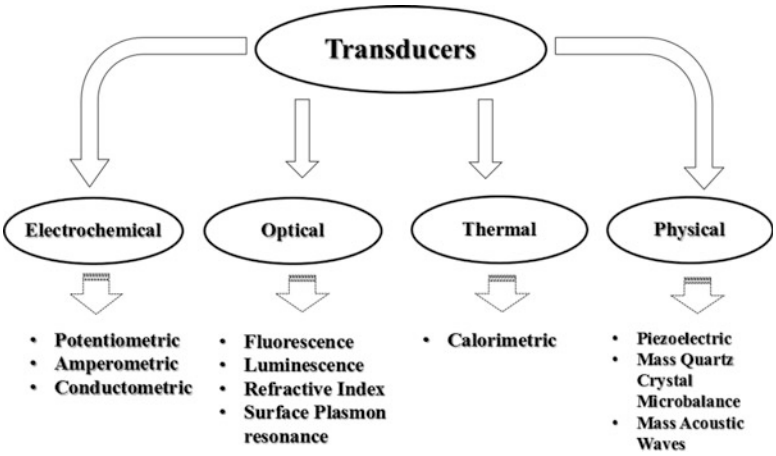

Fig. 16.2 Different categories of transducer used for constructing biosensors

the environment (Rogers 2006). Other than analysis of specific chemical, rapid estimation of general quality parameters, such as the BOD, toxicity, and assessment of contamination by pathogenic organisms, has also been a concern for the development of biosensor. Therefore, a range of biosensors have already been developed and applied for their environmental determination. The following section describes biosensors developed for monitoring variety of different environmental concerns.

16.4.1 Biochemical Oxygen Demand (BOD)

Biochemical oxygen demand (BOD) is defined as the oxygen required oxidizing the organic wastes over 3 days at 27 °C; BOD is a widely used parameter to indicate the amount of biodegradable organic material in water. Although conventional method has certain benefits like being a universal method, measure wide variety of wastewater samples and no expensive instrument is required; it has limitation like time consumption, unsuitable for online monitoring. With the help of biosensor, rapid determination of BOD has been achieved.

Several BOD biosensors have been developed based on amperometric oxygen electrode transducer modified with microorganisms that degrade or metabolize organic pollutants. Microbial strains that have been used for developing amperometric BOD biosensors include *Torulopsis candida*, *Trichosporon cutaneum*, *Pseudomonas putida*, *Klebsiella oxytoca* AS1, *Bacillus subtilis*, *Arxula adeninivorans* LS3, *Serratia marcescens* LSY4, *Pseudomonas* sp., *P. fluorescens*, *P. putida* SG10, thermophilic bacteria, *Hansenula anomala*, and yeast (Lei et al. 2006). Because any given strain has a limited substrate spectrum, single-strain BOD biosensor has limitations in analyzing complex samples (Reshetilov et al. 2013). BOD biosensors require microorganisms of low selectivity and high bio-oxidation activity for a wide range of organics, so that they can be used to monitor process effluent and

wastewater from different sources (Jia et al. 2003). This bottleneck is improved by using a mixture of two or more microorganisms to broaden the substrate spectrum and thereby analyte spectrum to have a stable performance (Tan and Wu 1999; Rastogi et al. 2003).

For the development of electrochemical biosensor for BOD measurement, microbial cells are immobilized with different methods onto a membrane; the membrane is placed in close proximity to the oxygen-sensing electrode (e.g., Clark electrode), and thereby the change in oxygen concentration (as a result of decomposition of organic pollutants by microbial film) is sensed by the electrode; this type of operating principle is known as oxygen electrode-based film biosensor (Reshetilov et al. 2013). Various examples of biosensors developed for estimation of BOD are summarized in Table 16.1.

Optical transducers have also been used to develop BOD biosensor; Kwok et al. (2005) immobilized microbes on a film (microbial film), placed in close proximity

Table 16.1 Biosensors developed for estimation of BOD

S. no.	Biocomponent (microorganism)	Transducer type	Limit of detection (LOD) (mg/l)	References
1.	*S. cerevisiae*	Electrochemical (screen-printed carbon electrode)	6.6	Nakamura et al. (2007)
2.	Activated sludge FIA5–25 mg/L	Electrochemical (Clark oxygen electrode)	0.2	Kumlanghan et al. (2008)
3.	Seawater microorganisms	Optical (fluorescence)	4	Dai et al. (2004)
4.	*P. putidas* and optical fiber sensor from ASR Co. Ltd.	Optical (fluorescence)	0.5	Chee et al. (2000)
5.	*B. subtilis*	Optical (luminescence)	25	Kwok et al. (2005)
6.	Activated sludge	Optical (luminescence)	60	Kwok et al. (2005)
7.	Microbial consortium	Electrochemical (microbial biofuel cell)	Successfully measure BOD below 240 mg/l in real wastewater samples	Hsieh and Chung (2014)
8.	Microbial consortium	Electrochemical (Clark oxygen electrode)	1	Dhall et al. (2008)
9.	*P. syringae*	Electrochemical (dissolved oxygen electrode)	5	Kara et al. (2009)
10.	BIOSEED	Electrochemical	5	Tan and Wu (1999)
11.	Yeast *SPT1* and *SPT2*	Electrochemical	2	Rastogi et al. (2003)

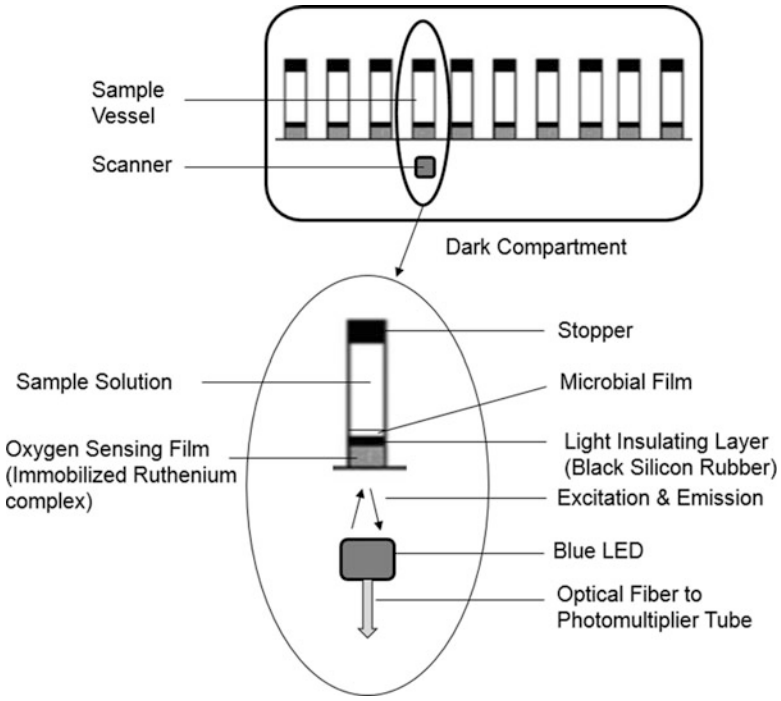

Fig. 16.3 Schematic diagram showing working mechanism and the oxygen-sensing part of the BOD biosensor developed by Kwok et al. (2005)

to an oxygen-sensing film comprised of tris(4,7-diphenyl-1,10-phenanthroline) ruthenium(II) dye (Ru(dpp)) the luminescence intensity of which varies with oxygen concentration; luminescence intensity is thereby correlated to BOD of the sample. Figure 16.3 is a schematic representation of biosensor developed by Kwok et al. (2005).

16.4.2 Commercial BOD Biosensors

Most of the BOD biosensor designs described in the literature are not commercialized and remained breadboard models. Nisshin Electric Co., Ltd. in 1983 marketed first commercial biosensor; till date several models of BOD biosensors have been commercialized by different companies, e.g., Central Kagaku Corp. (Japan), Dr. Lange GmbH, Aucoteam GmbH, and Prufgeratewerk Medingen GmbH (Europe). Models of BOD biosensor marketed by Central Kagaku Corp. (Japan) include HABS-2000, Quick BODα1000, and BOD-3300. BOD-3300 measures BOD in the range 0–500 mg/l within 30–60 min. The weight of BOD-3300 model is around 210 kg. Quick BODα1000 model of BOD biosensor is able to determine BOD in the range of 2–50 mg/l in 60 min, and the weight of the

device is 16 kg. The cost of BOD-3300 model and Quick BODα1000 model are around 80,000 USD and 30,000 USD, respectively (Reshetilov et al. 2013).

16.5 Pesticides

According to Environment Protection Agency (EPA), "Pesticides are substances meant for attracting, seducing, and then destroying or mitigating any pest." They are a class of biocide. On the basis of chemistry, pesticide includes a huge number of diversified chemicals and is most commonly used for crop protection. Use of pesticides in plant protection is so common that the term pesticide is treated synonymously with "plant protection product" despite the fact that pesticide is a broader term that includes herbicide, insecticide, insect growth regulator, nematicide, termiticide, molluscicide, piscicide, bactericide, insect repellent, avicide, rodenticide, predacide, animal repellent, antimicrobial, fungicide, disinfectant (antimicrobial), and sanitizers (Sassolas et al. 2012).

Many pesticides can be grouped into chemical families. Prominent insecticide families include organochlorines, organophosphates, and carbamates. Organochlorine hydrocarbons (e.g., DDT) operate by disrupting the sodium/potassium balance of the nerve fiber, forcing the nerve to transmit continuously. Their toxicities vary greatly, but they have been phased out because of their persistence and potential to bioaccumulate (Rodriguez-Mozaz et al. 2004). Widespread application of pesticide resulted in environment contamination. European Drinking Water Directive (DWD), Council Directive 98/83/EC on the quality of water for human consumption, has set a limit of 0.1 μg/l for individual pesticides and of 0.5 μg/l for total pesticides.

Although conventional techniques such as HPLC/MS and GC/MS can be used to estimate pesticide with satisfaction, they have certain limitations like being costly, laboratory bound, etc. Biosensors have been developed for a cheaper, faster, and on-site analysis of pesticides. Bioassay principle has been used for enzymatic pesticide. Biosensor can broadly be classified as inhibition-based biosensors and catalytic biosensor based on the hydrolytic activity of certain enzymes, e.g., OPH and glutathione S-transferase (Sassolas et al. 2012). Enzyme inhibition is the most applied bioassay principle for pesticide biosensor and is based on the inhibition of a selected enzymatic biocomponent (Rodriguez-Mozaz et al. 2004).

Two types of natural cholinesterase (ChE) enzymes are known, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE), both use different substrate; AChE preferentially hydrolyzes acetyl esters, whereas BChE hydrolyzes butyrylcholine to give rise to choline, acetic acid and butyric acid, respectively. The resulting pH change can be measured potentiometrically (Liu et al. 2005) or optically by pH-sensitive spectrophotometric methods (Andreou and Clonis 2002a; Wong et al. 2006) or by using fluorescent indicators (Tsai and Doong 2005), and thereby inhibition of enzyme activity can be correlated to the concentration of analyte. Based on the inhibition of acetyl cholinesterase (AChE) and choline oxidase, various biosensors have been developed for the detection of

organophosphorous and carbamate pesticides such as those described by Choi et al. (2001), Andreou and Clonis (2002a) and Andres and Narayanaswamy (1997). Other than cholinesterase, tyrosinase, alkaline phosphatase, peroxidase, and acid phosphatase enzymes have been used to develop biosensors for different pesticides (Sassolaset al. 2012).

Biosensor based on catalytic activity has also been developed using enzymes like organophosphorus hydrolase (OPH) and glutathione S-transferase (Andreou and Clonis 2002b; Pedrosa et al. 2010; Lee et al. 2010).

Mulchandani et al. (2005) developed amperometric microbial biosensors for detection of p-nitrophenol. The biosensor takes advantage of the ability of *Moraxella* sp. to specifically degrade p-nitrophenol to hydroquinone, a more electroactive compound than p-nitrophenol. The electrochemical oxidation current of hydroquinone formed in biodegradation of p-nitrophenol was measured at *Moraxella* sp.-modified carbon paste electrode and correlated to p-phenol concentrations.

To develop these biosensor microbial cells and OPH, either free (Lei et al. 2004) or expressed, the cell surface of other organisms (Lei et al. 2006) are co-immobilized. For the detection, pesticide is hydrolyzed by OPH to release p-nitrophenol; released p-nitrophenol is either degraded by the immobilized microbe, e.g., *Pseudomonas putida* JS444 resulted in electroactive compound that can be detected amperometrically and correlated with pesticide concentration (Lei et al. 2004), or the release p-nitrophenol is oxidized by the immobilized microbe, e.g., *Arthrobacter* sp. JS443 (Lei et al. 2006), and consumed oxygen is measured by Clark electrode and correlated with pesticide concentration.

Nowicka et al. (2010) developed DNA-based biosensor, used to study DNA damage caused by pesticides; a biotinylated DNA probe was immobilized on an electrode surface modified with streptavidin. This DNA probe was hybridized with biotinylated complementary DNA target analyte. The voltammetric transduction was achieved by coupling ferrocene moiety to streptavidin linked to biotinylated target DNA. The close proximity of ferrocene to the electrode surface induced a current signal. The presence of pesticides caused an unwinding of the DNA, and thus a decrease of the ferrocene oxidation current observed in voltammetric experiments. Paraoxon-ethyl and atrazine caused the fastest and most severe damage to DNA (Table 16.2).

16.6 Heavy Metals

Heavy metals are naturally occurring elements that have a high atomic weight and a density at least five times greater than that of water. Their multiple industrial, domestic, agricultural, medical, and technological applications have led to their wide distribution in the environment, raising concerns over their potential effects on human health and the environment (Turdean 2011). Heavy metals are not biodegradable which make them a serious environmental problem and have a high environmental persistence, enter into the food chains. Their toxicity depends on

Table 16.2 Biosensors developed for pesticides estimation

Analyte	Biocomponent	Transducer	Limit of detection (LOD)	References
Organophosphorus	AChE	Optical	2 µg/l	Choi et al. (2001)
Organophosphorus	AChE	Electrochemical	1 nM	Stoytcheva et al. (2009)
Carbaryl	ChE	Optical	108 µg/l	Andreou and Clonis (2002a)
Dichlorvos	ChE	Optical	5.2 µg/l	Andreou and Clonis (2002a)
Carbofuron	AChE	Optical	0.15 nM	Andres and Narayanaswamy (1997)
Paraoxon	AChE	Optical	41 μM	Andres and Narayanaswamy (1997)
Atrazine	Glutathione S-transferase	Optical	0.84 μM	Andreou and Clonis (2002b)
Paraoxon	OPH	Amperometric	0.1 nM	Pedrosa et al. (2010)
Paraoxon	OPH	Amperometric	12 µM	Lee et al. (2010)
Methyl parathion	OPH	Amperometric	3.8 nM	Du et al. (2010)
Parathion	OPH	Optical	2 µM	Mulchandani et al. (1999)
Carbaryl	Tyrosinase	Amperometric	5 µM	Campanella et al. (2007)
Atrazine	Immunosensor	Amperometric	17 nM	Zacco et al. (2007)
Diuron	Immunosensor	Amperometric	4.3 pM	Sharma et al. (2011)
Naphthalene	Immunosensor	Amperometric	62 nM	Zhang and Zhuang (2010)
2,4-D	Immunosensor	Optical (spectroscopic)	20 nM	Navratilova and Skladal (2004)
Chlorpyrifos	Immunosensor	Optical (SPR)	14 nM	Mauriz et al. (2006)
2,4-D	Immunosensor	Optical (SPR)	0.36 pM	Kim et al. (2007)
Paraoxon	*Arthrobacter* JS443	Amperometric	10 nM	Lei et al. (2004)
Methyl parathion	*Arthrobacter* JS443	Amperometric	20 nM	Lei et al. (2004)

several factors including the dose, route of exposure, and chemical species, as well as the age, gender, genetics, and nutritional status of exposed individuals. Because of their high degree of toxicity, arsenic, cadmium, chromium, lead, and mercury rank among the priority metals that are of public health significance. These metallic elements are considered systemic toxicants that are known to induce multiple organ

damage, even at lower levels of exposure (Tchounwou et al. 2012). Thereby, precise estimation of heavy metal at different levels is indispensable. Various biosensors have been developed for heavy metal using a variety of biocomponents from enzymes to whole cells, DNA, etc.

For heavy metal detection, different enzymes, such as acetylcholinesterase (AChE), alkaline phosphatase, urease, invertase, peroxidase, L-lactate dehydrogenase, tyrosinase, and nitrate reductase, have been used. The inhibition of the immobilized enzyme can be detected via electrochemical (amperometric, potentiometric, and conductometric) or optical measurements (Turdean 2011).

The principle of detection can be based on solo enzyme activity as well as bienzymatic combinations, e.g., urease enzyme hydrolyze urea as a substrate releasing ammonia and carbon dioxide; the released ammonium ion can directly be analyzed with ion-selective electrode and correlated to analyte concentration (Verma et al. 2011) or in a bienzymatic approach; the reaction can be coupled with glutamate dehydrogenase which utilized these ammonium to convert α-ketoglutarate to glutamate; in this reaction one NADH is oxidized to NAD that can be measured potentiometrically (Rodriguez et al. 2004); estimation of NADH concentration is correlated with inhibition of enzyme activity and thereby the concentration of analyte. Table 16.3 summarizes the biosensors based on enzymes for the detection of heavy metals.

Other than enzymes, recombinant DNA technology has also been used for developing heavy metal biosensors. Various specific and nonspecific promoters responding to different heavy metals have been used for making different promoter reporter gene combinations; variety of specific and nonspecific heavy metal biosensor has been developed (Table 16.4).

Heavy metals show a high affinity for DNA, and they can interact with nucleic acids. Based on this property of interaction, DNA has been used as biocomponent for heavy metal biosensors. For heavy metal studies, double-stranded DNA (dsDNA) as well as single-stranded DNA (ssDNA) has been used (Wong et al. 2007; Tencaliec et al. 2006). An electrochemical DNA biosensor is an integrated receptor-transducer device that uses DNA as a biomolecular recognition element to measure specific binding processes with DNA, through electrochemical (especially carbon electrodes) transduction (Tencaliec et al. 2006). DNA or its components (such as nucleotides, nucleosides, purine, and pyrimidine bases) is immobilized on the electrode surface, and the strategies developed to get the result of interactions electrochemically. Tencaliec et al. (2006) developed DNA biosensor by immobilizing double-stranded calf thymus DNA onto the surface of a disposable carbon screen-printed electrode. The oxidation signal of the guanine base, obtained by a square wave voltammetric scan, is used as analytical signal to detect the DNA damage; the presence of low molecular weight compounds with affinity for nucleic acids is measured by their effect on the guanine oxidation peak.

Wong et al. (2007) developed biosensor using detection strategy of underpotential deposition (UDP) of cadmium onto gold electrode surface modified with single-stranded DNA (ssDNA) and could detect Cd^{2+} up to 10pM. Long et al. (2011) developed biosensor for Hg^{2+} by immobilizing a DNA probe onto a fiber

Table 16.3 Enzymatic biosensor developed for different heavy metals

Enzyme	Heavy metal ions detected	Respective detection limits	Detection method	References
AChE	Hg^{2+}, Cd^{2+}, Zn^{2+}	0.1 nM, 0.5 mg/l, 2 mg/l	Amperometric	Stoytcheva and Sharkova (2002)
AChE	Cu^{2+}	50 μM	Amperometric	Evtugyn et al. (2003)
ALP	Co^{2+}, Ni^{2+}, Pb^{2+}	2 μg/l, 5 μg/l, 40 μ/l	Conductometric	Berezhetskyy et al. (2008)
GOx	Hg^{2+}, Cu^{2+}	2.5 μM, 2.5 μM	Amperometric	Malitesta and Guascito (2005)
GOx	Hg^{2+}	1 ng/l	Amperometric	Mohammadi et al. (2002)
HRP	Hg^{2+}	0.1 ng/ml	Amperometric	Han et al. (2001)
Phosphatase	Hg^{2+}, Cd^{2+}	10^{-20} M, 10^{-20} M	Conductometric	Tekaya et al. (2014)
Tyrosinase	Cr^{3+}	50 μM	Amperometric	Domınguez Renedo et al. (2004)
Urease	Hg^{2+}, Cu^{2+}, Cd^{2+}	10 nM, 150 μM, 200 μM	Optical	Tsai and Doong (2005)
Urease	Ag^{+}, Ni^{2+}, Cu^{2+}	0.35 nM, 0.7 μM, 2 μM	Potentiometric	Soldatkin et al. (2000)
Urease	Pb^{2+}	9.6 μM	Potentiometric	Kaur et al. (2014)
Urease	Cd^{2+}	0.1 μg/l	Optical	Verma et al. (2010)
Urease	Cd^{2+}	1 μg/l	Potentiometric	Verma et al. (2011)
Urease/GLDH combination	Hg^{2+}, Cu^{2+}, Cd^{2+}, Zn^{2+}	7.2 μg/l, 8.5 μg/l, 0.3 mg/l, 0.2 mg/l	Amperometric	Lee and Lee (2002)

Table 16.4 Recombinant biosensors for heavy metals

Promoter/ reporter combination	(Host organism)	Detection method	Heavy metal ions / LOD	References
Ars pR773/lacZ	*E. coli*	Chemiluminescence	As^{3+}/50 μM; Sb^{3+}/ 1fM	Ramanathan et al. (1997)
CUP 1/lacZ	*S. cerevisiae*	Amperometry	Cu^{2+} /50 μg/l	Tag et al. (2007)
CadA and CadC/LucFF	*S. aureus* (RN4220)	Bioluminescence	CD^{2+}/10 nM Pb^{2+}/ 33 nM Sb^{2+}/1 nM	Tauriainen et al. (1998)
copA/lux	*E. coli* (W3110)	Bioluminescence	Cu^{2+}, Ag^{+}, Au^{3+}	Stoyanov et al. (2003)
Cad/rsGFP	*E. coli* (DH5α)	Fluorescence	Cd^{2+}/0.1 nMPb^{2+}/ 10nMSb^{3+}/0.1 nM	Liao et al. (2006)
mer/lux	*E. coli* (CM2624)	Bioluminescence	Hg^{2+}	Bontidean et al. (2004)

optic sensor containing short common oligonucleotide sequences that can hybridize with a fluorescently labeled complementary DNA. The DNA probe also comprises a sequence of T–T mismatch pairs that bind with Hg^{2+} to form a T–Hg^{2+}–T complex by folding of the DNA segments into a hairpin structure. With a structure-competitive mode, a higher concentration of Hg^{2+} leads to less fluorescence-labeled cDNA bound to the sensor surface and thus to lower fluorescence signal. Developed biosensor could detect Hg^{2+} up to 2.1 nM within 6 min. Chang et al. (2005) used DNAzyme construct, selective for cleavage in the presence of Pb^{2+} and derivatized with fluorophore (quencher) at the 5′ (3′) end of the substrate and enzyme strands, respectively, forms a molecular beacon that is used as the recognition element. The developed biosensor could detect Pb^{2+} up to 11 nM.

16.7 Toxicity

Toxicity is the degree to which a substance can damage an organism (such as an animal, bacterium, or plant) or a substructure of the organism (such as cell, tissues, or organ). Bulich and Isenberg (1981) proposed a simple and rapid method for monitoring general toxicity of aquatic samples based on inhibition of light production by naturally luminescent bacteria, commercialized soon after (Microtox test). Nonspecific general biosensor faces certain limitations, e.g., any decrease in metabolic activity will also result in decreased luminescence by these nonspecific biosensor cells (Unge et al. 1999), resulting in false-positive results; moreover, ions like Na, K, Mg, and Ca have been shown to influence light emission by *V. fischeri* (Sorensen et al. 2006). Okochi et al. (2004) developed an automated water toxicity biosensor using *Thiobacillus ferrooxidans* and oxygen electrode for monitoring cyanides in natural water. The bioassay principle is based on the monitoring of a current increase by addition of toxicoids, which is caused by the inhibition of bacterial respiration and decrease in oxygen consumption. A toxicity biosensor based on mammalian cells as the biological recognition agent (A549 human lung epithelial cells) has been developed by Hara et al. (2015). Bioassay principle has been based on cellular enzyme activity (acid phosphatase – AP) following 24 hours exposure. AP catalyzes the dephosphorylation of 2-naphthyl phosphate to 2-naphthol (determined using chronocoulometry) and is indicative of metabolically active cells. Immobilized living cells exposed to pentachlorophenol, cadmium chloride, and nickel chloride exhibited a decrease in AP activity which enabled IC50 (50% reduction in enzyme activity) values of toxic chemicals to be reliably and conveniently determined using electronic detection.

Various stress responsive promoters have been exploited in the construction of biosensors for the detection of conditions or compounds eliciting a stress response. Alkorta et al. (2006) developed biosensor for heavy metal toxicity by fusing lux gene of *Vibrio fischeri* with transcriptionally active machinery of heavy metal resistance. Biosensor for sodium dodecyl sulfate (SDS) toxicity detection was built by immobilizing recombinant *Escherichia coli* expressing GFP in

k-carrageenan matrix. The fabricated *E. coli* GFP toxicity biosensor has a wide dynamic range of 4–100 mg/l, with LOD of 1.7 mg/l (Ooi et al. 2015). To develop biosensor for genotoxic substances, *recA*, *sulA*, *umuCD recN*, *uvrA*, and *cda* have been fused with reporter genes and expressed in *E. coli* and *Salmonella* sp., etc. (Sorensen et al. 2006).

16.8 Bioremediation

Bioremediation is the process of complex organic compounds to simpler inorganic constituents using the capacity of different microbes. Bioremediation process is influenced by different parameters, such as temperature, pH, nutrient availability, metal ions, available oxygen, etc.; monitoring these parameters will help to better control the process. Biosensors have been developed to monitor the process of bioremediation. Different molecular biosensors applied to monitor bioremediation process have been reviewed by Purohit (2003); most of biosensor used the strategy of expressing reporter gene, e.g., luciferase under the control of specific promoter sensitive to the target analyte. Dawson et al. (2008) used lux-based luminescent biosensors to monitor changes in contaminant toxicity and bioavailability in aqueous extracts from BTEX-impacted soils as degradation of proceeded (benzene, toluene, ethylbenzene, and xylene collectively referred to as BTEX). Zhou et al. (2014) developed tyrosinase-based biosensor simultaneous determination of catechol (CC) and hydroquinone (HQ) in compost bioremediation of municipal solid waste (Table 16.5).

16.9 Others

Biosensors have been developed for various other categories of environmental pollutants, e.g., polychlorinated biphenyls (PCBs), phenols, surfactants, polycyclic aromatic hydrocarbons (PAHs) inorganic phosphates, nitrates, etc.

16.10 Conclusion and Future Trends

Since its inception, biosensors have been anticipated to play a significant analytical role in medicine, agriculture, food safety, homeland security, and environmental and industrial monitoring. Nevertheless, commercialization of biosensor technology has significantly lagged behind the research output as reflected by a number of publications and patenting activities. The rationale behind the slow and limited technology transfer could be attributed to cost considerations and some key technical barriers (Luong et al. 2008). Successful commercial biosensors have to have several properties on different fronts (summarized in Table 16.6) to meet the analytical requirements of the modern world.

Table 16.5 Biosensors for different categories of environmental pollutants

Analyte	Biocomponent	Transducer	Limit of detection (LOD)	References
E. coli O157:H7	Antibody	Electrochemical (impedance)	10 CFU/ml	Joung et al. (2013)
Vibrio cholerae	DNA	Optical	5 ng of target DNA	Chua et al. (2011)
Yersinia pestis	Antibody	Luminescence	10^4CFU/ml	Yan et al. (2006)
E. coli O157:H7	Antibody	Electrochemical	61 CFU/ml	Luo et al. (2010)
Salmonella typhi	Antibody	Optical	2 × 10 (Andres and Narayanaswamy 1997) cells/ml	Jain et al. (2012)
Staphylococcus aureus	Nanocomposite of antibody	Optical	1.5 × 10 (Andres and Narayanaswamy 1997) CFU in PBS 1.5 × 10 (Bontidean et al. 2004) CFU in milk	Sung et al. (2013)
Bacillus cereus	B1–7064 phage	Electrochemical (amperometric)	10 CFU/ml	Kretzer et al. (2007)
E. coli O157:H7	T4 phage	Optical (SPR)	10^3CFU/ml	Tawil et al. (2012)
Naphthalene	Recombinant *Pseudomonas putida* (sal/luxAB)[a]	Optical	50 nM	Werlen et al. (2004)
Fluorene	Recombinant *Sphingomonas* sp. (mini-Tn5:luxAB-tet)[a]	Optical	200 µl/g	Bastiaens et al. (2001)
Phenanthrene	Recombinant *Burkholderia* sp. RP037 (phnS:GFP)[a]	Optical	NA	Tecon et al. (2006)
Naphthalene	Recombinant *E.coli* (nahR/lacZ)[a]	Optical	NA	Cho et al. (2014)

Nitrite	Copper-containing nitrite reductase (Cu-NiR, from *Rhodopseudomonas sphaeroides* forma sp. *denitrificans*)	Electrochemical	40 nM	Quan and Shin (2010)
Nitrite	Cytochromec nitrite reductase (ccNiR) from *Desulfovibrio desulfuricans*	Hj	4 nM	Chen et al. (2007)
Nitrite	Hemoglobin (Hb) protein	Electrochemical (amperometric)	0.1 μM	Saadati et al. (2014)
Nitrite and nitrate	Copper, zinc superoxide dismutase (SOD1), and nitrate reductase (NaR)	Electrochemical	200 nM	Madasamy et al. (2014)
Polycarbonated biphenyl (Delor103, 2,4,4′-trichlorobiphenyl)	*Pseudomonas* sp. P2	Optical	0.5 mg/l, 0.2 mg/l respectively	Gavlasova et al. (2008)
Sodium dodecyl sulfate (model surfactant)	*Pseudomonas* sp. and *Achromobacter* sp.	Electrochemical (amperometric)	1 μM	Taranova et al. (2002)
Aflatoxin B_1 (AFB_1)	Aptamer	Electrochemical	0.4 nM	Castillo et al. (2015)

[a]Promoter/reporter combination used for developing recombinant biosensor

Table 16.6 Summary of requirements for a commercial biosensor

Limit of detection (LOD)	Lowest possible, far lower than the permissible limits in case of toxic chemicals
Assay protocol	Free of need for reagent addition
Time of assay	As minimum as possible, preferably in seconds/minutes
Size	Compact and complete to allow portability for on-site assessment of analytes
Automaticity	Highly automated (single button device)
Prerequisite	Preferably should not require any pretreatment of sample, skilled operator

According to the report on "Biosensor Market 2014–2020" by Transparency Market Research, the world market for biosensors is estimated to be above 21 thousand million USD by the year 2020. Most of biosensors designed today are to detect single or a few target analytes; in future we have to have biosensors supporting interchangeable biocomponents to detect various analytes on the same platform "lab-on-a-chip." The fields of study to progress in that direction involve microfluidics nanotechnology to integrate sensing system and signal processing. The ongoing development of integrated lab-on-a-chip devices will employ various elements of nanotechnology, though nanotechnology have certain issues to be addressed on the front of increased cost, lack of consensus on toxicity analysis, etc. (Vashist et al. 2012). With 200 (Salgado et al. 2011) to 500 (Mongra and Kaur 2012) companies worldwide working in the field of biosensors, market potential is there to accept biosensor if the said hurdles are covered.

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