Energy, Environment, and Sustainability

Sunita J. Varjani
Avinash Kumar Agarwal
Edgard Gnansounou
Baskar Gurunathan *Editors*

Bioremediation: Applications for Environmental Protection and Management

Springer

Energy, Environment, and Sustainability

This books series publishes cutting edge monographs and professional books focused on all aspects of energy and environmental sustainability, especially as it relates to energy concerns. The Series is published in partnership with the International Society for Energy, Environment, and Sustainability.
The books in these series are editor or authored by top researchers and professional across the globe. The series aims at publishing state-of-the-art research and development in areas including, but not limited to:

- Renewable Energy
- Alternative Fuels
- Engines and Locomotives
- Combustion and Propulsion
- Fossil Fuels
- Carbon Capture
- Control and Automation for Energy
- Environmental Pollution
- Waste Management
- Transportation Sustainability

More information about this series at http://www.springer.com/series/15901

Sunita J. Varjani · Avinash Kumar Agarwal
Edgard Gnansounou · Baskar Gurunathan
Editors

Bioremediation: Applications for Environmental Protection and Management

Springer

Editors
Sunita J. Varjani
Gujarat Pollution Control Board
Gandhinagar, Gujarat
India

Avinash Kumar Agarwal
Department of Mechanical Engineering
Indian Institute of Technology Kanpur
Kanpur, Uttar Pradesh
India

Edgard Gnansounou
Bioenergy and Energy Planning Research Group (BPE)
Swiss Federal Institute of Technology Lausanne (EPFL)
Lausanne
Switzerland

Baskar Gurunathan
Department of Biotechnology
St. Joseph's College of Engineering
Chennai, Tamil Nadu
India

ISSN 2522-8366 ISSN 2522-8374 (electronic)
Energy, Environment, and Sustainability
ISBN 978-981-10-7484-4 ISBN 978-981-10-7485-1 (eBook)
https://doi.org/10.1007/978-981-10-7485-1

Library of Congress Control Number: 2017959893

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Preface

Varieties of pollutants are released into the environment day by day due to increase in population and urbanization which leads to environmental pollution, viz. air, land and water. Pollutants may be organic or inorganic. Environmental Protection and Management is a burning issue for different industrial sectors. Many conventional engineering-based physicochemical decontamination methods for remediation of these pollutants are expensive. The increasing costs and limited efficiency of traditional physicochemical treatments have spurred the development of green technologies which ultimately leads to the sustainable development of the environment.

The first international conference on 'Sustainable Energy and Environmental Challenges' (SEEC-2017) was organized under the auspices of 'International Society for Energy and Environmental Sustainability' (ISEES) by the 'Center of Innovative and Applied Bioprocessing' (CIAB), Mohali, held from 26–28 February 2017. ISEES was founded at IIT Kanpur in January 2014 with the aim of spreading knowledge in the fields of energy, environment, sustainability and combustion. The society's goal is to contribute to the development of clean, affordable and secure energy resource and a sustainable environment for the society and to spread knowledge in the above-mentioned areas and awareness about the environmental challenges, which the world is facing today. ISEES is involved in various activities such as conducting workshops, seminars and conferences in the domains of its interest. The society also recognizes the outstanding works done by the young scientists and engineers for their contributions in these fields by conferring them awards under various categories.

This conference provided a platform for discussions between eminent scientists and engineers from various countries including India, the USA, South Korea, Norway, Malaysia and Australia. In this conference, eminent speakers from all over the world presented their views related to different aspects of energy, combustion, emissions and alternative energy resource for sustainable development and cleaner environment. The conference started with four mini-symposiums on very topical themes, which included (i) New Fuels and Advanced Engine Combustion, (ii) Sustainable Energy, (iii) Experimental and Numerical Combustion and

(iv) Environmental Remediation and Rail Road Transport. The conference had 14 technical sessions on topics related to energy and environmental sustainability and a panel discussion on 'Challenges, Opportunities and Directions of Technical Education & Research in the Area of Energy, Environment and Sustainability' to wrap up the three-day technical extravaganza. The conference included 2 plenary talks, 12 keynote talks, 42 invited talks from prominent scientists, 49 contributed talks and 120 posters. A total of 234 participants and speakers attended this three-day conference, which hosted Dr. V. K. Saraswat, Member, NITI Aayog, India, as a chief guest for the award ceremony of ISEES. This conference laid out the road map for technology development, opportunities and challenges in this technology domain. The technical sessions in the conference included Advances in IC Engines and Fuels; Conversion of Biomass to Biofuels; Combustion Processes; Renewable Energy: Prospects and Technologies; Waste to Wealth—Chemicals and Fuels; Energy Conversion Systems; Numerical Simulation of Combustion Processes; Alternate Fuels for IC Engines; Sprays and Heterogeneous Combustion of Coal/Biomass; Biomass Conversion to Fuels and Chemicals—Thermochemical Processes; Utilization of Biofuels; and Environmental Protection and Health. All these topics are very relevant for the country and the world in the present context. The society is grateful to Prof. Ashok Pandey for organizing and hosting this conference, which led to the germination of this series of monographs, which included 16 books related to different aspects of energy, environment and sustainability. This is the first time that such a voluminous and high-quality outcome has been achieved by any society in India from one conference.

The editors would like to express their sincere gratitude to the authors for submitting their work in a timely manner and revising it appropriately at short notice. We would like to express our special thanks to reviewers for reviewing various chapters of this monograph and provided their valuable suggestions to improve the manuscripts. We acknowledge the support received from various funding agencies and organizations for successfully conducting the first ISEES conference SEEC-2017, where these monographs germinated. These include Department of Science and Technology, Government of India (special thanks to Dr. Sanjay Bajpai); TSI, India (special thanks to Dr. Deepak Sharma); Tesscorn, India (special thanks to Sh. Satyanarayana); AVL India; Horiba, India; Springer (special thanks to Swati Mehershi); CIAB (special thanks to Dr. Sangwan).

Due to some limitations such as cost and efficiency, conventional remediation methods of polluted sites, there is a need for the development of alternative technologies for in situ applications, particularly based on biological remediation capabilities of plants and microorganisms. Green technologies for clean-up of pollutants by biological means are used for Environmental Protection and Management. Hence, bioremediation technology is referred as an efficient, economic, versatile and environmentally sound technique. The monograph is primarily focused on every aspect of bioremediation technology practiced globally. It provides concise and updated literature on bioremediation technologies as a tool for Environmental Protection and Management, which can be used by engineers, scientists and academicians working in the field of bioremediation. Every chapter

of the book contains recent information and is clearly illustrated with tables, figures and pictures in a more simple and scientific way.

The book shall include chapters on different aspects of recent advances in bioremediation of different environmental pollutants. Some of the topics covered in this book are: evaluation of next-generation sequencing technologies for environmental monitoring in wastewater abatement; genetically modified organisms and its impact on the enhancement of bioremediation; bioremediation of industrial wastewater using bioelectrochemical treatment; phenol degradation from industrial wastewater by engineered microbes; bioremediation of heavy metals; pesticides bioremediation; mathematical modelling in bioremediation; application of microbes in remediation of hazardous wastes: a review; phytoremediation of textile dyes and effluents; biosorption strategies in the remediation of toxic pollutants from contaminated water bodies; phytoremediation technique for the removal of dye in wastewater; role of nanofibers in bioremediation; bioremediation of volatile organic compounds using biofilters; bioremediation of industrial and municipal wastewater using microalgae; role of biosurfactants in enhancing the microbial degradation of pyrene and so on.

Gandhinagar, India — Sunita J. Varjani
Kanpur, India — Avinash Kumar Agarwal
Lausanne, Switzerland — Edgard Gnansounou
Chennai, India — Baskar Gurunathan

Contents

1 **Introduction to Environmental Protection and Management** 1
Sunita J. Varjani, Avinash Kumar Agarwal, Edgard Gnansounou and Baskar Gurunathan

2 **Mathematical Modeling in Bioremediation** 7
Parthasarthy Vijay and Margavelu Gopinath

3 **Evaluation of Next-Generation Sequencing Technologies for Environmental Monitoring in Wastewater Abatement** 29
P. Senthil Kumar, S. Suganya and Sunita J. Varjani

4 **Genetically Modified Organisms and Its Impact on the Enhancement of Bioremediation** 53
Narasimhan Manoj Kumar, Chandrasekaran Muthukumaran, Govindasamy Sharmila and Baskar Gurunathan

5 **Integration of Lignin Removal from Black Liquor and Biotransformation Process** 77
Krishnamurthi Tamilarasan, Periyar Selvam Sellamuthu and Baskar Gurunathan

6 **Role of Nanofibers in Bioremediation** 99
Sekar Aiswarya Devi, Muthukumar Harshiny and Manickam Matheswaran

7 **Bioremediation of Industrial Wastewater Using Bioelectrochemical Treatment** 115
N. Samsudeen and Manickam Matheswaran

8 **Biosorption Strategies in the Remediation of Toxic Pollutants from Contaminated Water Bodies** 127
P. Senthil Kumar and K. Grace Pavithra

9 Bioremediation of Heavy Metals 165
P. Senthil Kumar and E. Gunasundari

10 Pesticides Bioremediation 197
P. Senthil Kumar, C. Femina Carolin and Sunita J. Varjani

11 Application of Microbes in Remediation of Hazardous Wastes: A Review 223
Moni Kumari, Pooja Ghosh and Indu Shekhar Thakur

12 Phytoremediation Techniques for the Removal of Dye in Wastewater 243
B. Bharathiraja, J. Jayamuthunagai, R. Praveenkumar and J. Iyyappan

13 Phenol Degradation from Industrial Wastewater by Engineered Microbes 253
Ravichandran Rathna and Ekambaram Nakkeeran

14 Insect Gut Bacteria and Their Potential Application in Degradation of Lignocellulosic Biomass: A Review 277
Rajesh Kumar Prasad, Soumya Chatterjee, Sonika Sharma, Pranab Behari Mazumder, Mohan G. Vairale and P. Srinavas Raju

15 Bioremediation of Volatile Organic Compounds in Biofilters 301
Margavelu Gopinath, Rose Havilah Pulla, K. S. Rajmohan, Parthasarthy Vijay, Chandrasekaran Muthukumaran and Baskar Gurunathan

16 Bioremediation of Industrial and Municipal Wastewater Using Microalgae 331
Baskar Gurunathan, I. Aberna Ebenezer Selvakumari, R. Aiswarya and S. Renganthan

17 Phytoremediation of Textile Dye Effluents 359
Shanmugaprakash Muthusamy, Dhilipkumar Govindaraj and Karthik Rajendran

18 Role of Biosurfactants in Enhancing the Microbial Degradation of Pyrene 375
Gauri Gupta, Avantika Chandra, Sunita J. Varjani, Chiranjib Banerjee and Vipin Kumar

19 Bioremediation of Nitrate-Contaminated Wastewater and Soil 387
K. S. Rajmohan, Margavelu Gopinath and Raghuram Chetty

Author Index 411

Editors and Contributors

About the Editors

Dr. Sunita J. Varjani is Scientific Officer at Gujarat Pollution Control Board, Gandhinagar, Gujarat, India. She holds M.Sc. degree in Microbiology (2009) and Ph.D. in Biotechnology (2014). Her major areas of research are Industrial and Environmental Microbiology/Biotechnology and Molecular Biology. She has authored 35 publications, including 1 book, 19 book chapters/reviews and 15 original research papers. She has won several awards and honours, including Young Scientist Award at AFRO-ASIAN Congress on Microbes for Human and Environmental Health, New Delhi (2014), and Best Paper Awards for oral presentations in national and international conferences in 2008, 2012 and 2013. She is a member of the editorial board of Journal of Energy and Environmental Sustainability.

Prof. Avinash Kumar Agarwal joined IIT Kanpur in 2001 and is currently a Poonam and Prabhu Goyal Endowed Chair Professor. He was at ERC, University of Wisconsin, Madison, USA, as a Postdoctoral Fellow (1999–2001). His areas of interest are IC engines, combustion, alternative fuels, hydrogen, conventional fuels, lubricating oil tribology, optical diagnostics, laser ignition, HCCI, emission and particulate control and large bore engines. He has published more than 160 peer-reviewed international journals and conference papers. He is Associate Editor of ASME Journal of Energy Resources Technology and International Journal of Vehicle Systems Modelling and Testing. He has edited 'Handbook of Combustion' (5 volumes; 3168 pages), published by Wiley VCH, Germany. He is a Fellow of SAE (2012), a Fellow of ASME (2013) and a Fellow of INAE (2015). He is the recipient of several prestigious awards such as NASI-Reliance Industries Platinum Jubilee Award-2012; INAE Silver Jubilee Young Engineer Award-2012; Dr. C.V. Raman Young Teachers Award-2011; SAE International's Ralph R. Teetor Educational Award-2008; INSA Young Scientist

Award-2007; UICT Young Scientist Award-2007; INAE Young Engineer Award-2005. He is the recipient of prestigious Shanti Swarup Bhatnagar Award-2016 in Engineering Sciences. He is the first combustion/IC engine researcher to get this honour.

Prof. Edgard Gnansounou is a Professor of Modelling and planning of Energy Systems at the Swiss Federal Institute of Technology Lausanne (EPFL) where he is Director of the Bioenergy and Energy Planning Research Group. His current research works comprise techno-economic and environmental assessment of bio-refinery schemes based on the conversion of agricultural residues. He is leading research projects in that field in several countries including Brazil, Colombia and South Africa. He is credited with numerous papers in high-impact scientific journals. He is a member of the editorial board of Bioresource Technology Journal. He graduated with an M.S. in Civil Engineering and Ph.D. in Energy Systems at the Swiss Federal Institute of Technology Lausanne. He was Visiting Researcher at the Thayer College, Dartmouth School of Engineering, with Professor Charles Wyman, USA, at Polytech of Clermont-Ferrand, University Blaise Pascal, France, and at the Center of Biofuels, the National Institute for Interdisciplinary Science and Technology, with Prof. Ashok Pandey, Trivandrum, India. He was also Visiting Professor of the African University of Science of Technology, Abuja, Nigeria. He is a citizen of Benin, Africa, and Switzerland.

Prof. Baskar Gurunathan is currently working as Professor of Biotechnology in St. Joseph's College of Engineering, Chennai, India. He has set an example for scholarly research work during the past 11 years. Since his postgraduation, he has published his research work in 92 reputed national and international journals. To his credit, he has presented more than 100 papers in national and international forum and published 5 chapters in books. Currently, his research works have got h-index of 14 and i10 index of 19 in Google Scholar with a total citation of 483. His current research expertise in biofuels, therapeutic proteins, microbial enzymes nanomedicine and nanocatalysis clearly indicates his thirst towards research and development in multi-disciplinary areas. Currently, he is the Management Council Member of Biotechnology Research Society, India (2017–19). He is also the Honorary Secretary of Indian Institute of Chemical Engineers, Chennai Regional Centre (2016–17). He is also an active life member of various professional bodies to keep abreast of latest trends and developments. He has organized various training programmes to enhance the knowledge of students, faculties and researchers. He has received two funded projects of total worth Rs. 55 lakhs from DST (Food Processing) and DBT

(Cancer Biology), GOI, during the academic year 2015–16. He has received Outstanding Reviewer Award from Bioresource Technology in 2013, Materials science and Engineering-C in 2015 and Biochemical Engineering Journal and Energy Conversion and Management in 2016. The International Bioprocessing Association (International Forum on Industrial Bioprocesses) has conferred him with Young Scientist Award-2015 in recognition of his contributions in the area of Bioprocess Technology. The Indian Society for Technical Education has conferred him with ISTE-Syed Sajid Ali National Award-2016 for his outstanding research work in the area of Renewable Energy.

Contributors

Avinash Kumar Agarwal Department of Mechanical Engineering, Indian Institute of Technology Kanpur, Kanpur, India

R. Aiswarya Department of Biotechnology, St. Joseph's College of Engineering, Chennai, India

Sekar Aiswarya Devi Department of Chemical Engineering, National Institute of Technology, Tiruchirappalli, India

Chiranjib Banerjee Department of Environmental Science & Engineering, Indian Institute of Technology (ISM), Dhanbad, Jharkhand, India

B. Bharathiraja Vel Tech High Tech Dr. Rangarajan Dr. Sakunthala Engineering College, Chennai, Tamilnadu, India

Avantika Chandra Department of Environmental Science & Engineering, Indian Institute of Technology (ISM), Dhanbad, Jharkhand, India

Soumya Chatterjee Defence Research Laboratory, DRDO, Tezpur, Assam, India

Raghuram Chetty Department of Chemical Engineering, Indian Institute of Technology Madras, Chennai, Tamil Nadu, India

C. Femina Carolin Department of Chemical Engineering, SSN College of Engineering, Chennai, India

Pooja Ghosh Centre for Rural Development and Technology, Indian Institute of Technology, New Delhi, India

Edgard Gnansounou Bioenergy and Energy Planning Research Group (BPE), Swiss Federal Institute of Technology Lausanne (EPFL), Lausanne, Switzerland

Margavelu Gopinath Department of Chemical Engineering, University of Petroleum and Energy Studies, Dehradun, Uttarakhand, India

Dhilipkumar Govindaraj Downstream Processing Laboratory, Department of Biotechnology, Kumaraguru College of Technology, Coimbatore, India

K. Grace Pavithra Department of Chemical Engineering, SSN College of Engineering, Chennai, India

E. Gunasundari Department of Chemical Engineering, SSN College of Engineering, Chennai, India

Gauri Gupta Laboratory of Applied Microbiology, Department of Environmental Science & Engineering, Indian Institute of Technology (ISM), Dhanbad, Jharkhand, India

Baskar Gurunathan Department of Biotechnology, St. Joseph's College of Engineering, Chennai, Tamil Nadu, India

Muthukumar Harshiny Department of Chemical Engineering, National Institute of Technology, Tiruchirappalli, India

J. Iyyappan Vel Tech High Tech Dr. Rangarajan Dr. Sakunthala Engineering College, Chennai, Tamilnadu, India

J. Jayamuthunagai Centre for Biotechnology, Anna University, Chennai, Tamilnadu, India

Narasimhan Manoj Kumar Department of Genetic Engineering, School of Bioengineering, SRM University, Kattankulathur, Tamilnadu, India

Vipin Kumar Laboratory of Applied Microbiology, Department of Environmental Science & Engineering, Indian Institute of Technology (ISM), Dhanbad, Jharkhand, India

Moni Kumari School of Environmental Sciences, Jawaharlal Nehru University, New Delhi, India

Manickam Matheswaran Department of Chemical Engineering, National Institute of Technology, Tiruchirappalli, India

Pranab Behari Mazumder Department of Biotechnology, Assam University, Silchar, Assam, India

Chandrasekaran Muthukumaran Department of Industrial Biotechnology, Government College of Technology, Coimbatore, Tamilnadu, India

Shanmugaprakash Muthusamy Downstream Processing Laboratory, Department of Biotechnology, Kumaraguru College of Technology, Coimbatore, India

Ekambaram Nakkeeran Research Laboratory, Department of Biotechnology, SriVenkateswara College of Engineering (Autonomous), Sriperumbudur, Tamil Nadu, India

Rajesh Kumar Prasad Defence Research Laboratory, DRDO, Tezpur, Assam, India; Department of Biotechnology, Assam University, Silchar, Assam, India

R. Praveenkumar Department of Biotechnology, Arunai Engineering College, Tiruvannamalai, Tamilnadu, India

Rose Havilah Pulla Department of Chemical Engineering, College of Engineering Studies, University of Petroleum and Energy Studies, Dehradun, India

Karthik Rajendran MaREI Center, Environmental Research Institute, University College Cork, Cork, Ireland; School of Engineering, University College Cork, Cork, Ireland

K. S. Rajmohan Department of Chemical Engineering, College of Engineering Studies, University of Petroleum and Energy Studies, Dehradun, India; Department of Chemical Engineering, Indian Institute of Technology Madras, Chennai, Tamil Nadu, India

P. Srinavas Raju Defence Research Laboratory, DRDO, Tezpur, Assam, India

Ravichandran Rathna Research Laboratory, Department of Biotechnology, Sri Venkateswara College of Engineering (Autonomous), Sriperumbudur, Tamil Nadu, India

S. Renganthan Department of Biotechnology, A.C.Tech., Anna University, Chennai, India

N. Samsudeen Department of Chemical Engineering, National Institute of Technology, Tiruchirappalli, India

Periyar Selvam Sellamuthu Department of Food Process Engineering, SRM University, Chennai, Tamilnadu, India

I. Aberna Ebenezer Selvakumari Department of Biotechnology, St. Joseph's College of Engineering, Chennai, India

P. Senthil Kumar Department of Chemical Engineering, SSN College of Engineering, Chennai, India

Sonika Sharma Defence Research Laboratory, DRDO, Tezpur, Assam, India

Govindasamy Sharmila Department of Industrial Biotechnology, Government College of Technology, Coimbatore, Tamilnadu, India

S. Suganya Department of Chemical Engineering, SSN College of Engineering, Chennai, India

Krishnamurthi Tamilarasan Department of Chemical Engineering, SRM University, Chennai, Tamilnadu, India

Indu Shekhar Thakur School of Environmental Sciences, Jawaharlal Nehru University, New Delhi, India

Mohan G. Vairale Defence Research Laboratory, DRDO, Tezpur, Assam, India

Sunita J. Varjani Gujarat Pollution Control Board, Gandhinagar, Gujarat, India

Parthasarthy Vijay Department of Chemical Engineering, College of Engineering Studies, University of Petroleum and Energy Studies, Dehradun, India

Chapter 1
Introduction to Environmental Protection and Management

Sunita J. Varjani, Avinash Kumar Agarwal, Edgard Gnansounou and Baskar Gurunathan

Abstract Man's environment consists of natural resources like air, land, water, plants, and animals. With the progress of industrialization and civilization, man has interacted with his surroundings and disturbed the nature. It leads to environmental pollution, which cannot be eradicated by nature's self-acting process, i.e., various biogeochemical cycles. Environmental problems stem from two main categories of human activities: (a) resources utilization at unsustainable levels and contamination of the environment through pollution and (b) discharge of wastes at levels beyond the earth's and environment's capacity to absorb them or render them harmless which results in ecological damage and degradation of the environment. Environmental damage around includes pollution of water and air and consequent health problems, biodiversity loss, deterioration of buildings and monuments, soil fertility loss, desertification, ozone depletion, and many more. Environmental protection and management has become one of the foremost concerns of the world community. International concern for environmental protection and management has gained momentum with Stockholm Declaration in 1972. It is considered as Magna Carta of environmental protection and sustainable development. Then a series of global efforts have been undertaken internationally for protection of the environ-

S. J. Varjani (✉)
Gujarat Pollution Control Board, Paryavaran Bhavan, Gandhinagar, India
e-mail: drsvs18@gmail.com

A. K. Agarwal
Department of Mechanical Engineering, Indian Institute of Technology Kanpur, Kanpur, India
e-mail: akag@iitk.ac.in

E. Gnansounou
Bioenergy and Energy Planning Research Group (BPE), Swiss Federal Institute of Technology Lausanne (EPFL), Lausanne, Switzerland
e-mail: edgard.gnansounou@epfl.ch

B. Gurunathan
Department of Biotechnology, St. Joseph's College of Engineering, Chennai, Tamil Nadu, India
e-mail: baskarg@stjosephs.ac.in

S. J. Varjani et al. (eds.), *Bioremediation: Applications for Environmental Protection and Management*, Energy, Environment, and Sustainability,
https://doi.org/10.1007/978-981-10-7485-1_1

ment. Hence, environmental protection has become not only local, regional, or national importance but also a global concern. Over the past several decades, growing public awareness regarding threats to the environment, informed by warnings from scientists, has led to demands that law protects the natural surroundings on which human well-being depends. Under growing pressure from national and international public opinion, governments began to demonstrate concern over the general state of the environment introduced legislation to combat pollution of inland waters, ocean, and air.

Keywords Biodegradation · Bioleaching · Genetically modified microorganisms · Microalgae · Molecular tools · Nanofibers · Next-generation sequencing

The waste generation and their disposal in natural water bodies become a serious topic of concern. Consequently, there is a demand for new strategies and technologies to address wastewater treatment and subsequent recycle and reuse. Biological treatment process is advantageous and constitutes tools to biodegrade organic matter, transfer toxic compounds into harmless products, and nutrient removal in wastewater microbiology. Biomonitoring employs sentinel or indicator species in water bodies to infer water quality, ecosystem health status, and to protect public health from waterborne risks. Next-generation sequencing is one of the most leveraging studies focussed on the ecology of microbial-mediated processes that influence freshwater quality such as algal blooms, contaminant biodegradation, and pathogen dissemination. One of the chapters in this book discusses next-generation sequencing technologies for environmental monitoring in wastewater abatement. However, essential hypothesis and utilization of contaminant transport demonstrated by numerical methods have been discussed in other chapter.

Bioremediation is a process to degrade environmental pollutants, which are introduced accidentally or deliberately, and causes a hazardous effect on the earth and harms the normal life process. The conversion of these pollutants into less toxic forms is the goal of bioremediation process that can be achieved by the use of microorganisms. The bioremediation approaches have more advantages when compared with traditional methods as it can be directly implemented at the targeted pollutant site. Sometimes, bacteria and fungus when employed to decompose chemical compounds showed limited ratio to metabolize the pollutants on their own. The genetically modified organisms as well as immobilized microbes/their products are applied nowadays in bioremediation process for effective removal of pollutants, where the indigenous microbes cannot work efficiently. Genetically modified microorganisms (GMOs) play an important role in remediating the industrial waste, reducing the toxicity of some hazardous compounds, and helping in removal of pollutants efficiently. A variety of molecular tools used for construction of GMOs, pros and cons, ethical issues, and laws governing the application of GMOs are explained.

Bioremediation of pollutants released in environment from various industries, viz. paper and pulp, dye and dyes intermediates, metal, pesticide manufacturing, pharmaceuticals, chemical manufacturing, petroleum refineries, petrochemical, coal gasification operations, and tannery by various biological means, viz. microorganisms, plant, and microalgae is discussed in-depth by authors of different chapters. Heavy metals, radioactive waste, hydrocarbons, pesticides, phenol, and nitrate are some of the leading toxic pollutants in the environment. Challenges are faced in decontamination of these types of pollutants in soil and water for a long period of time. A number of methods such as membrane technology, electro-Fenton reaction, advanced oxidation process, nanotechnology play a major role in removing toxic pollutants, but difficulties are seen in degradation of toxic sludge, additional side reactions, high cost in initial installment and in maintenance, etc. Roles of microorganisms and plants to remediate the organic and inorganic are discussed in chapters of this book. Recent bioremediation technologies and methods for pollutant removal are also discussed.

Phenol and its derivatives are the most pondered substantial pollutant generated from various industrial processes. Accumulation of phenol even at a lower concentration may be fatal to all living beings in the ecosystem. Overview of phenol pollution, deleterious effects of phenol in ecosystem, biodegradation of phenols, and significance of engineered microbes for phenol degradation are discussed. Pyrene is a high-molecular-weight polycyclic aromatic hydrocarbon (PAH) with a symmetrical structure, commonly found as a pollutant of air, water, and soil. Being one of the most abundant high-molecular-weight pericondensed PAH and having its structure similar to several carcinogenic PAHs, it is being used as a model compound to study the degradation of high-molecular-weight PAHs. Therefore, its removal from the environment is a challenging task for scientists. Microbial degradation of pyrene by pure microbial cultures and microbial consortium has been discussed by authors, which simultaneously emphasizes the role of surfactants in enhancing degradation process.

Microbes such as bacteria and fungi are involved in biodegradation of lignin present in the effluent of paper and pulp industry. Degradation of lignin by white-rot fungi may be helpful for the biotechnical applications like biopulping, biobleaching and pulp mill effluents treatment, and soil bioremediation. The abundance and renewability of lignin potentially converted to valuable bioproduct may eventually replace the existing technology in manufacturing industries.

Scarcity of pure water is a threatening issue worldwide. Water is essential for human survival and all activities on the earth. The effluent water from industries containing recalcitrant pollutants causes dangerous impacts to the environment and human health. Immobilized nanofibers possessing enhanced catalytic activity, high stability, and very good reusability of novel nanobiocomposites have remarkable potential for the treatment of water and wastewater. It also plays a major role in safe preservation of bioremediating bacteria for potential wastewater treatment applications. Nanofibers have become a popular carrier matrix for immobilization of specific microorganisms. Simple, versatile, and cost-effective properties of nanofibers made them a promising tool for microbial integration which enhances the

bioremediation by efficient removal of contaminants such as dyes and heavy metals from wastewater. One chapter of this book describes immobilization of specific bacteria on electrospinning nanofibers and its application in bioremediation process. However, the other chapter summarizes the research on bioelectrochemical systems for bioremediation of organic matter as well as recovery of heavy metal ions from the wastewater.

Biosorption is a process involving solid and liquid phases in which dissolved species need to be sorbed. Low cost, high efficiency, and reusability of biosorbent are some of the advantages of biosorption. Biosorption involves removal of toxic pollutants by biomass. Some microorganisms are targeted for the removal of single pollutant alone. Algae, bacteria, fungi, yeast, waste materials from agricultural and food industries, etc., are used as biosorbents. Different mechanisms like precipitation, absorption, adsorption, ion exchange are combined with biosorption in order to treat toxic pollutants. Collective ideas of various pollutant removal techniques in combination with biosorption and their applications to remediate water streams are discussed.

The applications of certain microorganisms have gained importance in applied environmental microbiology. Amongst them, biomineral processing is a field that deals with metal mining from ores, concentrates, industrial wastes, overburdens, etc., under the impact of microorganisms and/or their metabolites. Metals from poor quality ore and mineral compounds are removed by bioleaching process which is simple and low cost valuable technology. Metal recovery technique is widely practiced for the recovery of copper, gold, iron, manganese, and lead. Treatment of mineral industry effluents by microorganisms with incidental recovery of some metal values constitutes a similarly important area of biomineral processing. Microbial metal-leaching processes offer a possibility to recover metal values from mineral resources not accessible by conventional mining. Microbes act as biocatalysts to convert metal compounds into their water-soluble forms in leaching processes. Therefore, bioleaching has possible effect on metal retrieval and detoxification of waste products of industry, coal mine, sewage sludge, and heavy metal-contaminated soil.

In the developing countries, the usage of pesticides in the production of crops, fruits, and vegetables increases the economic status which establishes the major success in this field. Although the pesticide is an important aspect of the agricultural practices, the vast handling of harmful pesticides is an ultimate concern to the air, water, soil, and public health. Due to high impacts on human health, their application has been limited and different scenarios are developed to clean up the stubborn pesticides at different contaminated sites. Properties of polluted sites, temperature, pH, and nature of the pollutants are important factors which play a major role in the bioremediation process. Bioremediation technologies for cleaning up the pesticides at polluted sites additionally, their fundamentals, advantages, limitations, and the pesticides treated are summarized.

One of the major reasons behind the growing environmental pollution is illegal disposal of waste. Due to the toxicity of waste, establishing efficient and environmentally friendly method to degrade and detoxify these wastes represents an

important research challenge. Various physiochemical methods are applied all over the world for solid waste management. The application of microbes to degrade waste is gaining attention due to its environmental and economic benefits. Application of microbes and factors affecting the bioremediation of hazardous wastes are discussed. Authors have also discussed in detail about the prospects of waste valorization for production of biopolymers, biofuels, biocompost, and industrial enzymes.

Phytoremediation attempts the application of plants and microbes associated with plant root systems to protect the environment by removal of pollutants. Phytoremediation is capable of treating pollutants of dyes waste, which are derived from various sources. Adaptation in genetic levels is basic attitude behind plants that are able to manage the contaminants from the polluted site. The phytoremediation techniques are classified in detail. Treatment of textile dyes using plant remains an unfamiliar area of research. Mechanisms of uptake of different dyes by plants have also been proposed by authors. Selection criteria of plants for achieving high efficiency for treatment of dye contaminant wastewater have been projected.

Lignocellulosic biomass is most abundant in the environment. Enzymatic breakdown of lignocellulose, an important component of common waste materials, can be an essential step toward mitigating the wastes and generating biofuel. The diverse microbial community is maintained within the insect gut as per their food habit and ecological niche. Certain insects have enzymatic potential as they feed on lignocellulosic materials for their nutrition. In this context, scientific community has become interested to explore different insect gut microbial diversity through advent of new technologies. Potential role of insect gut bacteria, aspects of colonization, and role in degradation of lignocellulosic biomass are discussed. Further, the significance of potential bacteria for harnessing the enzymes and appropriateness of application in lignocellulosic wastes degradation are also discussed in one chapter.

Industrial and municipal wastewater contains numerous ingredients, and interestingly, some of the compounds in wastewater, like nitrogen and phosphorus, are identified as beneficial ingredients for microalgae cultures. Therefore, algal bioremediation can be considered as a feasible alternate technology for treating the wastewater in a cost-effective and assertable way compared to conventional water treatment process. These microalgal cultures are autotrophs, and they play a notable role in remediation of wastewater by their photosynthetic ability. A win-win situation of using microalgae in the bioremediation of wastewater provides tertiary biotreatment of wastewater coupled with production of potentially valuable biomass as bioresource for biofuel or high-value by-products.

Water utilization is on a steep hike due to the urbanization and population increase. On the other hand, pollution of freshwater due to human activities is increasingly a major concern as it affects economy and growth of a nation. Among various water pollutants, nitrogen compounds form a significant role in wastewater contamination due to the increase in anthropogenic sources like agriculture. Nitrate contamination in water and soil has become a growing environmental concern. According to USEPA standards, the maximum contamination level for nitrate is 45 mg L^{-1}, and the same standard is adopted by the Bureau of Indian Standards

(BIS). Among various technologies employed for treating nitrate-contaminated water, biological denitrification is one of the more versatile and promising methods widely being employed. The treatment of NO_3^- using bacteria referred as denitrification or bioremediation of nitrate has high separation efficiency. Bio-denitrification processes, immobilization of microorganism and different reactors employed for removing the nitrate from wastewater as well as reactor designs ranging from fixed-bed reactors to biological-aerated filters have been demonstrated for effective denitrification.

Volatile organic compounds (VOCs) are major air pollutants which are released into the environment. Stationary sources such as petrochemical and pharmaceutical industries release VOCs like toluene in the environment. In addition to it, VOCs pollute air, soil, and water which are a growing environmental concern. Several biological methods ranging from biotrickling filters to biofilters have been demonstrated, and they are found to be economical. The biofilters are simple to construct, easy to operate, and cost-effective. Major advantage of this method is that the pollutant is converted into biodegradable waste which can decompose within a moderate time frame, thus producing no secondary pollutants. Authors have focused on bioremediation of volatile organic compounds in biofilters.

Chapter 2
Mathematical Modeling in Bioremediation

Parthasarthy Vijay and Margavelu Gopinath

Abstract Roughly 98% of the available freshwater is represented by the groundwater on the planet. Protecting and re-establishing groundwater quality is of great importance. A major threat to the resources of groundwater is soil and aquifer pollution by hazardous wastes. This pervasive issue represents an important practical and cost-effective challenge because underground environmental pollution is tough to locate and eliminate by conventional extraction and excavation methods. Thus, there is a necessity for a broader investigation of efficient, in situ remediation approaches that uses the benefit of natural phenomena, such as bioremediation and natural attenuation. This chapter gives a prologue to the essential hypothesis and utilization of contaminant transport demonstrating by numerical methods. In the wake of perusing this section, the reader ought to have the capacity to choose a proper numerical model for the circumstance under thought, run the code, and adjust it. The utilization of numerical methods to tackle groundwater contaminant transport issues has turned into a broadly utilized strategy in view of the intensive enthusiasm for groundwater quality and the quick improvement of processing innovation, which has made numerical simulations accessible to hydrogeologists and civil engineers. If suitably applied, numerical models can give answers to the following questions.

- What is the relationship between solute concentration and location?
- What will be the time taken to reach a target level of solution concentration due to remediation process?
- Will a remedial measure reach a targeted concentration (reduced) in a certain time?
- How might one recreate the historical data of contamination to discover the relationship between population, time of exposure, and concentration?

The chapter starts with fundamentals of groundwater flow. The concepts of groundwater such as hydraulic head, Darcy's Law, hydraulic conductivity are discussed. Basics of transport processes, such as diffusion, advection that are involved in groundwater contamination, are also discussed. Later, theory of the model equations, assumptions considered, initial and boundary conditions are presented. In the latter

P. Vijay (✉) · M. Gopinath
Department of Chemical Engineering, University of Petroleum and Energy Studies, Dehradun 248007, India
e-mail: pvijay@ddn.upes.ac.in

S. J. Varjani et al. (eds.), *Bioremediation: Applications for Environmental Protection and Management*, Energy, Environment, and Sustainability,
https://doi.org/10.1007/978-981-10-7485-1_2

sections, analytical and numerical models are discussed in detail with few recent advances in bioremediation modeling. The discussion presented here is considered to be basics yet should provide ample background for the reader concerned with the detailed workings of a numerical model.

Keywords Bioremediation · Fate and transport equations · FEM · Modeling Solute transport

2.1 Basics of Flow of Groundwater and Transport of Contaminants

2.1.1 Introduction

Groundwater includes the part of the underground water that fills the pore space completely. Formations that carry groundwater are divided into aquifers, high-permeability hydraulic conductors, aquicludes, and low-permeability hydraulic isolators. There is no sharp boundary between aquifer and aquiclude. Sometimes even an transitional formation termed as the aquitard is formed, whose permeability is in between the aquifer and aquiclude.

Groundwater arises in porous media, like rock, sediments, and soil, underneath the ground surface. Once the entire pore space in a rock or soil is occupied with water, the material is termed to be saturated. However, a medium is termed as unsaturated when the pore space is partially filled with air and water. Water from snowmelt, rainfall, lakes, and rivers penetrates through the pores and cracks of rocks and soil, and it passes through vadose zone, as shown in Fig. 2.1. Water in the saturated and vadose zones are termed as groundwater. Shallow root zones of the plants mainly utilize soil water.

Groundwater is fundamentally held by capillary forces in between grains in the vadose zone. Many pores are completely saturated even within the vadose zone. The root zone reaches full saturation during substantial rainfall when the penetration capacity is reached. Pores present within the capillary fringe are also fully saturated; however, this zone has negative water pressure.

Ratio of volume of water and the volume of voids of the soil is termed as water saturation.

At the water table or phreatic surface, the pressure of water (p) is atmospheric pressure (1 atm). However, the water pressure becomes negative, above the water table. Due to hydrostatic forces, it increases with depth in the saturated zone.

An *aquifer* (from Latin, *aqua*—water, *fer*—to bear) is defined as an underground layer of water-bearing porous rock, rock fractures, or loosely packed constituents (gravel, sand, or silt) from which groundwater can be extracted using a water well. *Discharge* is termed as water moving out from an aquifer, whereas water moving in to an aquifer is termed *recharge*. Aquifers which are entirely saturated, and under

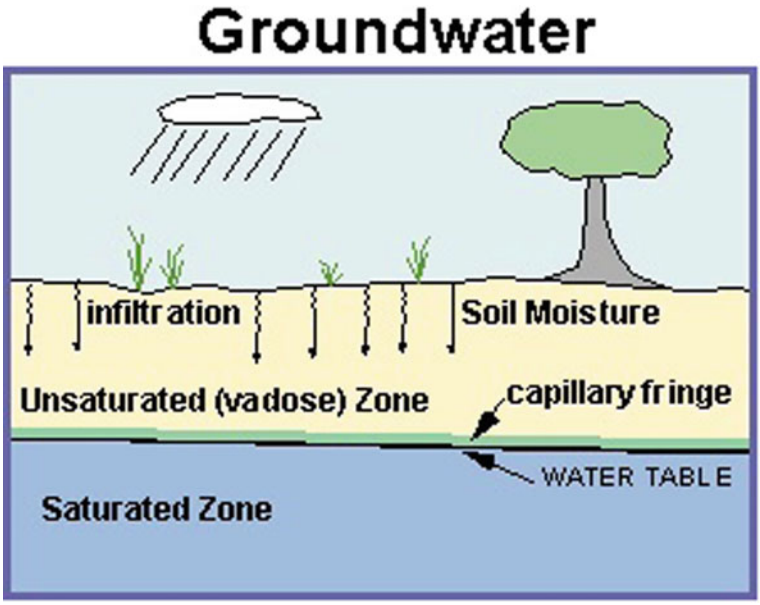


Fig. 2.1 Schematic representation of the vadose and saturated zones (http://www.westfield.ma.edu/personalpages/draker/edcom/final/webprojects/sp07/watercycle/hydro_cycle.gif)

pressure from the layers above are called as confined. Unconfined aquifers, instead, have the water table as an upper edge. Since unconfined aquifers are shallow, they are exposed more to contamination from events on the ground (Fig. 2.2). Although confined aquifers are believed to be protected by the aquitards. Yet, contamination of a confined aquifer may happen through a constructed path such as a well or pollution resulting in a recharge area.

Modeling of dissolved contaminant movement in groundwater includes two different processes: (1) flow of groundwater and (2) transport of contaminant. In the dominion of flow of groundwater, parameters describing water flow in the aquifer are focussed. In the domain of transport of contaminant, parameters depicting the pore network of aquifer and interactions between different contaminants, the aquifer solid, and contaminant concentrations in air or water are of interest. In this chapter, fundamentals in both these domains are discussed.

This data forms the origin for the governing fate and transport equations described later in this chapter. The dimensions of all physical and chemical terms will be expressed as mass [M], length [L], and time [T].

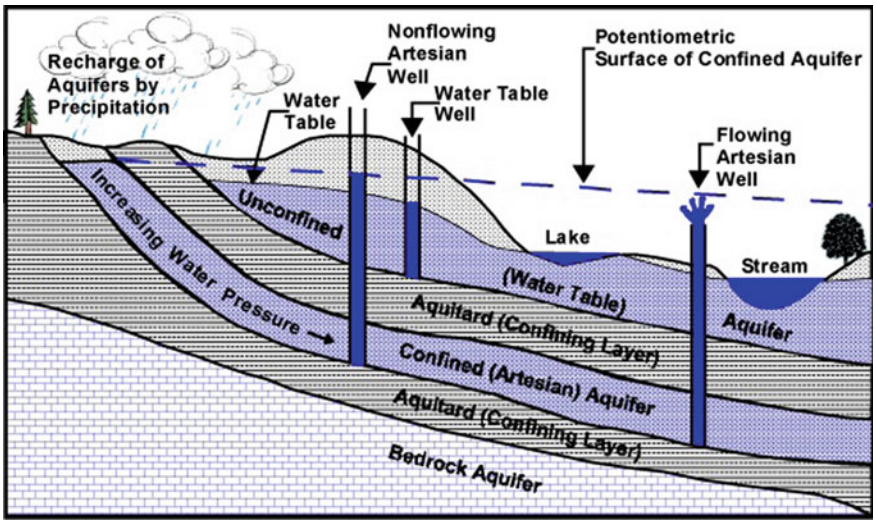


Fig. 2.2 Aquifer types and groundwater movement (http://www.in.gov/dnr/water/7258.htm)

2.1.2 Concepts of Groundwater

When following the flow paths of groundwater from a hill to an adjacent stream, water discharges into the stream from all probable directions. This curving path can be elucidated as a compromise amongst the force of gravity and the tendency of water to flow laterally in the direction of the slope of the water table. The propensity toward lateral flow is the result of the movement of water toward an area of lower pressure, the stream channel. The resulting movement is neither directly downward nor directly toward the channel, but relatively along the curving paths toward the stream. Groundwater can move upwards or downwards.

2.1.2.1 Hydraulic Head

Hydraulic head is defined as the elevation to which water can rise naturally in a well. Observation well or a monitoring well or a piezometer is an artificially created well for measuring hydraulic head. For unconfined aquifers, the hydraulic head is the same as the water table. However, for confined aquifers, it is not.

Fluid flow through porous and fractured media could be a mechanical method within which the driving forces liable for the fluid flow basically ought to overcome the resistance caused by viscous and resistance forces.

The general form of Phenomenological law is given by

$$\frac{\partial(\rho g)}{\partial t} + \nabla.(\rho \underline{v} g) = -\nabla.\underline{j}_g + \phi_g \tag{2.1}$$

G—Property
G/mass ≡ g
g—Scalar or vector
$\underline{j}_g$ —flux
$\nabla.\underline{j}_g$ —you get a "minus" sign because area normal points outwards.

2.1.2.2 Continuity Equation

In particular, we have G = mass; therefore g = G/mass ≡ 1
By definition, our system is of constant mass, and therefore $\underline{j}_g$ is zero.
No generation of mass ⟶ $\phi_g = 0$, we get

$$\frac{\partial \rho}{\partial t} + \nabla.(\rho \underline{v}) = 0 \tag{2.2}$$

$$\underbrace{\frac{\partial \rho}{\partial t} + \underline{v}.\nabla\rho}_{\frac{D\rho}{Dt}} + \rho \nabla.\underline{v} = 0 \tag{2.3}$$

Substituting Eq. 2.2 in Eq. 2.1, we get

$$\rho\frac{\partial g}{\partial t} + g\frac{\partial \rho}{\partial t} + g\nabla.(\rho\underline{v}) + \rho\underline{v}.\nabla g = -\nabla.\underline{j}_g + \phi_g \tag{2.4}$$

$$\rho\frac{\partial g}{\partial t} + g\underbrace{\left(\frac{\partial \rho}{\partial t} + \nabla.(\rho\underline{v})\right)}_{0} + \rho\underline{v}.\nabla g = -\nabla.\underline{j}_g + \phi_g \tag{2.5}$$

$$\boxed{\rho\frac{\partial g}{\partial t} + \rho\underline{v}.\nabla g = -\nabla.\underline{j}_g + \phi_g} \tag{2.6}$$

The above equation is used widely where density is constant.

$$\frac{\partial(\rho g)}{\partial t} + \nabla.(\rho\underline{v}g) = -\nabla.\underline{j}_g + \phi_g \tag{2.7}$$

G = Total momentum = $m\underline{v}$; g = $\underline{v}$

$$\frac{\partial(\rho\underline{v})}{\partial t} + \nabla.(\rho\underline{v}\underline{v}) = -\nabla.\underline{\underline{\sigma}} + \rho\underline{b}$$

$$\underline{\underline{\sigma}} = \underbrace{p\underline{\underline{I}}}_{\text{normal stress}} + \underbrace{\underline{\underline{\tau}}}_{\text{tangential shear stress}} \tag{2.8}$$

$$\rho\frac{\partial \underline{v}}{\partial t} + \underline{v}\frac{\partial \rho}{\partial t} + \rho\underline{v}.\nabla\underline{v} + \underline{v}.\nabla(\rho\underline{v}) = -\nabla p - \nabla.\underline{\underline{\tau}} + \rho\underline{b} \tag{2.9}$$

For constant density system, we get

$$\underbrace{\rho\left(\frac{\partial \underline{v}}{\partial t} + \underline{v}.\nabla\underline{v}\right)}_{\frac{D\underline{v}}{Dt}} = -\nabla p - \nabla.\underline{\underline{\tau}} + \rho\underline{b} \tag{2.10}$$

The above equation is the "Equation of motion" for constant density system (Bird et al. 1960).

Mechanical energy equation = $\underline{v}$.(Equation of motion)

$$\frac{\partial}{\partial t}\left(\frac{\rho v^2}{2}\right) + \underline{v}.\nabla\left(\frac{\rho v^2}{2}\right) = -\underline{v}.\nabla p - \underline{v}.\nabla.\underline{\underline{\tau}} + \rho\underline{b}.\underline{v} \tag{2.11}$$

$$\boxed{\frac{\partial}{\partial t}\left(\frac{\rho v^2}{2}\right) + \nabla\left(\frac{\rho v^2\underline{v}}{2}\right) = -\nabla.(p\underline{v}) + p\nabla.\underline{v} - \nabla.(\underline{\underline{\tau}}.\underline{v}) + \underline{\underline{\tau}} : \nabla\underline{v} + \rho\underline{b}.\underline{v}} \tag{2.12}$$

Omiting, time derivative term, replacing the last term (on the right-hand side) by $-g\nabla h$, where h is the elevation, in the above equation, and then dividing by ρ and then form the dot product with the unit vector $s = v/|v|$ in the flow direction. When this is completed, the term involving the curl of the velocity field can be shown to vanish (a good exercise in vector analysis), and $(s \cdot \nabla)$ can be replaced by d/ds, where s is the distance along a streamline. Thus, we get

$$\frac{\mathrm{d}}{\mathrm{d}s}\left(\frac{1}{2}v^2\right) = -\frac{1}{\rho}\frac{\mathrm{d}p}{\mathrm{d}s} - g\frac{\mathrm{d}h}{\mathrm{d}s} \tag{2.13}$$

Integrating the above equation between two points leads to *Bernoulli equation*, given as below.

$$\frac{1}{2}\left(v_2^2 - v_1^2\right) + \int_{p_1}^{p_2}\frac{1}{\rho}dp + g\left(h_2 - h_1\right) = 0 \tag{2.14}$$

Hydraulic head is the total of elevation and also the pressure head. The hydraulic head reflects the elevation of the top of a water column inside the aquifer relative to some point of reference.

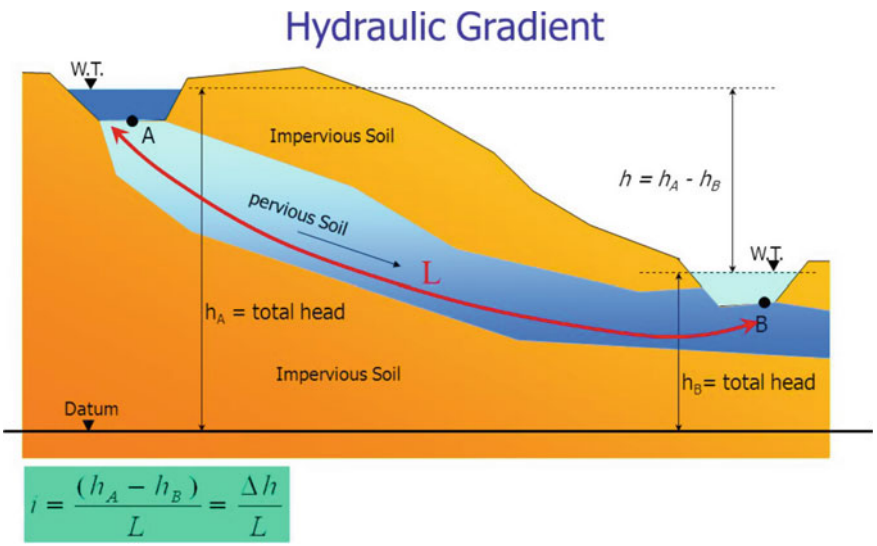


Fig. 2.3 Schematic representation of hydraulic gradient in an aquifer (http://slideplayer.com/slide/6104388/)

Hydraulic gradient could be a measure of the energy potential inflicting groundwater to flow between two points in a formation. This is schematically represented in Fig. 2.3.

2.1.2.3 Flow Through Porous Media

Wide range of engineers and scientists found interest in flow through porous media, who identified the importance of groundwater flows. In 1856, Darcy had performed numerous experiments based on the practical applications of flow through porous media. Darcy's experiment can be performed in laboratory using a similar experimental setup shown in Fig. 2.4.

Started as one-dimensional flow, extended to three-dimensional flow which covers most of the issues encountered in groundwater flow and oil recovery process. He found that the volumetric flow rate of discharged water flow is directly proportional to the *hydraulic gradient* $\Delta h/\Delta x$, cross-sectional area A. The proportionality constant is termed as the *hydraulic conductivity* (which we will discuss in detail in the following sections).

The empirical relationship of Darcy's law can be mathematically (Whitaker 1986; Greenkorn 1981; Permeability 2017) represented for 1-D case as,

$$Q = -KA\frac{\Delta h}{\Delta x} \tag{2.15}$$

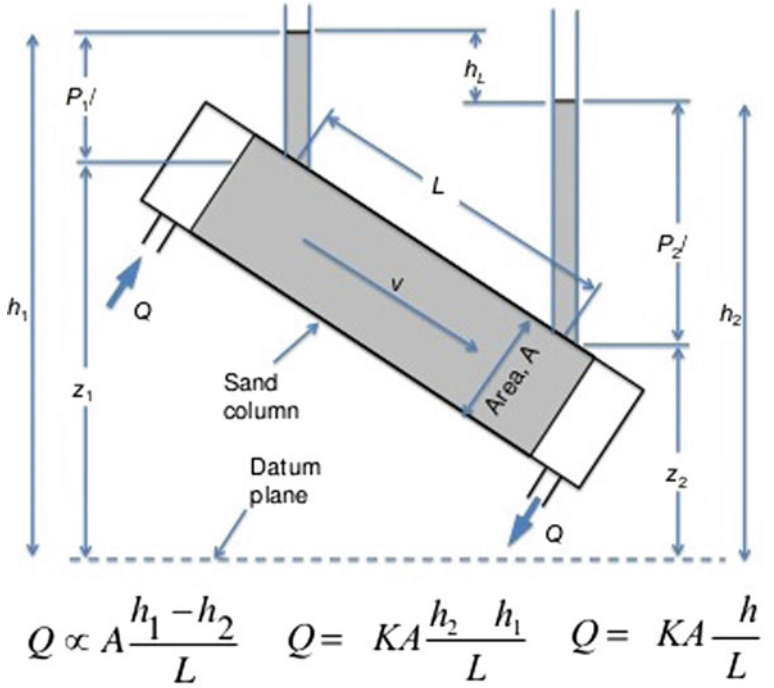


Fig. 2.4 Experimental set up used to show the relationship between volumetric flow rate of discharged water, hydraulic gradient and hydraulic conductivity

The dimensions of volumetric flow rate of discharged water, Q, hydraulic gradient, $\Delta h/\Delta x$, and hydraulic conductivity, K, are $[L^3t^{-1}]$, $[LL^{-1}]$, and $[Lt^{-1}]$, respectively.

2.1.2.4 Conductivity—Hydraulic

The hydraulic conductivity, K, defined by Darcy's law, Eq. 2.15, is dependent on the properties of the fluid as well as the pore structure of the medium.

Since the properties of the fluid such as density, viscosity are temperature-dependent, the hydraulic conductivity is also temperature-dependent.

Hydraulic conductivity can be written in terms of the intrinsic permeability and the properties of the fluids as

$$K = \frac{k\rho g}{\mu} \tag{2.16}$$

where k is the intrinsic permeability of the porous medium and is a function only of the pore structure. However, the intrinsic permeability is not temperature-dependent (Greenkorn 1981).

Darcy's law when expressed in three dimensions, permeability k will be a second-order tensor dependent on the directional properties of the pore structure. If hydraulic conductivity does not vary in space from one point to another, the hydraulic conductivity of that formation is homogeneous. Conversely, if the hydraulic conductivity varies from one point to another, then the hydraulic conductivity of a formation becomes heterogeneous.

Hydraulic conductivity is termed isotropic, if it does not exhibit directional dependence, while it is anisotropic if it varies from one direction to other. In most of the contaminant transport studies, hydraulic conductivity is treated as a constant, denoted as K_h since it can be economically not feasible to determine it at various locations.

Groundwater contamination has become a global ecological problem in several parts of the world and since the 1970s this has become an active research area. Substantial advances have been accomplished in our understanding of the intricate processes of fluid flow and contaminant transport in natural environmental media. Multiscale heterogeneity of the geological media makes it difficult to describe the system completely. Hydraulic conductivity (and permeability) for an aquifer increases with the dimensions of the test in a variety of geological locations (Illman 2004).

For those readers, who wish to learn more about hydraulic conductivity in detail, can refer the literature (Whitaker 1986; Greenkorn 1981; Freeze and Cherry 1979).

2.1.3 Concepts of Contaminant Transport Processes

Diffusion and advection are the basic processes involved in the transport of contaminants (whether soluble, miscible, or particulate) in the groundwater. *Transport* processes are defined as the process involved in the transport of contaminant, whereas the processes involved in decay, reaction, or volatilization of the contaminant are termed as *fate* processes. Amid advective process, dispersion, and diffusion cause the contaminant to spread and ends up in dilution.

Matrix particles, such as carbon, clay, or oxides of metals, adsorb organic contaminants thus decreasing the flowing contaminant mass and reducing its advancement relative to the advection of groundwater.

Transport process will also be get affected by reaction of organic contaminants or destruction by bioprocesses. However, vaporization of a volatile contaminant into air-filled pores will decrease its concentration in groundwater.

Dissolution of salts, namely sodium chloride in an aquifer, and exchange of slackly bound ions between matrix particles and water are categorized as inorganic reactions. Ion exchange may also occur in the groundwater, especially for sodium cations. Radioactive contaminants will decay according to their half live periods when transported.

The following sections describe the fate and transport factors in detail.

2.1.3.1 Diffusion

The general form of Phenomenological law is given by

$$\frac{\partial(\rho g)}{\partial t} + \nabla.(\rho \underline{v} g) = -\nabla.\underline{j}_g + \phi_g \tag{2.17}$$

Mass of component i $\quad G = m_i$, then $g = w_i$ (mass fraction of i)

By definition, $\underline{j}_i \equiv \rho_i(\underline{v}_i - \underline{v}) =$ function(dependent and independent variables)

ϕ_g—Generation of mass by reaction in terms of moles is given by

$$\sum_{i=1}^{n} r_i \nu_{ik} M_i$$

Therefore, we get

$$\underbrace{\frac{\partial(\rho w_i)}{\partial t} + \nabla.(\rho \underline{v} w_i)}_{\rho \frac{Dw_i}{Dt}} = -\nabla.\underline{j}_i + \sum_{i=1}^{n} r_i \nu_{ik} M_i \tag{2.18}$$

The rate at which a component is transferred from one phase to other depends on *mass transfer coefficient* and on the degree of deviation from equilibrium. Once equilibrium is attained, transfer stops. The mass transfer coefficients of various components in a given phase, under molecular diffusion prevails, will differ from each other to a greater extent. However, under turbulence, where molecular diffusion is relatively unimportant, the transfer coefficients become alike for all components.

Rates are conveniently described using molar flux (mol/(area)(time)). The area measured in the direction normal to the diffusion. The diffusivity or diffusion coefficient, D_{AB}, of a constituent A diffusing in B, measures the diffusive mobility. This is defined as the ratio of its flux (j_A) to its concentration gradient. The following expression is termed as "Fick's law" of diffusion. The direction of decreasing concentration is indicated by a negative sign.

$$j_A = -D_{AB} \nabla c_A = -c D_{AB} \nabla x_A \tag{2.19}$$

Rewritting Eq. 2.19 in z-direction, we get

$$j_A = -D_{AB} \frac{\mathrm{d}c_A}{\mathrm{d}z} = -c D_{AB} \frac{\mathrm{d}x_A}{\mathrm{d}z} \tag{2.20}$$

Diffusivity [L^2T^{-1}] is a characteristic of a constituent and its environment, such as temperature, pressure, concentration, direction, and nature of the other constituents.

Diffusivity of selected cations, anions in water, organic compounds in air and water are explained in the literature (Schwartz and Zhang 2003).

In systems, when the concentration changes with direction or space and with time (usually referred as unsteady state process), the mathematical expression requires second-order differential equation to explain the physics. Fick's second law of diffusion includes time as well as the second-order derivative of concentration with respect to space coordinates. The following expression (Eq. 2.21) denotes unsteady state diffusion equation in three dimensions (assuming diffusivity is constant in all directions).

$$\frac{\partial c}{\partial t} = D_{AB}\nabla^2 c \tag{2.21}$$

If we consider the diffusion occurs in only one direction, namely z, then the above equation will reduce to

$$\frac{\partial c}{\partial t} = D_{AB}\frac{\partial^2 c}{\partial z^2} \tag{2.22}$$

The analytical solution to this equation is

$$X(Z) = \frac{1 - \text{erf}(Z - \phi)}{1 + \text{erf}\ \phi} \tag{2.23}$$

where $X = \dfrac{x}{x_A}$, $Z = \dfrac{z}{\sqrt{4D_{AB}t}}$, and $\phi = v_z^*\sqrt{\dfrac{t}{D_{AB}}}$

For further details, one can refer Chap. 20 of *Transport Phenomena* by Bird et al. (1960).

2.1.3.2 Advection

Advection is the process of bulk transport of mass, heat, or momentum. This transport could be atmospheric flow, boundary layer flow, buoyant flow, pipe flow, etc.

It is considered to be the major driving force for the transport of solutes from one location to another lower location. This infers contaminants move at an indistinguishable speed from the streaming groundwater. So, information of flow patterns of groundwater offers the required information for forecasting solute transport under advection.

The advection process can be mathematically represented by advection equation as

$$\frac{\partial c}{\partial t} + u \cdot \nabla c = 0 \tag{2.24}$$

2.1.3.3 Solute Transport in Saturated Media

Solute transport in saturated media is given by advection-diffusion equation (ADE). We will discuss the derivation of this equation with the following assumptions: homogeneous, isotropic, fully saturated with water, steady groundwater flow (independent of time), and finally, Darcy's law is valid.

Consider a control volume as shown in Fig. 2.5, with the sides dx, dy, and dz, through which the solute flux occurs. Solute flux in and out of the control volume is controlled by two mechanisms, viz., advection and diffusion.

The mass of solute per unit volume of the porous medium can be defined as $\phi_e C$; thus, advective transport in the x-direction can be defined as

$$v_x n_e C dydz$$

Similarly, transportation by diffusion in x-direction is given as

$$\phi_e D_x \frac{\partial C}{\partial x} dydz$$

where v_x is the average linear velocity in the x-direction, C is the solute concentration (mass per unit volume of solution), dy dz is the cross-sectional area of control volume, and D_x is the hydrodynamic dispersion coefficient in the x-direction.

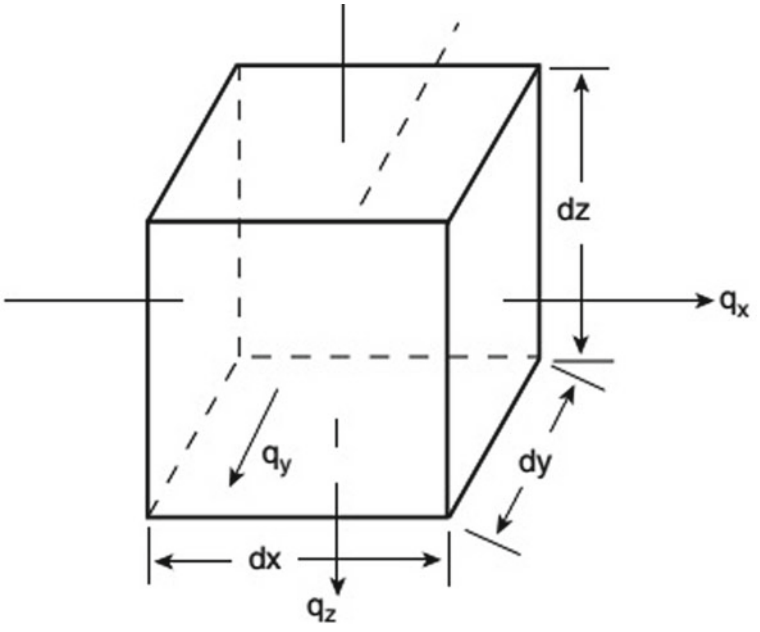


Fig. 2.5 Schematic representation of a control volume

The total mass of the solute per unit cross-sectional area transported by means of advection and dispersion processes in the x-direction per unit time is F_x and is given as

$$F_x = v_x \phi_e C - \phi_e D_x \frac{\partial C}{\partial x} \tag{2.25}$$

The negative sign before the second term shows that transport by diffusion takes place from higher to lower concentration. Diffusion transport is primarily based on Fick's Law, and this means that the dispersive mass flux is directly proportional to the concentration gradient. The proportionality constant is replaced by hydrodynamic dispersion coefficient.

Considering law of conservation of mass, the mass flux in the control volume entering and leaving is given as

$$\nabla \cdot F = -\phi_e \frac{\partial C}{\partial t} \tag{2.26}$$

Substituting Eq. 2.25 in the above equation, with the assumption of constant D_x, D_y, and D_z, we get

$$\left[D_x \frac{\partial^2 C}{\partial x^2} + D_y \frac{\partial^2 C}{\partial y^2} + D_z \frac{\partial^2 C}{\partial z^2} \right] - \left[\frac{\partial (v_x C)}{\partial x} + \frac{\partial (v_y C)}{\partial y} + \frac{\partial (v_z C)}{\partial z} \right] = \frac{\partial C}{\partial t} \tag{2.27}$$

The above equation is termed as "advection-diffusion equation (ADE)".

2.1.4 Other Terminologies

There are many terminologies used in the hydrology and in bioremediation of groundwater contamination. A few are dilution, dissolution, volatilization, adsorption, retardation, and degradation processes.

The most common degradation processes are microbial degradation, natural exponential decay, hydrolytic, photochemical, redox reactions.

2.2 Model Equations for Bioremediation

2.2.1 Theory

Fate and transport models are based on mass balances that include processes such as advection, dispersion, chemical reactions, and biodegradation of the contaminants as

a function of time. From the first principles and as discussed in the previous section, the unsteady state expression of concentration of contaminant may be given as

$$\frac{\partial C}{\partial t} = \frac{1}{R_f}\left[D\nabla^2 C - v\nabla C - \lambda C\right] \tag{2.28}$$

where C is the concentration of a dissolved contaminant at any given point at time t, R_f is the retardation factor for instantaneous, linear sorption, D is the hydrodynamic dispersion coefficient tensor, v is the groundwater velocity, and λ (lambda) is the first-order decay coefficient.

2.2.1.1 Assumptions

The unsteady state expression given in Eq. 2.28 explains the change of contaminant concentration with respect to time as a function of its diffusing in all directions (first term on right-hand side), the amount of contaminant transportation in advective flow of the groundwater (second term on the right-hand side), and the amount of contaminant degraded (last term on the right).

The above equation assumes, the hydrodynamic dispersion coefficient, D is constant and independent of space coordinates. However, earlier works states that dispersion coefficient is scale-dependent (Zhang and Neuman 1990; Neuman 1990). The decay coefficient, λ, which describes the degradation processes, includes microbial degradation, natural exponential decay, hydrolytic, photochemical, redox reactions (Domenico 1987).

Except in the case of very shallow groundwater, volatilization is not expected to contribute significantly to the overall dilution of the contaminant. Therefore, it is neglected (Chiang et al. 1989). The major assumption in the above equation is that the degradation occurs only in the aqueous phase.

2.2.1.2 Initial and Boundary Conditions

Eq. 2.28 can be solved analytically and numerically using various techniques. In order to obtain a unique solution irrespective of the technique used to solve, material properties of the domain and the driving function consist of initial and boundary conditions.

In steady-state process, an initial condition is not required. However, for unsteady state process, initial condition is a must. Initial distribution of the contaminant (solute) is specified using initial condition. For one-dimensional flow, in x-direction, the initial condition for the concentration is represented as

$$C(x, 0) = C_0(x) \tag{2.29}$$

This represents the initial concentration of solute at time t = 0, throughout x-direction.

Boundary conditions are used to specify the conditions/restrictions at the physical boundaries that help in solving the differential equations. There are three different types of boundary conditions, viz., Dirichlet, Neumann, and Cauchy conditions. We will consider the unsteady state concentration distribution along x-direction to explain different boundary conditions.

Dirichlet boundary condition:

This boundary condition specifies the concentration along the boundary for all time t > 0. This is represented as

$$C(x,t) = c(x) \tag{2.30}$$

Here, c(x) is the concentration that is specified along the boundary. In few cases, the latter may also be time-varying boundary condition.

Neumann boundary condition:

This type of boundary condition involves the relationship between the concentration gradient with space as well as time. This specifies the concentration gradient normal to the boundary, usually represented as a normal vector. Neumann boundary condition can be represented as

$$-D_x \frac{\partial C}{\partial x} = f(x) \tag{2.31}$$

where f(x) represents the dispersive flux normal to the boundary. If f(x) = 0, the above boundary condition becomes no-flux boundary conditions, indicating no mass transfer at the boundary.

Cauchy boundary condition:

This boundary condition is also termed as mixed boundary condition. Cauchy or mixed boundary condition includes fluxes of both concentration and its gradient along the boundary. This may be represented as

$$-D_x \frac{\partial C}{\partial x} + v_x C = k(x) \tag{2.32}$$

where k(x) represents the dispersive and advective flux normal to the boundary. For impermeable boundary, k(x) = 0 and there is no dispersive-advective flux throught the boundary.

Similarly, we can write all the above different boundary conditions for all space coordinates.

One of the most important steps in the modeling groundwater flow and solute transport is the choice of specifying the correct boundary condition. Wrong choice of boundary condition will lead to erroneous results of concentration profile of the contaminant solute.

Boundary conditions for different loading scenarios, such as pulse-type loading with constant concentration, constant input flux, exponential decay zero, and fixed

concentration at source, are explained in the literature (Domenico and Schwartz 1998).

2.2.2 Analytical Models

Considering there is a source zone of contaminant, we are concerned in expecting the concentration of the contaminants in the dissolved phase that might appear at a receptor or point of compliance (POC) some distance downgradient from the source zone. In order to make the predictions of the change of contaminant gradients with time and space, we use different analytical models. As in any mathematical modeling of any process, the initial and boundary conditions to be clearly stated. In the following sections, we will discuss the transport equations for steady and unsteady state process considering the following assumptions, initial and boundary conditions.

1. Unidirectional transport (only in x-direction);
2. Initial concentration of the contaminant is zero (C(x,0) = 0);
3. Homogeneous and isotropic aquifer;
4. Flow field of groundwater is uniform and unidirection, i.e., v_x is constant and $v_y = v_z = 0$;
5. Molecular diffusion is neglected, assuming groundwater flow is fast;
6. Diffusion follows Fick's law of diffusion;
7. Biodegradation kinetics is of first order, with biodegradation kinetic rate coefficient, λ, as constant.

2.2.2.1 Transport Equation in Unsteady State

Based on the assumptions mentioned above, the analytical solution for all dimensions transport may be obtained using Eq. 2.28. The solution is as follows (Domenico and Schwartz 1998).

$$C(x,y,z,t) = \left(\frac{C_0}{8}\right) exp\left[\frac{x}{2\alpha_x}(1-A)\right] erfc\left[\frac{x - v_c t\sqrt{A}}{2\sqrt{\alpha_x v_c t}}\right] .B.K \qquad (2.33)$$

where $A = \sqrt{1 + \frac{4\lambda\alpha_x}{v_c}}$, $B = \left[erf\left(\frac{y + Y/2}{2\sqrt{\alpha_y x}}\right) - erf\left(\frac{y - Y/2}{2\sqrt{\alpha_y x}}\right)\right]$,

$K = \left[erf\left(\frac{z + Z}{2\sqrt{\alpha_z x}}\right) - erf\left(\frac{z - Z}{2\sqrt{\alpha_z x}}\right)\right]$, C—contaminant concentration, C_0—initial contaminant concentration at the source, x—distance downgradient of source, y—distance from the centerline of the source, z—vertical distance from the

groundwater surface to the measurement point, Y—source width, Z—source depth, α_x—longitudinal dispersivity, α_y—horizontal transverse dispersivity, α_z—vertical transverse dispersivity, λ—site-specific first-order decay coefficient, t—time, v_c—contaminant velocity in groundwater, erf(x)—error function, and erfc(x)—complementary error function $= 1 - \text{erf(x)}$.

2.2.2.2 Transport Equation in Steady State

Under steady-state conditions, concentration is independent of time or in other words, Concentration does not change with time. Therefore, left-hand side term in Eq. 2.28 will become zero.

At steady-state conditions, $\text{erfc}(-2) = 2$, and therefore, Eq. 2.33 becomes

$$C(x,y,z) = \left(\frac{C_0}{4}\right) exp\left[\frac{x}{2\alpha_x}(1-A)\right] erfc\left[\frac{x - v_c t\sqrt{A}}{2\sqrt{\alpha_x v_c t}}\right] .B.K \tag{2.34}$$

where $A = \sqrt{1 + \frac{4\lambda\alpha_x}{v_c}}$, $B = \left[erf\left(\frac{y + Y/2}{2\sqrt{\alpha_y x}}\right) - erf\left(\frac{y - Y/2}{2\sqrt{\alpha_y x}}\right)\right]$, $K = \left[erf\left(\frac{z + Z}{2\sqrt{\alpha_z x}}\right) - erf\left(\frac{z - Z}{2\sqrt{\alpha_z x}}\right)\right]$, C—contaminant concentration, C_0—initial contaminant concentration at the source, x—distance downgradient of source, y—distance from the centerline of the source, z—vertical distance from the groundwater surface to the measurement point, Y—source width, Z—source depth, α_x—longitudinal dispersivity, α_y—horizontal transverse dispersivity, α_z—vertical transverse dispersivity, λ—site-specific first-order decay coefficient, t—time, v_c—contaminant velocity in groundwater, erf(x)—error function, and erfc(x)—complementary error function $= 1 - \text{erf(x)}$.

Time taken for concentration to become half, i.e., 50% from initial concentration level, can be calculated with ASTM 1998 as

$$t_{1/2} = \frac{x}{Av_c} \tag{2.35}$$

2.3 Recent Advances in Mathematical Modeling in Bioremediation

A quantitative framework supported a group of dimensionless numbers was developed to capture the results of competitors for surface and biokinetic processes and dene limits on the applying of in situ bioremediation. An integrated numerical mod-

eling and experimental approach was used to judge the quantitative framework. Experiments were conducted to look at the transport and biodegradation of hydrocarbon in an exceedingly saturated, heterogeneous intermediate-scale ow cell with two layers of different hydraulic conductivities.The experiments were administered in two phases: clinical test, simulating intrinsic biodegradation; and clinical test, simulating associate designed in place bioremediation (Song and Seagren 2008).

In Phase I, the dispersion was identied because the overall rate-limiting method supported the projected quantitative framework. Two designed perturbations to the system were selected in the clinical test to look at their skills to reinforce in place biodegradation. Within the rst perturbation, chemical element and phosphorus were spiked into the inuent resolution in way over the specified ratio amounts. This perturbation did not have a signicant impact as a result of dispersion, not biokinetics, was the rate-limiting method. However, within the second perturbation, temperature change was accrued, resulting in increased longitudinal and vertical crosswise dispersion, thereby assuaging the rate-limiting method and enhancing the biotransformation rate (Song and Seagren 2008).

Mathematical types of cometabolic biodegradation kinetics can improve our knowledge of the relevant microbial reactions and invite us to create in situ or in-reactor applications of cometabolic bioremediation. A number of models can be found, but their capacity to spell it out experimental data is not systematically assessed for a number of functional/experimental conditions. Here five the latest models of were considered: first order; Michaelis-Menten; reductant; competition; and blended models. The models were evaluated on their potential to match data from simulated batch tests covering an authentic selection of experimental conditions.

The simulated observations were made utilizing the most complicated model composition and parameters predicated on the books, with an extra experimental errors. Three standards were used to judge model fit: a capacity to match the simulated experimental data, identifiability of guidelines by using a collinearity research, and suitability of the model size and difficulty using the Bayesian and Akaike information conditions. Results show that no model suits data well for a variety of experimental conditions. The reductant model achieved best results but required completely different parameter collections to simulate each test. Parameter nonuniqueness was apt to be because of the parameter relationship. These results claim that the cometabolic models must be further developed if they're to reliably simulate experimental and functional data (Liu et al. 2015).

Vapor intrusion from volatile underground contaminants may be mitigated by aerobic biodegradation. Laboratory column studies with contamination sources of chlorobenzene and a combination of chlorobenzene, 1,2-dichlorobenzene, and 1,4-dichlorobenzene showed that contaminants were quickly degraded in thin reactive zones with high biomass and low substrate concentrations within the section of the capillary fringe. Such behavior was well characterized by a model that includes oxygen-, substrate-, and biomass-dependent biodegradation kinetics on with dispersive transport processes.

Associate degree analytical answer was derived to produce theoretical support for the simplification of reaction kinetics and also the approximation of reactive zone

location and mass flux relationships at steady state. Results demonstrate the potential of aerobic natural attenuation within the capillary fringe for preventing material migration within the unsaturated zone. The solution indicates that increasing contaminant mass flux into the column creates a thinner reactive zone and pushes it toward the oxygen boundary, leading to a shorter distance to the oxygen supply and a bigger oxygen mass flux that balances the contaminant mass flux. As a consequence, the aerobic biodegradation will reduce high material concentrations to low levels inside the capillary fringe and unsaturated zone. The results are in line with the observations of thin reactive layers at the interface in unsaturated zones. The model considers biomass whereas together with biodegradation within the capillary fringe and unsaturated zone and clearly demonstrates that microbial communities capable of exploitation the contaminants as electron donors might result in instant degradation kinetics in the capillary fringe and unsaturated zone (Luo et al. 2015).

There are two other ways to model reactive transport: spontaneous and innovative reaction-based approaches. The former, like the Kd simplification of sorption, has been wide utilized by practitioners, whereas the latter has been primarily employed in scientific communities for elucidating mechanisms of biogeochemical transport processes. It is believed that innovative mechanistic-based models might function protocols for environmental remediation also (Yeh et al. 2013).

Four example issues previously applied are used to demonstrate however numerical experimentation can be accustomed assess the feasibleness of various remediation approaches. The primary one involved the application of a 56-species uranium tailing drawback to the Melton Branch Subwatershed at Oak Ridge National Laboratory (ORNL) exploitation the parallel version of the model. Simulations were created to demonstrate the potential mobilization of uranium and different chelating agents within the planned waste disposal site. The second downside simulated laboratory-scale system to analyze the role of natural attenuation in the potential off-site migration of uranium from uranium mill tailings once restoration. It showed the inadequacy of employing a single Kd even for a homogeneous medium. The third example simulated laboratory experiments involving very high concentrations of uranium, technetium, aluminum, nitrate, and harmful metals (e.g., Ni, Cr, Co). The fourth example modeled microbially mediated immobilization of uranium in an unconfined aquifer using acetate modification during a field-scale experiment. The needs of those modeling studies were to simulate varied mechanisms of mobilization and immobilization of radioactive wastes, and for instance, how to apply reactive transport models for environmental remediation (Yeh et al. 2013).

The prosperous application of models can rely on several factors (Song et al. 2014):

- how flexibly they incorporate numerical and experimental tools to boost their predictive power;
- how effectively they resolve the issues of educational, industrial, and social interest;
- how considerably they surpass rival models, whereas one might like a particular model to others.

It is advantageous to require an integrative approach that by selection, combines multiple relevant methods, as mentioned within the preceding section.

Supra-organismal approaches specialize in the interactions of the entire community with the surroundings without considering cell boundaries. These models are applied to several domains of life including insect colonies and viruses, further as microbial species, and are significantly applicable for the analysis of large-scale complicated communities, like those found in human, soil, and marine microbiomes, the quantity of whose member species is tremendous. A crucial advantage of taking supra-organismal approaches is that various modeling approaches developed for analyzing single organisms are promptly applicable that embrace stoichiometric and dynamic modeling frameworks (Yoo et al. 2014; Song et al. 2013; Borenstein 2012; Orth et al. 2010; Ramkrishna and Song 2012).

Biomass-spreading rules utilized in previous cellular automation ways to simulate multispecies biofilm introduced intensive combining between completely different biomass species or resulted in spatially discontinuous biomass concentration and distribution; this caused results supported the cellular automation ways to deviate from experimental results and those from the additional computationally intensive continuous methodology (Tang and Valocchi 2013).

To beat the issues, we tend to propose new biomass-spreading rules during this work: Excess biomass spreads by pushing a line of grid cells that are on the shortest path from the supply grid cell to the destination grid cell, and also the fractions of various biomass species within the grid cells on the trail modification attributable to the spreading. To judge the new rules, 3 two-dimensional simulation examples are accustomed compare the biomass distribution computed using the continuous methodology and 3 cellular automaton methods, one based on the new rules and also the different 2 based on rules given in two previous studies. The link between the biomass species is syntrophic in one example and competitive within the different two examples. Simulation results generated using the cellular automaton technique based on the new rules agree far better with the continuous technique than do results using the other two cellular automaton strategies. The new biomass-spreading rules are not any more complicated to implement than the present rules (Tang and Valocchi 2013).

References

Bird RB, Stewart WE, Lightfoot EN (1960) Transport phenomena. Wiley, New York

Borenstein E (2012) Computational systems biology and in silico modeling of the human microbiome. Brief Bioinform 13(6):769–780

Chiang CY, Salanitro JP, Chai EY, Colthart JD, Klein CL (1989) Aerobic biodegradation of benzene, toluene, and xylene in a sandy aquiferdata analysis and computer modeling. Ground Water 27(6):823–834

Domenico PA (1987) An analytical model for multidimensional transport of a decaying contaminant species. J Hydrol 91(1):49–58

Domenico P, Schwartz FW (1998) Physical and chemical hydrogeology, 2nd edn. Wiley

Freeze AR, Cherry JA (1979) Groundwater. Pearson
Greenkorn RA (1981) Steady flow through porous media. AIChE J 27(4):529–545
Liu L, Binning PJ, Smets BF (2015) Evaluating alternate biokinetic models for trace pollutant cometabolism. Environ Sci Technol 49(4):2230–2236
Luo J, Kurt Z, Hou D, Spain JC (2015) Modeling aerobic biodegradation in the capillary fringe. Environ Sci Technol 49(3):1501–1510
Neuman SP (1990) Universal scaling of hydraulic conductivities and dispersivities in geologic media. Water Resour Res 26(8):1749–1758
Orth J, Thiele I, Palsson B (2010) What is flux balance analysis? Nat Biotechnol 28(3):245–248
Permeability (2017) http://slideplayer.com/slide/6104388/
Potentiometric surface mapping (1:48,000) overview. http://www.in.gov/dnr/water/7258.htm
Ramkrishna D, Song H-S (2012) Dynamic models of metabolism: review of the cybernetic approch. Bioeng Food Nat Prod 58(4):986–997
Schwartz FW, Zhang H (2003) Fundamentals of ground water. Wiley
Song HS, DeVilbiss F, Ramkrishna D (2013) Modeling metabolic systems: the need for dynamics. Current Opin Chem Eng 2(4):373–382
Song X, Seagren EA (2008) In situ bioremediation in heterogeneous porous media : dispersion limited scenario. Environ Sci Technol 42(16):6131–6140
Song H-S, Cannon W, Beliaev A, Konopka A (2014) Mathematical modeling of microbial community dynamics: a methodological review. Processes 2(4):711–752
Tang Y, Valocchi AJ (2013) An improved cellular automaton method to model multispecies biofilms. Water Res 47(15):5729–5742
WA Illman (2004) Analysis of permeability scaling within single boreholes. Geophys Res Lett 31(5):n/a–n/a. L06503
What is water cycle? http://www.westfield.ma.edu/personalpages/draker/edcom/final/webprojects/sp07/watercycle/hydro_cycle.gif
Whitaker S (1986) Flow in porous media I: a theoretical derivation of Darcy's law. Transp Porous Media 1(1):3–25
Yeh G-T, Gwo J-P, Siegel MD, Li M-H, Fang Y, Zhang F, Luo W, Yabusaki SB (2013) Innovative mathematical modeling in environmental remediation. J Environ Radioact 119:26–38
Yoo SJ, Kim JH, Lee JM (2014) Dynamic modelling of mixotrophic microalgal photobioreactor systems with time-varying yield coefficient for the lipid consumption. Bioresour Technol 162:228–235
Zhang Y-K, Neuman SP (1990) A quasi-linear theory of non-fickian and fickian subsurface dispersion: 2. Application to anisotropic media and the borden site. Water Resour Res 26(5):903–913

Chapter 3
Evaluation of Next-Generation Sequencing Technologies for Environmental Monitoring in Wastewater Abatement

P. Senthil Kumar, S. Suganya and Sunita J. Varjani

Abstract The waste generation and disposal into natural water bodies become a serious topic to be concerned by researchers today. Consequently, there is a demand for new strategies and technologies to address wastewater treatment and subsequent recycle and reuse especially in arid/semiarid areas. The harmful microbial load in raw sewage, toxic chemicals, and nutrients may cause pollution and can render water utilities unfit for human consumption or recreational activities. Biological treatment process is advantageous and constitutes tools to biodegrade organic matter, transfer toxic compounds into harmless products, and remove nutrient in wastewater microbiology. Bio-monitoring employs sentinel or indicator species in water bodies to infer water quality, ecosystem health status, and to protect public health from waterborne risks. Next-Generation Sequencing is one of the most leveraging studies focus on the ecology of microbial-mediated processes that influence freshwater quality such as algal blooms, contaminant biodegradation, and pathogen dissemination. Sequencing methods targeting small subunit (SSU) rRNA hypervariable regions have allowed for identification of microbial species which serve as bioindicators for sewage contamination in raw, treated, semi-treated water utilities. In addition, hidden diversity of unknown or uncultured microorganisms reveals the genetic capabilities for biodegradation of toxins and other contaminants. This chapter aims to provide brief knowledge about the development of bioindicators for sewage pollution and microbial source tracking, characterizing the distribution of toxin and antibiotic resistance genes in water samples. The assessment of biological risk, suitability, and unfairness inherent in the application of Next-Generation Sequencing may be a prior concern.

P. Senthil Kumar (✉) · S. Suganya
Department of Chemical Engineering, SSN College of Engineering,
Chennai 603110, India
e-mail: senthilchem8582@gmail.com

S. J. Varjani
Gujarat Pollution Control Board, Sector-10A, Gandhinagar, Gujarat 382010, India

S. J. Varjani et al. (eds.), *Bioremediation: Applications for Environmental Protection and Management*, Energy, Environment, and Sustainability,
https://doi.org/10.1007/978-981-10-7485-1_3

Keywords Biodegradation · Organic matter · Microbial consortia
Next-Generation Sequencing · Water quality · Fecal indicator

3.1 Introduction

The dangerous disposal practice of industrial and human waste creates unacceptable damage to natural environment. The reuse of treated wastewater for the purposes such as agricultural and landscape irrigation may reduce the amount of wastewater discharge to environment. Indeed, wastewater is an innumerable water source confronting water challenges and shortages in arid and semiarid areas by recycling and reuse in agriculture (Drechsel and Evans 2010). How the different forms of wastewater affect the ecosystem is a topic of serious concern. First, domestic or sanitary wastewater that comes from residential sources mainly contains body waste such as urine (urea and salt, toxins) that triggers the surveillance of intestinal disease-causing organisms (https://thewaterproject.org/Wastewater%20Pathogens.pdf) (*Escherichia* coli, Salmonella, Shigella and Rotavirus and Ascaris). Second, the industrial wastewater which discharges from manufacturing processes and commercial enterprises is a main source of residual acids, plating metals, volatile organic compounds, and other toxic and nontoxic chemicals. According to the US Environmental Protection Agency, fish populations are negatively affected by untreated wastewater that contains endocrine-disrupting chemicals (EDCs) (http://news.nationalgeographic.com/2016/02/160203-feminized-fish-endocrine-disruption-hormones-wildlife-refuges/). It creates two of the primary danger zones including feminization and disruption of fish populations. Thus, addressing wastewater treatment is must to get rid of wastewater pathogens, waste particles, and nutrients, but potential EDCs (Kidd et al. 2007) are more difficult to eliminate. A variety of ways is employed for those pollutants to present in pharmaceutical products, detergents, soaps, plastics, food, and personal care products such as perfume. For instance, the reproductive development of both the male and female minnows led to a near extinction of the fish from the experimental lake area, USA, (Lake 442 and Lake 260) during the fall of 1999–2005. It concludes that the EDCs present in wastewater have the potential to disrupt wild fish populations (Kidd et al. 2007; Besner et al. 2011). In many parts of the world especially in developing countries, health problems, burden of diseases, and mortality have often been caused by discharging untreated wastewater mixes with groundwater or inadequately treated wastewater. In the case of irrigation, soil conditions, the adoptable system of effluent distribution, and some degree of required quality of effluent that is to be irrigated on the crops that must be feasible in aquaculture systems and more reliance.

On the other hand, the discharge of wastewater to surface water, such as rivers, ocean or to groundwater must assure the acceptable overall water quality. Especially, the subsequent use of wastewater must improve the purity of water, removing contaminants, making it fit for reuse. Consequently, the most arid and

semiarid areas must be excused with water shortages (Blumenthal et al. 2000). Such discharges must be free from spreading diseases, disruption of aquatic life, or destruction of other forms of aquatic life, no or less impact on all living creatures. The problem lies in choosing appropriate wastewater treatment which must produce an effluent meeting the recommended microbiological and chemical quality standard including acceptable level of risk. Such standards in drinking water, wastewater, and recreational water are considered in availing any treatment strategies and risk-based approach to monitor and assess quality with minimal operational conditions (Microbiological water quality guidelines 2014). The difficulty of operating complex systems must be reliable and acknowledged by all private and government bodies. Rather than advanced treatment, a simple low-grade effluent reuse system must be designed that should meet stringent quality standard. A higher-grade effluent system is necessary in some cases to meet the essential wastewater treatment technology (Beale et al. 2013).

The wastewater treatment plant must be designed in order to reduce organic or inorganic origin of suspended solids and microbial loads to limit the pollution. Wastewater constituents including pathogens may be toxic or harmful to ecosystem. Besides, the occurrence of microbial population in wastewater is natural and which requires energy for respiration using dissolved oxygen and metabolizes them to compensate respiratory demand organic carbon food sources in order to synthesize new cells (Rattan 2016). The presence of natural organic food sources in the water helps releasing energy through the biodegradation of carbon matter with oxygen. During this process, nitrogen, phosphorus, and sugar are used as nutrients as help to create new microbial cell tissue and which organize them into plant tissue. The by-products of this phenomenon end in more microbial cells and carbon dioxide. In a clean or improved water environment, the limitation in organic food supplies leads to the periodic fluctuations with respect to the periods of microbial population swings or declines in the ecosystem. It aims to achieve an equilibrium condition over time (George 2004) on the basis of consistent amount of organic food present.

On the contrary, if raw or untreated sewage (water or human urine or solid waste) is discharged into surface water, the occupiers of waterborne microbes get elevated in response to the availability of high organic carbon content (hydrogen, oxygen, and nitrogen) that provides magnificent supply of food. This causes the aforementioned biodegradation reaction to precede fast as well oxygen consumption that results in the depletion of the treating water's oxygen. The presence of maximum amount of dissolved oxygen in receiving water is considered as a function of temperature, atmospheric pressure, salinity, elevation, and the solid content of the water. However, temperature is a main factor that influences the saturation value of dissolved oxygen. The saturation point of dissolved oxygen is observed at 0 °C which is relatively small and high at 30 °C. This poses high biodegradation reaction rate to the oxygen depletion (Seviour et al. 2000). Hence, summertime is more critical than during the cold winter for the maintenance of dissolved oxygen conditions in receiving or treating waters. Free dissolved oxygen holds for aerobic, and the depleted dissolved oxygen holds for anoxic or anaerobic ecosystem

Table 3.1 Summary of types of biodegradation system and their products

System type	Oxidizing compound	By-products	Problems associated with by-products
Aerobic	O_2	CO_2	None
Anoxic	NO_3	N_2 gas	Creating more solids
Anaerobic	SO_4	H_2S	Toxic, odorous, and corrosive in nature
Anaerobic	CO_2	CH_4	Odorous, toxic, and explosive in nature

Table 3.1 shows that some of the by-products produced via alternate reaction seem less favorable than the formation of carbon dioxide during oxygen consumption. Oxygen as oxidizing agent can be used in microbial biodegradation to obtain high energy and by-products without affecting the reaction system and ingredients. Nitrate can even attain the same energy level but produces nitrogen gas at the end of reaction that able solids floating in receiving waters or treatment systems. Under septic conditions, sulfur or carbon dioxide compounds lead to biodegradation of organic matter; however, extremely low energy yields result in gas (methane) and hydrogen sulfide production. These gases are toxic, corrosive, odorous, and explosive. They lead to acid formation and pH reductions in natural environment as well create unsafe environmental conditions (http://www.searo.who.int/entity/world_health_day/2015/whd-what-you-should-know/en/). The discussion on microbes' biodegrading organic compounds, also often cause extra problems and waterborne diseases to human from harmful human enteric microbes. In such case, some microbes (*E. coli* or fecal coliform bacteria) act as indicator of upstream human contact and human waste contamination. It is evident for the presence of harmful disease-causing pathogens.

3.2 Wastewater Treatment Mechanism

Generally, a wastewater treatment plant consists of sequential unit processes that receives raw sewage in headworks of the plant, directly allowing them for primary treatment process, in which debris/undesirable constituents are removed to make the water conductive and easier for the subsequent downstream process. Sedimentation process performs under quiescent conditions as preliminary treatment for letting pollutants settle at bottom of the primary treatment reactors' surface. Meanwhile, screening, shredding, grit removal, equalization, and pH neutralization may be preferred as per requirements. Whereas screening or shredding refines incoming wastewater to achieve uniform particles size, grit removal system is to remove abrasive substances; however, equalization is approached to reduce the variability of boulder waste loads, dampen influent flow, total suspended solids (TSS), and biological oxygen demand (BOD) fluctuations and minimize pH and temperature (Arora et al. 2014). Next, secondary treatment system employs the grown microbes in which biodegradation of non-settleable organic pollutants occurs. Disinfection follows to

kill pathogens, cleaning them to a certain degree where it is ready for the safety discharge to receiving water. Moreover, sludge processing facilities must be implanted, licensed to treat sludge residual forms at each unit process. Anyway, overall treatment plant optimization must be often encouraged to promote the downstream process efficiency and stability (Regan et al. 2002).

3.2.1 Biological Wastewater Treatment

The aim of a biological wastewater treatment plant is to force the microbe's biodegrade all of the polluted sewage's organic pollutant content as treated effluent. Most importantly, secondary aeration reactor designed in a way to contain numerous types of species and microbes to offer stable end-products by consuming organic compounds and pollutants as their nutrients. BOD (lbs/day) in a reactor is to be considered as a function of biodegradable organic content. TSS content is used to estimate the amount of bacteria in the treatment system. These solids are referred as mixed liquor, and their solids content is named MLSS (Kumar et al. 2014) after the mixed liquor suspended solids (MLSS). Indeed, the organic portion of the MLSS is estimated by measuring the volatile or combustible portion of the MLSS in a muffle furnace. This value is further called as the mixed liquor volatile suspended solids (MLVSS).

Limitations

- To prevent from over stimulating the growth of microorganisms in sewage, it is advised to limit the amount of available organic matter that is being discharged to the receiving water.
- To overcome the excess amount of dissolved oxygen, the depletion of discharged organic matter can be triggered in the environment which tends to biodegradation.
- Disinfect the wastewater discharge by minimizing the pathogenic consortium in the water.

3.2.2 Types of Microbes in Wastewater Treatment Plant

Availing the amount of food (F) to the microbes is fairly known as bacteria's expected growth rate. Food-to-microorganism ratio (F/M) of a biological treatment system is written as

F/M ratio = lbs/day of influent BOD into aeration/lbs MLVSS under aeration

Based on the amount of microbes present in the system, the amount of food consumed can be estimated. The following microbes can be seen in any wastewater treatment system. They are followed as,

Facultative Bacteria: Facultative bacteria are easily adaptable to survive and multiply in both anaerobic and aerobic conditions. An individual facultative bacterium possesses anaerobic nature unless there is a sort of mechanical or biochemical process involved in order to achieve more addition of oxygen to the wastewater. As the environment of bacteria is being transferred for a moment, the metamorphosis from anaerobic to aerobic state (and vice versa) takes place within a couple of hours.

Anaerobic Bacteria: Anaerobic bacteria can survive and reproduce themselves in the absence of free oxygen. They utilize nutrients from compounds such as sulfates and nitrates for enhancing metabolic activities and gaining energy which is later as seen as substantially reduced. As the bacterial consortium increases, the reduction in organic matter increases when the organic material in an anaerobic treatment system exposed to a significantly higher quantity of bacterial population and/or detained for a much longer period of time. This happens in a residential septic tank whereas slower metabolism of the anaerobic bacteria is allowed as wastewater is held for several days in order to perform 50% of reduction in organic material. It is coupled with some type of effluent treatment process. Anaerobic bacteria begin to act in the collection lines of a sewer system, releasing extremely hydrogen sulfide as well as explosive methane gas, both of which can be life threatening.

Aerobic Bacteria: Aerobic bacteria are susceptible to live and multiply in the presence of free oxygen. In case of aerobic bacteria, the primary source of energy is dissolved oxygen. Compare to anaerobes, the metabolism of aerobic bacteria is much higher with 90% less time. The removal of organic matter is rapid and leads to by-product (carbon dioxide and water) formation. Floc is a colonial form aerobic bacterium, kept in suspension by mechanical action to expose it to the organic material for the purpose of introducing oxygen into the wastewater. A gravity clarifier is used to separate and settle out the floc as the digestion starts.

Activated Sludge: It is a healthy state of aerobic floc. Rather than septic tank, activated sludge tank can also reduce the equivalent amount of organic material in approximately 3–6 h. This occupies a higher degree of overall process efficiency with respect to high removal levels. Additionally, less/no downstream treatment components are encouraged or totally eliminated.

Filamentous Organisms: The bacteria, algae, fungi, or other vertebrates are called filamentous organisms. These are often present in activated sludge tank. Filamentous organisms present in the form of beneficial and detrimental. The low-concentrated filamentous organisms serve to strengthen the floc particles which certainly reduce the amount of shear force during mechanical action and increase the floc particles in terms to size. While large floc particles may settle in the

clarifier, they tend to accumulate smaller particulates. In contrary, if the high-concentrated filamentous activated sludge will not settle well organisms, due to rising surface area (https://www.watertechonline.com/microorganisms-in-activated-sludge/). The floc particles tie to another to form an interfloc bridging that occurs to form a filamentous mat of extra-large size. When an excess concentration reaches, they are able to absorb a higher percentage of the organic matter and inhibit the growth of more desirable organisms.

Ciliates: Ciliate kind of animals is microscopic attributes to the organism great mobility to swim around in search of food. When the BOD supply in wastewater is declining, the water becomes clean as the large amounts of free swimming ciliate present in it.

Protozoans and Metazoans: Protozoans are single-celled organisms present high next to the bacteria in wastewater treatment system. Since they are larger in size, screening, identification, and characterization are easy to carry out. They indicate both the biomass health and effluent quality. But, metazoans are multi-celled organisms. Excludingly, they are similar to protozoans. Macro-invertebrates such as nematodes and rotifers are typically abundant only in a well-developed biomass. The abundance of certain species is a predictor of operational changes within a treatment plant system. Monitoring the changes in protozoans' population, one can minimize negative operational effects in a plant.

Amoeba: Amoebas are single-celled microbes that highly depend on bacteria for food since they cannot swim. In alter, they have pseudopods or "fake feet" which helps them to reach and entrap bacteria. It is an indication that large amount of microscopic presence of pseudopods or "fake feet" refers to the higher bacterial population.

Flagellates: Flagellates have a poorly developed tail called flagella, which helps to move slowly to search for food. They even depend on bacteria to graze up since they are impotent to swim for the necessity of food.

3.2.3 Water Quality Analysis

Important aquatic ecosystems including rivers, lakes, and streams are a source of drinking water which is being contaminated by increase in human population, urbanization, and the utilization of natural water resources for human consumption and recreation. Water gets unfit for human consumption when toxins, nutrients, and resultant algal blooms contaminate the water quality up to certain degree. Recently, Water Management Authorities use bio-monitoring tools including sentinel or indicator species in the form of microbes and aquatic macro-invertebrates (Clara et al. 2005). It provides information about the water quality, risk associations, and ecosystem health status to protect ethnic group less susceptible to waterborne risks. The abundance of indicator organisms can be measured easily as a function of quality of water and being served as proxies which are then correlated to often unknown agents that directly mediate waterborne risk such as pathogens, biotoxins, and chemicals.

Emerging molecular methods have ushered in bringing opportunities to assess waterborne microbial communities for the development of indicators, new markers for microbial source tracking and sentinels, and observation of microbiologically mediated processes. Though nucleic acid-based methods are having biases, uncertainties, associated with NGS techniques, are highly recommendable now. Culture-dependent enumeration and detection of fecal indicator bacteria (FIB) serve improved quality of drinking water. For instance, total coliforms, Escherichia coli, or Enterococci are being used under the "gold standard" in the assessment of microbial safety of water. Such presence of FIB's in freshwater acts as a proxy for risk-associated pathogens, where risks of exposure have been seen as incidence rate of disease in exposed populations. In some cases, these proxies are detrived from non-human sources which are imperfect to predict ecological interactions. The predictive power (contamination from sewage) of microbial proxies can find the origin of sewage since fecal material of human-origin transmits human pathogens. It offers the most significant risk to water quality.

Nowadays, "source tracking" enables at least two species within the same genus bacteroides as indicators of human-origin. Finally, direct detection and enumeration of specific pathogens such as waterborne *Leptospira*, *Campylobacter*, *Legionella*, viruses (e.g., *Norovirus*), and parasites (e.g., *Cryptosporidium*) enable quantitative microbial risk assessment whereby environmental concentrations of pathogens are compared to models of infectivity to characterize the risk to exposed human populations.

Some of the main well-waterborne diseases are caused while consuming untreated well-water where waterborne pathogens such as Legionella and Campylobacter present. Other causative agents are mostly parasites such as Cryptosporidium parvum, Toxoplasma gondii, Cyclospora cayetanesis, Giardia lamblia and viruses such as norovirus. Studies reported that some potential pathogens' DNAs (*Legionella*, *Mycobacterium*, *Pseudomonas*, and *Leptospira*) serve as indicator and virulence factors during water treatment processes, and NGS shed light on the fate of microbial populations.

Studies of microbial population require microbial biomass from large amount of drinking water. But in real-time studies, the microbial abundance (e.g., $<10^5$ cells per ml) is less where isolated DNA concentration is very low to allow for sequencing (Cai and Zhang 2013).

3.3 Infrastructure of Wastewater Treatment Plant

3.3.1 Drinking Water Distribution System

Drinking water is assumed to be safe by consumers, yet the microbial ecology of drinking water distribution systems (DWDS) is not easily accessible that creates challenging environments for microbial life as well for human beings. It is traditionally believed that DWDS is available for diverse microbial ecosystems in order to introduce molecular-based methods to improve the quality of drinking water.

Such ecosystems contain favorable to non-favorable microbial abundance from a variety of viruses to protozoa. Preventive measures of water must be taken right from the origin to treatment plant through complex water distribution infrastructure. In order to achieve safe, reliable, efficient, sterile water with high quality, researchers focus on the microbial contamination, the disinfection residuals in the majority of DWDS, treatment methodology, and alteration in water quality. During installation of any treatment plant, it is possible for microorganisms to obtain various nutrients developed within DWDS (Boe-Hansen et al. 2003). Once microorganisms enter DWDS, they will face and encounter a challenging environment, with limited nutrient supply and changing water flow and pressure fluctuations at non-steady-state conditions. It results in the survival prone of microbes in pipe walls forming biofilms where they are protective from external adverse factors including temperature, pressure, etc. As a consequence, this survival mechanism raises few questions to be answered by researchers. They are (a) which type of microorganism is present and their abundance? (b) how their activities influence other organisms and reshape the environment including any possible effects on human health? and (c) how is an environment affected by its structure and the presence of microbial population and their function?

Enormous methods have been attempted to analyze these questions as per the regulations drawn by Environmental Protection Agency. The application of this study offers brief idea about culture-dependent and culture-independent techniques. According to water companies, culture-dependent methods are encouraged to assess the drinking water quality by detecting, enumerating fecal coliforms as indicator for monitoring drinking water at a reasonable cost. However, they provide limited information about total microbial community (TMC) (encompassing <1% of the diversity) and a slight change therein. To overcome this, culture-independent techniques have been improved to study completely about the microbial world in DWDS. Investigating microbial communities by water utilities is slow, since they require more specialized equipment, trained personnel, and are more expensive than the culture-dependent techniques. However, expectation lies on a number of culture-independent methods routinely used in the near future (Hamonts 2012).

This chapter provides fundamental information about microbial ecology of DWDS to preserve and guarantee safe and good quality of potable drinking water. Better insights into microbial ecology of drinking water prove more reliable risk assessments and improve current control and management strategies.

3.3.2 Types of Water Sampling

3.3.2.1 Bulk Water Sampling

International organizations and water companies draw special attention to appropriate sampling procedures that account for microbiological parameters. In accordance with bulk water sampling (BWS), the World Health Organization (WHO) has

certain standards and guidelines for drinking water quality (DWQ). According to these standards, a guide is developed at a national level to help collect water samples. Despite stringent guidelines are framed for regulatory purposes, there is no enough detailed scientific literature available about sampling methodologies, making the evaluation, comparison of data across systems and research difficulties. To avoid contamination during collection is prior concern and is appreciated by choosing suitable sampling containers for transport and storage. The modern approach of molecular based (DNA/RNA) or based on proteomics or metabolomics does not have any specific protocol guidance (Bai 2012). There are no standards found to represent sampling volume to focus on microbial biomass for downstream molecular analysis. It must answer for how, where, and when samples are taken for the suitability of advanced microbial analysis. As a consequence, the results of molecular work from various laboratories are unable to compare and become difficult due to the lack of standards.

3.3.2.2 Biofilm Water Sampling

On the other hand, biofilm sampling is one of the key components in DWDS microbial studies. It is applicable to bench-top laboratory including any kind of biofilm reactors such as the rotating disk reactor (RDC), the biofilm annular reactor (BAR), the propeller reactor (PR), and moving bed biofilm reactor (MBBR) but not in pipes since they are not readily accessible. Thus, one involves design and cutouts of pipes; the other one relies on extra installation or devices inserted into the pipe. Pipe cutout sampling protocols require man power, expensive and categorized as destructive sampling methods. In addition, the uncovering cutting processes often lead to concerns with contamination which hinders the entire reaction condition. The installation of devices allows to study about biofilm dynamics over time with respect to changing abiotic and biotic factors in situ. Sometimes, they twist hydraulic conditions in pipes, and in most cases, shear stress and turbulence regimes are different from those expected in real pipes, artificially influencing the way biofilms develop. Furthermore, some devices such as the "Biofilm Sampler" are directly connected to a DWDS to avoid such conditions. The in situ analysis of biofilms is able to extract nucleic acids for further characterization of microbial consortia. However, the numerous abiotic factors might strongly influence their formation and properties (Van Delft 2010).

3.4 Microbiological Techniques

To address the significance of maintaining potable water quality, conventional techniques are developed that detect, quantify, and characterize microbial consortia in drinking water-related samples. Therefore, microbial quality of water can be

analyzed using conventional microbial techniques. In this section, the existing techniques, applications, advantages, and limitations are discussed.

3.4.1 Microbial Detection and Enumeration

3.4.1.1 Culture-Dependent Techniques

This is the most advantageous technique followed by many water companies and analytical agencies to routinely monitor and assess microbial quality of drinking water, including the sensing and detection level of fecal contamination. Heterotrophic plate count (HPC) measurements are one of the most common reference methods for regular bacteriological monitoring in drinking water which letting only heterotrophic bacteria to form colonies on a solid medium at a specific temperature. After a defined incubation time, the number of colonies grown is counted to estimate the bacteriological biomass in the water samples. Incubation of plates attains decent microbial count during the temperature varies from 20 to 37 °C and over time from a few hours to several days as per standard operating procedure (SOP) defines. HPC method yields information about a small fraction of the whole microbial load at low cost (Fish 2013). It is widely accepted with relative simplicity that makes HPC a convenient tool for water utilities to assess the efficiency of water treatment and risk management and to infer regrowth of microorganism in the network.

Culture-dependent methods are also used to detect indicator microorganisms such as coliform bacteria. Coliform bacteria (e.g., *Escherichia* spp., *Enterobacter* spp., and *Citrobacter* spp.) are habitual inhabitants of animal feces. However, their presence beyond certain concentrations, established in specific legislations, is used to surmise fecal contamination in the water. The membrane filtration (MF) technique and the multiple tube fermentation (MTF) method are often used to detect coliforms in drinking water (Gagnon et al. 2005). The MF technique has an essential feature of filtering a water sample to concentrate cell biomass followed by incubation of filter on a specific solid medium, and after a given period of time, the developed colonies are enumerated. As well in the MTF technique, the bacteriological load is estimated by inoculating a series of tubes containing liquid medium with tenfold dilutions of water sample. As the medium provides supplements, the sample becomes turbid as an indicator of microbial growth. The most probable number (MPN) technique is used to express an estimation of the average number of bacteria in the sample. The tests are used to analyze the coliform bacteria which are relatively economic, easy, and safe to execute and convenient tool to assess risk of fecal contamination. Enzymatic reactions are alternate and more sensitive approach to detect coliforms using enzymes b-D galactosidase and b-D glucuronidase.

The ColilertR is an enzymatic test used widely to detect coliforms quantitatively followed by Quantity-Tray (QT) test. When specific medium is inoculated into the water sample contains specific enzyme substrates that are able to contact a specific microorganism to cause a quantifiable color change. These methods are easy to

detect non-culturable coliforms, but they are more expensive when compared with cultivation methods. Coliforms are indicators of fecal pollution monitored in water sample. Clostridium perfringens is the sulfite-reducing anaerobe bacterium, considered a good indicator of fecal contamination. The fecal origin is determined by the spore formation of this bacterium. Naturally, they are more resistant than vegetative cells and can survive disinfection. Consequently, *Clostridium* spp. is a better indicator than *Escherichia coli* of the presence of more long-lasting organisms such as viruses and protozoa because the survival conditions for both of them are similar. Therefore, culture-dependent methods are used as convenient diagnostic tool which are ease to perform, relatively low cost, and rapid detection in general microbial failures in the system. Yet, they are practically applicable for only specific fraction of microbial communities in water samples.

3.4.1.2 Culture-Independent Techniques

Microscopy-based methods are used traditionally to circumvent the limitations of culture-dependent techniques in representing the actual microbial diversity. For instance, epifluorescence microscopy monitors the quality of drinking water than traditional plate counts, which have long incubation times. Different fluorescent dyes can be used in biofilms and/or water samples for staining purposes, to estimate total cell counts using an epifluorescence microscope. In general, acridine orange (AO), 4, 6-di-amino-2 phenylindole (DAPI), and 5-cyano-2,3 Dytolyl Tetrazolium Chloride play a role as dye to quantify microorganisms in water and biofilm samples. LIVE/DEAD® Bacterial Viability Kit is widely used to check the viability of cells which fairly contains two nucleic acid stains: SYTO 9™ (green-fluorescent) and propidium iodide (PI) (red-fluorescent). The SYTO 9™ dye is able to penetrate all membranes while PI can only penetrate into cells with damaged membranes. Therefore, cells with compromised membranes will usually stain red, whereas cells with undamaged membranes will stain green (Ginige et al. 2004).

3.4.1.3 Fluorescent in situ Hybridization (FISH)

Fluorescent in situ hybridization is an advanced version of epifluorescence microscopy which allows detection rapidly and enumeration of specific microorganisms. In this method, fluorescent-labeled oligonucleotides probes (usually 15–25 bp) are used and bind to specific microbial DNA in the sample, allowing the visualization of cells in an epifluorescence or confocal laser scanning microscope (CLSM). It contributes to successful characterization of microorganisms within biofilms and to detect pathogens in drinking water samples. The catalyzed reporter deposition fluorescence in situ hybridization (CARD-FISH) is an improved version of FISH which allows oligonucleotide probes labeled with a horseradish peroxidase enzyme (HRP) to amplify the intensity of signal obtained from microbial load. It can enhance the fluorescent signal from cells in samples at low microbial

concentration. Using this, changes in *Legionella pneumophila* in DWDS can be investigated. This cannot be a standalone technique but can combine with other methods to characterize microbial consortia (Wagner et al. 1993). The higher affinity peptide nucleic acid (PNA)-FISH is useful to study pathogens in biofilms due to enhanced capability of the probe to penetrate through the Extracellular Polymeric Substance (EPS) matrix. Next, LIVE/DEADeFISH is combined with the cell viability kit with FISH to detect the efficiency of disinfection in DWDS.

Limitations:

(1) The little knowledge of the nucleotide sequence of the target organisms is required.

(2) The knowledge to design new probes specific to target spp.

(3) The knowledge to optimize the hybridization reaction conditions.

(4) The knowledge to interpret the efficiency of hybridization.

(5) The knowledge about the physiological state of the cells.

(6) The knowledge about signal emission by autofluorescence cells.

(7) The target of microorganisms must be prioritized.

Pros

- It helps for phylogenetic identification
- It visually differentiates non-cultivable microorganisms
- Highly sensitive and quantitative
- Single time detection of different microbes by using multiple fluorescent dyes

Cons

- Designing a probe is difficult unless sequence information is readily available for specific detection
- It is hardly difficult to differentiate between live and dead cells
- It possess difficult accessibility to target gene

3.4.1.4 Flow Cytometry (FC)

Flow cytometry is a rapid and reliable method to audit the bacterial abundance and viability of planktonic cells in suspension with the help of fluorescent dyes.

Through a capillary, the cell suspension is passed while it gets intersected by a laser beam, and the interaction between laser and cells occurs, causing the light to scatter and also excite the dye. Meanwhile, the fluorescence intensity and scattering generated can be quantified using different detectors. Whereby, various fluorescent dyes FC are employed to detect and measure total bacterial counts, virus-like particles, *Cryptosporidium,* and *Giardia* since dyes are more advantageous than traditional plate counts in order to produce much more realistic quantification of the total number of cells in water samples. However, when epifluorescence microscopy and flow cytometry are used to measure cell volume and/or estimate the viability or total cell counts of biofilms and sediments, both methods are susceptible to errors due to the formation of cell clusters and the attachment of cells to inorganic compounds (Metzker 2010).

3.4.1.5 PCR-Based Methods

The polymerase chain reaction (PCR) is a method used to amplify (i.e., obtain multiple copies) fragments of DNA. PCR-based methods involve in the extraction of nucleic acids (DNA/RNA), followed by the amplification of a target gene or genes via PCR and post-PCR analysis. This section discusses about the amplicons obtained from PCR and how it helps in all community fingerprinting techniques and Next-Generation Sequencing methods. Multiplex-PCR and quantitative real time (qePCR) is commonly used to detect microorganisms in drinking water.

Multiplex-PCR has been used in drinking water-related research to simultaneously detect several microorganisms using distinct oligonucleotide probes. q-PCR is a sensitive, quantitative tool used to detect environmental samples based on quantifying the number of target gene copies present in a sample. By quantifying a fluorescent reporter, the amount of PCR product obtained during the exponential phase of the PCR reaction can be determined. Then, the amount of detected reporter is correlated with the initial amount of target template allowing the quantification of the target organism. Despite the general limitations of the PCR-based techniques, it is used to detect viral indicators of human fecal contamination and pathogenic bacteria such as *Helicobacter pylori*, *Mycobacterium avium,* and *Legionella* sp., and to quantify *Giardia* and *Cryptosporidium* (Vesey et al. 1993).

Pros

- It is highly sensitive and quantitative
- It is accurate, rapid, and specific to gene quantification

Cons

- Real-time PCR fails to obtain enough and good-quality RNA

3.4.2 Microbial Community Composition

This section provides information about the microbial members present in drinking water-related samples. This information will help in order to study about the essential pathogens, microorganisms associated with corrosion, disruption, or water discoloration to monitor biofilm formation on pipes or any devices in the treatment plant, to assess the influence of abiotic factors on microbial communities and to compare diversity between different samples.

3.4.2.1 Phospholipid Fatty Acids

The microbial load in various environmental samples can be analyzed by Phospholipid fatty acids (PLFAs)-based methods. The membranes of microorganisms are made up of phospholipids which contain fatty acids used as fingerprints to study biofilms and to detect pathogens. However, it does not provide identification of specific species actually present in the samples.

3.4.2.2 Bioinformatic Tools

The characterization of natural microbial communities is highly dependent on the molecular techniques without the necessity of culturing microorganisms. It has introduced new insights into the microbial ecology of different ecosystems. Molecular analysis includes the extraction and purification of DNA and/or RNA followed by PCR amplification of "marker genes" to obtain taxonomic/phylogenetic information. Because DNA provides information about the entire microbial community of the samples while RNA-based analysis represents only the active part. The ribosomal RNA (rRNA) gene, 16S rRNA for prokaryotes, and 18S rRNA for eukaryotes are some of the common marker genes which help to find the transformed cells. The rRNA gene has different regions, some are highly conserved across all phylogenetic domains (i.e., bacteria, eukarya, and archaea), other regions are variable between related species, and this variability allows for inferring phylogenetic information from microorganisms inhabiting different ecosystems.

The databases of small (16S/18S) and large subunits (23S/28S) of rRNA sequences for bacteria, archaea, and eukarya have been established in recent years and are constantly expanding to identify the sequences of microbes recovered from environmental samples. SILVA rRNA database project provides good-quality, aligned ribosomal RNA sequence data which is regularly updated. Through the Ribosomal Database Project (RDP), Greengenes database, other databases are also accessible. An overview of the choice of primers pairs available for bacteria and archaea can be found there. It comprises of all the best available primers' pairs up for different amplicon sizes with respect to the SILVA 16S/18S rDNA non-redundant

reference dataset. In last, the resulting PCR products (i.e., amplicons) can be separated and analyzed using different techniques (Isabel et al. 2014).

3.4.2.3 Fingerprinting Techniques

The most commonly employed fingerprinting techniques are useful to simultaneously analyze multiple samples and to compare different microbial community structures. Denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) are two distinct fingerprinting techniques where specific fragments of the rRNA gene are amplified and then separated based upon their sequence composition in aforementioned techniques. It results with a pattern of bands which acts as a visual profile of the most abundant species in the studied microbial community. Semi-quantitative method is generally focused to study about the changes in microbial population in order to estimate species abundance and richness. In addition, specific bands on the gel can be excised and sequenced for subsequent taxonomic identification.

DGGE is the most powerful method to study the effect of stagnation in taps, corrosion on cast iron pipes, nitrification in drinking water networks, and occurrence of fungi in biofilms and their solid mediums and to assess bacterial water quality in real distribution systems.

This method also has drawbacks and difficulties in handling polyacrylamide gels; obtaining the optimal denaturing conditions is highly laborious; associating a single band with a particular species is complicated; and cloning and sequencing of particular bands are ultimately needed for confirmation of results. Despite the use of markers on the gels, comparison of patterns across gels, molecular weight, and the detection of rare members of the microbial community are challenging.

Pros

- It seems quick profiling of spatial temporal variability
- It can analyze large number of samples simultaneously
- Bands on DGGE/TGGE and SSCP gels can be excised

Cons

- Bias/unfairness associated with PCR
- Only predominant species can be detected successfully
- No direct taxonomic identification
- Time-consuming, requires post-PCR analysis of samples
- Analysis of short sequences (<500 bp)

- DGGE is difficult to compare between gels
- T-RFLPs and ARDRA are difficult for resolution of microbial profiles

3.4.2.4 Terminal Restriction Fragment Length Polymorphism (TeRFLP)

The amplification of short fragments of a marker gene using end-labeled primers can be studied by terminal restriction fragment length polymorphism (TeRFLP). Whereas restriction enzymes (e.g., Alu I, Cfo I, and Hae III) are used to digest amplicons, those fragments are further separated by capillary electrophoresis. Indeed, this method seems less technically laborious, appreciable only to identify protozoa in unchlorinated drinking water.

3.4.2.5 Amplified Ribosomal DNA Restriction Analysis (ARDRA)

It is one another fingerprinting tool in which amplicons of rRNA genes are digested with a set of restriction enzymes, producing a pattern of fragments representative of a given microbial community. ARDRA has been used to identify non-tuberculous Mycobacterium.

3.4.2.6 Automated Ribosomal Intergenic Spacer Analysis (ARISA)

This method is common to characterize fungal communities. In ARISA, the internal transcribed spacer (ITS) regions of nuclear DNA located between the 18S (SSU) and 28S (LSU) genes are amplified using fluorescent-labeled primers. The amplicons are then analyzed in a sequencer to determine their size and to ultimately obtain a fingerprint of the studied microbial consortia. The method fairly known as single-strand conformational polymorphism (SSCP) also separates amplicons as a result of variation in their sequence. Gel electrophoresis (GE) is used to separate single DNA strands obtained from amplicons. It has been used for in situ genotyping of *L. pneumophila*. In general, fingerprinting techniques are frequently used in combination with cloning and sequencing, in order to obtain specific phylogenetic information from selected samples. It requires specialized equipment which is very labor intensive (Fisher and Triplett 1999).

3.4.3 Sequencing-Based Approaches

The widespread genomic approach has started around the globe by cloning and sequencing whereas detailed and accurate phylogenetic information from

environmental samples is mandatory. Except the extraction of nucleic acids, the construction of clone libraries using sequencing vectors hardly depends on the amplification of the rRNA gene with suitable primers. In this way, selected clones are sequenced (Sanger-based method) and the nucleotide sequence of the rRNA gene retrieved, allowing estimates of the microbial diversity in the samples by comparison with sequences available in databases. Drinking water microbiology involves the generation of DNA clone libraries followed by sequencing to study long-term succession in biofilms and disinfection efficiency, nitrifier, and ammonia-oxidizing bacteria in biofilms, to characterize the microorganisms present in red water events and to detect Bacteroidetes in unchlorinated water. This approach is known as metagenomics which involves sampling the entire genome of an environmental sample in order to obtain sequence information from the microorganisms contained in it and to ultimately make taxonomic assignments to characterize them.

A sequencing-based approach is useful to sequence the entire genome and characterize microbial communities in environmental samples which are known as shotgun sequencing. Genomic DNA is cleaved into smaller fragments; these fragments can be sequenced, amplified individually, and then reassembled into their original order in the genome, based on sequence overlaps, to obtain the complete genome

3.5 Next-Generation Sequencing

Bioremediation has entered into genomics, proteomics, and metabolomics era to find the cutting-edge technologies which assumes noteworthy part in the field of molecular biology. Next-Generation Sequencing is a real revolution in environmental sciences which adds value to the advanced techniques such as metagenomics and metatranscriptomics. Present-day NGS principles are a real revolution creating gigabases of monoclonal and computerized DNA information. Environmental science ties up with the decreasing sequencing prices to produce convenient solutions for wastewater abatement.

Bioremediation studies with microbes have been hampering for a long-time investigation since most of the microbes are uncultivable in the laboratory condition. Nevertheless, metagenomic approach renders the knowledge of PCR amplification by 16S or 18S rDNA segments of huge number of individual microorganisms to be distinguished and determined in a single run. All the diagnostic industries focus on the species-level taxonomic characterization which consists of long-read genome sequencing of two hypervariable regions and 400 base pair (bp) chemistry with low sequencing charge per bps (Metzker 2010). Bioaugmentation or biostimulation regimes are the branches of NGS-based molecular ecology which help to serve microbiological datasets that enables multidimensional matrices. However, using the microbial key players, the coherent dynamics, association networks, and the synergistic interactions can be identified.

3.5.1 Stable Isotope Probing (SIP) Technique

SIP methodology in addition to concentrating on targeted sequence information focuses on the entire genomic data of the microbial degraders. The complex phenomena of microbial interactions can be studied in laboratory scale using SIP technique by isolating the heavy fraction of synthesized gDNA after adding the labeled substrate to the microcosm. While in the microbial distribution, the functionally enriched metagenome can also be determined in analogous way. Additionally, the catabolic enzymes involved in substrate depletion are to be identified during shotgun sequencing, assembly, and annotation. NGS technology enhances utilitarian genomic research, when the microorganism is obscure yet viable. Uniform sequencing is done followed by physical or enzymatic fragmentation of any microbes' DNA in short segment. Such aligned strings of bases are called reads which are being annotated in the absence of a reference genome. Thusly, metabolic pathways, transformations, and exceptional hereditary arrangements in chromosomes or in ambiguous plasmids are contemplated by the de novo sequencing. The bottleneck of NGS needs artificial intelligence bolster; the elite logical processing and big data research are still in developing state. Bioinformaticians and data mining experts are highly encouraged in the fields of bioremediation and biodegradation in order to increase dependable logical outcomes.

NGS-based strategies are sought after for water quality appraisal, including advancement of bioindicators for sewage contamination and tracking of microbial source, portraying the appropriation of toxin and antitoxin resistance qualities in water tests and examining systems of biodegradation of unsafe contaminations that undermine water quality. Rising patterns in NGS demonstrate their potential applications to the up and coming era of water quality evaluation tools.

This NGS technique presents major challenges associated with improvement and approval of strategies for identification of indicators or microorganisms. Initial step is to recognize waterborne markers which depend on particular refined and list of hypothetical isolates, trailed by species affirmation utilizing biochemical, serological, or genetic techniques. The institutionalization of straightforward and routine techniques for evaluation of FIB is important to culture them effectively, and although tedious, it is free from inability to identify a few life-forms because of critical development for a host. Yet, some indicators known to exist in a torpid yet infective condition cannot be measured by standard development-based techniques. In such cases, pathogens are Viable But Non-cultivable (VBNC) state (Zielińska et al. 2012).

Due to VBNC state, it is difficult to culture bacteria. But the molecular techniques detect and quantify specific bacteria. Here, target DNA is extracted from a variety of samples to subject subsequently for the analysis of genes from indicator species or microbes of interest. The small subunit ribosomal RNA gene (SSU rRNA), consisting of the 16S rRNA gene for bacteria and the 18S rRNA gene for fungi, etc., has risen as a standout among the most as often as possible utilized target qualities for molecular investigation. It is because of the nearness of

exceptionally rationed and variable/hypervariable areas to advance arrangement of the DNA grouping crosswise over different organisms and take into consideration all the more fine-scale ordered taxonomic identification. Polymerase Chain Reaction (PCR) targets the regions of the SSU rRNA and functional genes for microbial source and direct recognition and measurement of target indicator strains.

3.5.2 Challenges in NGS

1. Development autonomous investigations in light of direct recognition of nucleic acids or enhancement of target genes utilizing PCR go around a few issues related to culture-based techniques while raising extra difficulties.
2. To start with, discovery and evaluation of natural DNA from singular species are frequently thought to be gotten from living life-forms; notwithstanding, free DNA may likewise be recognized in such cultivation-autonomous investigations, making the connection between DNA duplicates and cell abundance flawed.
3. Second, DNA-based techniques utilized for measurement of microbial hazard operators might be bewildered by the very powerful and differentiated genomes of pathogens and predominance of strain-particular virulence factors. Along these lines, evaluation of pathogenic taxa in light of event of biomarker DNA, for example, the SSU rRNA, may not relate to general well-being hazard if the strain identified needs destructiveness qualities. In any case, PCR-based identification and measurement of living beings in ecological DNA have demonstrated helpful for specification of sewage markers, and one DNA-based technique is affirmed for evaluation of the fecal marker Enterococcus.
4. Third, the capacity of nucleic acid-based examinations to recognize as meager as one DNA target molecule; in this way, it is conceivable to identify follow levels of nucleic acids from microbial hazard specialists (e.g., pathogens or virulence factors). Hazard evaluation systems to characterize edges of "worthy hazard" will be vital before fusing measurement of microbial hazard operators by DNA-based methodologies into basic leadership for water quality administration.

3.5.3 NGS Technologies and Analysis Methods

Trends in NGS techniques are a parallel analysis of DNA sequence information retrieved from PCR amplicons or environmental nucleic acids. Clinicians believe that massively parallel sequencing (MPS) can be a screening instrument used to supplement or go around traditional indicative techniques for the recognition. MPS enables targeted sequencing of the hypervariable regions of SSU rRNA gene

(e.g., V1, V3, V4, and V6 regions) and large subunit (LSU) rRNA gene. Different qualities with ordered are signals, for example, nirS (denitrification) and nifH (nitrogen fixation) demonstrative of biochemical cycles. Aside from these genes, plastid SSU rRNA is additionally received for NGS-based microbial profiling. To study about the connection between waterborne microbial groups and water quality, 454 pyrosequencing (Roche) is favored.

3.5.4 Limitations in NGS

The determination of NGS techniques is at present too low to distinguish microorganisms to the species level. The most as often as possible utilized NGS platforms are Roche 454 and Illumina/Solexa. These days, Illumina is supplanting Roche 454 as the sequencing technique for decision for a large portion of microbial-related investigations. While Illumina yields shorter peruses than Roche 454, the sequencing blunder of the two stages is equivalent, and Illumina is considerably less expensive than 454. Bioinformatics programming and examination instruments are for the most part welcome to break down the various successions peruse acquired from NGS runs, the most helpful ones being MOTHUR, QIIME (Quantitative Insights Into Microbial Ecology). Pyrosequencing pipeline in the Ribosomal Database Project in drinking water is continually expanding, to describe bacterial groups from impellers recovered from client water meters, in layer filtration frameworks from a drinking water treatment plant, to evaluate the impact of pressure-driven administrations on bacterial group arrangement in an exploratory appropriation framework and to survey the impact of various disinfectant administrations on microbial group flow. With sequencing costs diminishing, NGS is empowering an expanding number of research facilities to systematically (and practically) characterize an extensive variety of the living beings that are available in drinking water.

3.5.5 Application of NGS in Water Quality Analysis

Multi-omic methodologies of genomics, metagenomics, metatranscriptomics, and MPS of focused qualities (e.g., SSU rRNA qualities) have been utilized to think about the capacity of microbial groups in an assortment of crisp water. For microbial water quality appraisal, the previously mentioned examinations have been consolidated with different procedures to research the microbial structure and their natural capacities (e.g., scattering of antimicrobial genes and degradation). These investigations yield bits of knowledge into the exercises intervened by microbial groups in seagoing frameworks and potential natural hazard factors as particular microbial populaces and their qualities for harmfulness, poison

bio-amalgamation, or antimicrobial resistance (AR), which can be imperative in microbial water quality administration.

- To date, coordinated molecular methodologies have utilized NGS to enhance the ebb and flow comprehension of variables intervening water quality in for the most part crisp water situations.
- To distinguish indicators of human sewage and human fecal defilement for water quality appraisal
- To analyze the transport of human pathogens in water and wastewater frameworks
- To comprehend the environmental drivers of destructive algal sprout diligence and toxin generation/debasement
- To watch the spread and dissemination of AR in freshwater conditions, and
- To examine components of regular and invigorated biodegradation of hurtful poisons that undermines water quality.

While NGS-based examinations are giving extraordinary bits of knowledge into the structure and capacity of waterborne microbial groups, challenge stays to change over patterns saw in the investigations which are enthusiastically capable information for deciding water quality (Tank et al. 2010)

3.5.6 Microbial Safety of Drinking Water

The microbial organization in drinking water dissemination frameworks including source water and end point taps is thought about by the Next-era sequencing. It is totally reliant on the different phases of drinking water treatment and appropriation process and biofilm grids related with circulation pipelines. Treated drinking water is protected up to certain degree, innocuous, free from ailment-causing specialists, yet at the same time pathogens can avoid treatment and purification process (Lu and Lu 2014). That enables them to hold on in water dispersion pipelines as biofilms.

Sources of further information

New methodologies are being created to set up confirmation of fecal sullying in surface freshwaters through option DNA-based pointers. MPS of the SSU rRNA quality has been utilized to describe the microbial arrangement in crude sewage entering wastewater treatment plants in a few unique nations. The sewage framework related with bacterial taxa is not quite the same as those present in human fecal and other ecological sources. From the outcomes recovered from Human Microbiome Project, it is noticed that 10–15% of sewage microbiomes were of a human fecal starting point. Microbiomes of human fecal beginning are broadly found in all sort of sewage treatment plants spoke to by Firmicutes (e.g., Lachnospiraceae and Ruminococcaceae), Bacteroidetes (e.g., Bacteroidaceae, Porphyromonadaceae, and Prevotellaceae), and anaerobic microorganisms (e.g., Bifidobacteriaceae, Coriobacteriaceae). From sequencing information, 27 human

fecal oligo-sorts (overwhelmingly Bacteroidaceae, Prevotellaceae, or Lachnospiraceae/Ruminococcaceae) were recognized from the municipal sewer communities and human tools. This finding represents higher abundance of microbial communities in the human gut microbiome.

References

Arora S, Rajpal A, Kumar T, Bhargava R, Kazmi AA (2014) Pathogen removal during wastewater treatment by vermifiltration. Environ Technol 35(17):2493–2499

Bai Y (2012) Bacterial communities in the sediments of Dianchi lake, a partitioned eutrophic waterbody in China. PLoS ONE 7:37796

Beale DJ, Barratt R, Marlow DR, Dunn MS, Palombo EA, Morrison PD, Key C (2013) Application of metabolomics to understanding biofilms in water distribution systems: a pilot study. Biofouling 29(3):283–294

Besner MC, Prevost M, Regli S (2011) Assessing the public health risk of microbial intrusion events in distribution systems: conceptual model, available data, and challenges. Water Res 45 (3):961–979

Blumenthal U, Mara D, Peasey A, Ruiz-Palacios G, Stott R (2000) Guidelines for the microbiological quality of treated wastewater used in agriculture: recommendations for revising WHO guidelines. Bull World Health Organ 78(9)

Boe-Hansen R, Martiny AC, Arvin E, Albrechtsen HJ (2003) Monitoring biofilm formation and activity in drinking water distribution networks under oligotrophic conditions. Water Sci Technol J Int Assoc Water Pollut Res 47:91–97

Cai L, Zhang T (2013) Detecting human bacterial pathogens in wastewater treatment plants by a high-throughput shotgun sequencing technique. Environ Sci Technol 47:5433–5441

Clara M, Kreuzinger N, Strenn B (2005) The solids retention time-a suitable design parameter to evaluate the capacity of wastewater treatment plants to remove micropollutants. Water Res 39:97–106

Drechsel P, Evans EV (2010) Wastewater use in irrigated agriculture. Irrig Drain Syst 24:1–3

Fish K (2013) The impact of hydraulic regime upon biofilms in drinking water distribution systems. University of Sheffield, Thesis

Fisher MM, Triplett EW (1999) Automated approach for ribosomal intergenic spacer analysis of microbial diversity and its application to freshwater bacterial communities. Appl Environ Microb 65:4630–4636

Gagnon GA, Rand JL, O'Leary KC, Rygel AC, Chauret C, Andrews RC (2005) Disinfectant efficacy of chlorite and chlorine dioxide in drinking water biofilms. Water Res 39:1809–1817

George B (2004) Marketing trends for organic food in the 21st century. Electronic books

Ginige MP, Hugenholtz P, Daims H, Wagner M, Keller J, Blackall LL (2004) Use of stable-isotope probing, full-cycle rRNA analysis, and fluorescence in situ hybridization micro auto radiography to study a methanol-fed denitrifying microbial community. Appl Environ Microbiol 70:588–596

Hamonts K (2012) Temporal variations in natural attenuation of chlorinated aliphatic hydrocarbons in eutrophic river sediments impacted by a contaminated groundwater plume. Water Res 46:1873–1888

http://news.nationalgeographic.com/2016/02/160203-feminized-fish-endocrine-disruption-hormones-wildlife-refuges/

http://www.searo.who.int/entity/world_health_day/2015/whd-what-you-should-know/en/

https://www.watertechonline.com/microorganisms-in-activated-sludge/

Isabel D, Joby BB, Peter D, Raju S, Katherine EF, Catherine AB (2014) Methodological approaches for studying the microbial ecology of drinking water distribution systems. Water Res 65:134–156

Kidd KA, Blanchfield PJ, Mills K, Palace VP, Evans P, Lazorchak J, Flick (2007) Collapse of a fish population after exposure to a synthetic estrogen. PNAS 104(21):8897–8901

Kumar K, Singh GK, Dastidar MG, Sreeekrishnan TR (2014) Effect of mixed liquor volatile suspended solids (MLVSS) and hydraulic retention time (HRT) on the performance of activated sludge process during the biotreatment of real textile wastewater. Water Res Ind 5:1–8

Lu XM, Lu PZ (2014) Characterization of bacterial communities in sediments receiving various wastewater effluents with high throughput sequencing analysis. Microb Ecol 67:1–12

Metzker ML (2010) Sequencing technologies—the next generation. Nat Rev Genet 11:31–46

Microbiological water quality guidelines for marine and freshwater recreational areas (2014)

Rattan L (2016) Soil health and carbon management. Food Energy Secur. 5(4):212–222

Regan JM, Harrington GW, Noguera DR (2002) Ammonia- and nitriteoxidizing bacterial communities in a pilot-scale chloraminated drinking water distribution system. Appl Environ Microbiol 68:73–81

Seviour RJ, Maszenan AM, Soddell JA, Tandoi V, Patel BK, Kong Y, Schumann P (2000) Microbiology of the 'G-bacteria' in activated sludge. Environ Microbiol 2:581–593

Snyder S Wastewater pathogens. https://thewaterproject.org/Wastewater%20Pathogens.pdf

Tank JL, Rosi-Marshall EJ, Griffiths NA, Entrekin SA, Stephen ML (2010) A review of allochthonous organic matter dynamics and metabolism in streams. J North Am Benthol Soc 29:118–146

Van Delft TJ (2010) Analysis of microbiological growth in drinking water networks and sediments. MSc thesis, Delft University of Technology, 18 June 2010

Vesey G, Slade JS, Byrne M, Shepherd K, Dennis PJ, Fricker CR (1993) Routine monitoring of Cryptosporidium oocysts in water using flow cytometry. J Appl Microbiol 75:87–90

Wagner M, Amann R, Lemmer H, Schleifer KH (1993) Probing activated sludge with oligonucleotides specific for proteobacteria: inadequacy of culture-dependent methods for describing microbial community structure. Appl Environ Microbiol 59:1520–1525

Zielińska M, Sobolewska J, Bułkowska K (2012) Removal of phenanthrene and 4-phenylphenanthrene from wastewater in an integrated technological system. Desalin Water Treat 50(1–3):78–86

Chapter 4
Genetically Modified Organisms and Its Impact on the Enhancement of Bioremediation

Narasimhan Manoj Kumar, Chandrasekaran Muthukumaran, Govindasamy Sharmila and Baskar Gurunathan

Abstract Bioremediation is a process of degrading the environmental contaminants, that are introduced accidentally or purposely which cause hazardous effect on earth and harm the normal life process. The conversion of these contaminants into less toxic forms is the goal of bioremediation process that can be achieved by the use of microorganisms. The bioremediation approaches have more advantages when compared with the traditional methods, as it can be directly implemented at the targeted contaminant site. Even though some bacteria and fungus were employed to decompose the chemical compounds, but they have only limited ratio to metabolize the hydrocarbons on their own. The genetically modified organisms are applied nowadays in bioremediation process for effective removal of contaminants, where the indigenous microbes cannot degrade. Genetically modified microorganisms (GMOs) play an important role in remediating the industrial waste, reduce the toxicity of some hazardous compounds, and also help in removal of pollution by hydrocarbons and petrol discharges. A variety of molecular tools such as molecular cloning, horizontal transfer of DNA in bacteria, electroporation, protoplast transformation, biolistic transformation, conjugation and transformation of competent cells are available for the successful construction of GMOs. Transfer of gene into the bacteria makes it as a novel strain, for eliminating the hydrocarbon contaminants from the environment in minimal time. Similarly, removal of compounds such as xylene, toluene, octane, naphthalene and salicylate is coded on bacterial plasmids for successful degradation of the environment. This chapter represents the applications of genetically modified organisms in bioremediation

N. M. Kumar
Department of Genetic Engineering, School of Bioengineering, SRM University, Kattankulathur 603203, Tamilnadu, India

C. Muthukumaran (✉) · G. Sharmila
Department of Industrial Biotechnology, Government College of Technology, Coimbatore 641013, Tamilnadu, India
e-mail: biopearl1981@gmail.com

B. Gurunathan
Department of Biotechnology, St. Joseph's College of Engineering, Chennai 600119, Tamilnadu, India

S. J. Varjani et al. (eds.), *Bioremediation: Applications for Environmental Protection and Management*, Energy, Environment, and Sustainability,
https://doi.org/10.1007/978-981-10-7485-1_4

processes, molecular tools used for construction of GMOs, pros and cons, ethical issues and laws governing the application of GMOs.

Keywords Bioremediation · GMO · Molecular cloning · Electroporation Protoplast transformation

4.1 Introduction

4.1.1 Bioremediation

Global pollution is a major potential threat to human health which has been witnessed from the past century. The major contribution to such pollution in the environment is mainly due to the contaminant release by accidental spillage or deliberate discharge of toxic chemicals from industries. This eventually disturbs the natural environment and thereby causes fatal effect on the biological system. Protection of environment and health of living system are a country's mandate today. Hence, there arises a growing demand in many countries for restoration of Mother Nature from diverse contaminants in the ecosystems. There are different conventional techniques employed to remove such chemical pollutants like land filling or to cap the contaminated site, high-temperature incineration and chemical decomposition. The landfill or to cap the contaminated site is a simple traditional method, which moves the contaminant to some other place and thus creates a notable risk while handling and transporting the harmful constituents. The conventional methods such as high-temperature incineration and chemical treatment process are considered to be effective method to remove contaminants, but they possess some drawbacks such as expensive small-scale operations, and expose more contaminants to labour and public nearby the site. The biological treatment of the contaminants using microorganisms to degrade the toxic chemicals into harmless compounds called bioremediation is the subject of research investigation nowadays. The bioremediation process uses the living organisms, like plants and microorganisms to detoxify environmental pollutants into minimal toxic forms. The microorganisms play a main role in transforming the toxic compounds through the biochemical reactions that take place as a part of their metabolism. Bioremediation is safest, economic and highly public acceptance way to remove many chemical pollutants which adversely affect the quality of the life and environment. Although the bioremediation has several advantages when compared with traditional and conventional methods of remediation, it also possesses a minimal drawback, which includes long processing time, lower level of degradation of contaminants, such as chlorinated organic or aromatic hydrocarbons. This can be successfully overcome by proper design and implementation, like assessing the suitability of contaminant site and optimizing the environmental conditions for microbial growth.

4.1.2 Types of Bioremediation

Bioremediation is classified into two major types:

(i) In situ bioremediation
(ii) Ex situ bioremediation.

4.1.2.1 In Situ Bioremediation

The use of metabolic activities of the microorganisms to remediate the pollutant at the pollutant site is known as in situ bioremediation (Stroo 2010). The efficacy of this type of bioremediation depends on the essential nutrients supplied to the microbial growth. Degradation of the oil spillage has been successfully applied by in situ bioremediation. In situ bioremediation is further classified into intrinsic in situ bioremediation and engineered in situ bioremediation.

4.1.2.2 Intrinsic In Situ Bioremediation

Biodegradation of the pollutant site occurs with the metabolic activity of indigenous microorganism is known as intrinsic in situ bioremediation. The nature of the microorganism or the pollutant site is not manipulated for bioremediation. The microorganisms are appropriately utilized after laboratory test to check the capability of indigenous microorganism to detoxify the pollutants. Intrinsic in situ bioremediation can be applied based on following methods.

(a) Bioventing
In Bioventing, oxygen is supplied by means of an air injection through residual contamination in the soil. The microorganism utilizes the oxygen and degrades the organic compounds, such as adsorbed fuel residuals in the soil. It also degrades the volatile organic compounds (VOCs) and the vapours that are released slowly into the biologically active soil. The efficiency of this method depends on the rate of oxygen supply and nutrients for microbial growth. Oxygen at low flow rate is supplied by means of an electric blower to the wells on the subsurface levels to ensure the sustainability of microbial activity. When the atmospheric pressure exceeds the pressure inside the well, it makes the valve to close, and thereby, it traps enough air in the well to maintain the degradation process.
(b) Bioaugmentation
Bioaugmentation refers to the addition of specific combination of microorganism at the pollutant site to augment the biodegradation (Andreolli et al. 2015; Bento et al. 2005). Inoculation of microbes enhances the metabolic activity of the native microorganism which is already present in the contaminant site. Use of such cocktail of microorganisms boosts the rate of

biodegradation, which may not be degraded by indigenous microbial community at the site of pollutant (Mrozik and Piotrowska-Seget 2010). Bioaugmentation is applied to degrade insecticides and pesticides, trinitrotoluene (TNT), poly vinyl compounds and aromatic hydrocarbons, etc.

(c) Phytoremediation
Phytoremediation is a green method to remediate the environmental pollutants mainly pesticides and petroleum hydrocarbons (Chuluun et al. 2014). It comprises the utilization of plants and its associated microbial flora which degrades the pollutants in an agronomic pattern. The plants remediate the pollutant in following ways, such as accumulation of pollutants in root, translocation of pollutant from root to leaves for evaporation, metabolism in rhizosphere, degradation of plant-associated microbial communities and degradation by metabolism through enzyme secreted by plants. Transgenic plants also can be employed, for example, *Arabidopsis thaliana* absorbs mercury from the soil by introduction of reductase enzyme-coding genes in plants.

(d) Biosparging
Biosparging is a technique to remove the petroleum products which are dissolved in ground water or absorbed in the soil. The method is similar to bioventing which utilizes the oxygen and nutrients to stimulate the metabolic activity of the wild microorganism localized in the contaminant-saturated zone.

(e) Biofilters
The major contribution of air pollution is due to discharge of the toxic substances into the atmosphere from industries and waste treatment which causes hazardous effect on human health. Traditionally, the air emission containing pollutants were controlled by various techniques such as scrubbing, adsorption and condensation. The disadvantages of these techniques include the difficulty in large-scale operation and expensive. Biofilters are a modernized method to degrade pollutant in the industrial air emission by immobilizing the microbial communities to grow on solid surface. When the air containing toxic pollutant is passed through the biofilters, it adsorbed onto microbes embedded biofilms and degraded. The filter media used for constructing the biofilters includes bark, peats, soil, compost, etc. Biofilters are successfully used to remove hydrogen sulphide, sulphur gases, terpenes, ethyl benzene, mercaptans, dimethyl sulphides, etc. from the air.

4.1.2.3 Ex Situ Bioremediation

Ex situ bioremediation, in which the pollutant substances collected from the polluted site are degraded using a consortium of microbes at controlled and designed area. Bioremediation of soil and water can be efficiently done using this method. The major advantages include better control over the process and less time-consuming. Ex situ bioremediation can be implemented using following methods

(a) Land farming
The bioremediation carried out above the ground for degrading the petroleum-contaminated soil can be done by the technique, land farming. The contaminated soil is taken from the contamination site, mixed with the required microorganism, nutrients and cultured in laboratory scale level. The rate of degradation can be improved by mixing a combination of efficient microbes degrading the pollutants or by the use of genetically modified microbes. After optimizing the conditions for enhanced degradation of pollutants, the biologically active soil is transferred to the pollutant site. Bioremediation of contaminated soil containing benzene, toluene and xylene can be efficiently degraded by this technique.

(b) Composting
In composting, the contaminants are degraded and converted into safely disposable end product using anaerobic microbes under a controlled temperature above 40 °C. The contaminated soil is mixed with bulking agents such as vegetable waste, animal waste and wood chips. The growth of microbes and degradation efficiency are monitored under controlled aeration, temperature and moisture level. Hazardous chemicals and explosives like TNT, RDX and HMX are remediated successfully using composting technique. Composting can be implemented using methods such as windrow, static pile and reactor vessel. It can also be used to treat the contaminated soil containing hydrocarbons.

(c) Bioreactors
In this approach, biodegradation is very rapid and efficient as the bioremediation occurs under controlled condition in specifically designed bioreactors with adequate nutrient supply, temperature, moisture, temperature and pH, etc. This method improves the contact between the microorganisms and the pollutants at optimum conditions. The major disadvantage of bioreactor-based biodegradation is very expensive.

4.2 Genetically Modified Microorganisms and Its Application in Bioremediation

Although a wide variety of microbial flora existing in the ecosystem to breakdown many toxic pollutants, the majority of these microorganisms exhibit slower degradation. The reason is due to various chemical elements in most of the pollutants are resistant to the biodegradation capability of the microbes. Due to lack of catabolic pathways for degrading the complex toxic pollutants, it is necessary to enhance the microbe capability to detoxify or degrade the specific contaminant. This can be achieved by genetic engineering approaches to construct the novel

strains of microbe possessing unique characteristics with broad spectrum of bioremediation potential compared with indigenous type microbes (Menn et al. 2000). A genetically engineered microorganism is a microorganism, in which the genetic engineering attempts have been made to improve its biodegradation potential. During the early 1980s, the cloning and characterization of genes responsible for coding catabolic enzymes for toxic chemical compounds were started. Nowadays, the need for genetic engineering in the field of bioremediation has been realized by many molecular biologists and microbiologists.

There are four main approaches for developing the genetically engineered microbes, which include

(i) Alteration of enzyme specificity and affinity
(ii) Gene construction and regulation pathways
(iii) Process development, monitoring and controlling of bioremediation
(iv) Application of sensor-based bioaffinity bioreporter for reducing toxicity, sensing chemicals and analysing end points.

The use of genetic-engineered microorganisms to enhance bioremediation has been reported in the literature and is listed in Table 4.1.

Table 4.1 List of Genetically engineered microbes used in bioremediation

Genetically engineered microbes	Gene cloned	Compound of interest	References
Escherichia coli AtzA	Atrazine chlorohydrolase	Atrazine	Strong et al. (2000)
Pseudomonas fluorescens HK4	*lux CDABE*	Naphthalene	Sayler and Ripp (2000)
Burkholderia cepacia L.S.2.4	pTOD plasmid	Toluene	Barac et al. (2004)
Pseudomonas fluorescens F113rifpcb*rrnB*P1::gfp- mut3	operon *bph*, *gfp*	Chlorinated biphenyls	Boldt et al. (2004)
Pseudomonas putida KT2442 (pNF142::TnMo-d-OTc)	pNF142 plasmid, *gfp*	Naphthalene	Filonov et al. (2005)
Burkholderia cepacia VM1468	pTOM-Bu61 plasmid	Toluene	Taghavi et al. (2005)
Rhodococcus sp.RHA1 (pRHD34::fcb)	*fcbABC* operon	2(4)-chlorobenzoate 2(4)-chlorobiphenyl	Rodrigues et al. (2006)
Pseudomonas putida PaW85	pWW0 plasmid	Petroleum	Jussila et al. (2007)
Comamonas testosteroni SB3	pNB2::*dsRed* plasmid	3-chloroaniline	Bathe et al. (2009)
Escherichia coli JM109 (pGEX-AZR)	azoreductase gene	Decolorize azo dyes, C.I. Direct Blue 71	Jin et al. (2009)
Pseudomonas putida PaW340 (pDH5)	pDH5 plasmid	4-chlorobenzoic acid	Massa et al. (2009)

Table adopted from Wasilkowski et al. (2012)

The tools offered by molecular biology are used to enhance the bioremediation capabilities of microorganisms, discovery and acceleration of novel biodegradation activities, assembling the catabolic segments from different microorganisms and constructing a new pathway for efficient bioremediation. For example, the genes for degrading environmental pollutants such as octane, hexane and decane (OCT plasmid), xylene and toluenes (XYL plasmid), camphor (CAM plasmid) and naphthalene (NAH plasmid) were constructed to produce a multiplasmid-rich *Pseudomonas* strain, which has potential of degrading aliphatic, aromatic, polyaromatic hydrocarbons and terpenes. Plasmid WWO, also known as TOL plasmid of *Pseudomonas putida* containing a set of plasmids, was the first organism subjected to intellectual property case. Hence, the use of GMOs clearly proved its biodegradation capability of environmental contaminants. The strain *Comamonas testosteroni* VP44 naturally possess the genes to degrade toxic chlorinated biphenyls into less toxic ortho-and para-chlorobenzoic acids. A complete mineralization of monochlorobiphenyls has been achieved by cloning the *ohb* operon and *fcb* operon from *P. aeruginosa* into the host *C. testosterone* VP44. Degradation pathway of DNT (2,4-dinitrotoluene) from *Burkholderia* sp. was engineered into *Pseudomonas fluorescens* ATCC 17400 (Monti et al. 2005). As a result the recombinant strain is superior in degradation of DNT at cold temperatures and also non-pathogenic to some plants. A novel partial reductive pathway was discovered by cloning the CNB-1 genes from *Comamonas* sp. into *E. coli* to detoxify 4-chloronitrobenzene and nitrobenzene (Wu et al. 2006). Desulphurization of dibenzothiophene a sulphur-rich compound present in fossil fuels was carried out by cloning *dsz* A, B and C gene segments from *Rhodococcus erythropolis* into *Pseudomonas* strain. The recombinant strain efficiently desulphurized the dibenzothiophene compared with native *R. Erythropolis* IGTS8 without affecting the active principles of fuel content.

Degradation of environmental pollutants by genetically modified *Comamonas testosteroni* VP44 by pathway modification and alteration of substrate specificity has been studied. The heavy metal removal efficiency of genetically engineered organism has been proved with *Alcaligenes eutrophys* AE104 for the removal of chromium in wastewater effluent from industries. Similarly, photosynthetic recombinant bacterium, *Rhodopseudomonas palustris*, proved the transport of metallothionein for mercury removal. Heavy metals in the form of hazardous radionuclide associated with organic compounds at pollutant site affect the human health as well as cause toxicity to most of the bacteria due to the emitted radiation from such contaminants. Cloning of organic toxic degrading enzyme gene into radiation-resistant organisms gives the solution to degrade such radionuclide-associated organic compounds. For example, engineering a high-radiation-resistant strain *Deinococcus radiodurans* by cloning with toluene dioxygenase gene obtained from *Pseudomonas putida* F1. The recombinant strain was effective in oxidizing toluene, chlorobenzene, trichloroethylene and 3,4-dichloro-1-butene present in extremely irradiating ecosystem. Similarly,

bioremediation of uranium was demonstrated by genetically engineered *Pseudomonas aeruginosa* (Renninger et al. 2004).

Application of genetically modified microorganisms has been stepped in the field of phytoremediation and rhizoremediation for efficient removal of contaminants from the environmental sites. For example, degradation of pollutants such as trichloroethylenes and polychlorinated biphenyls has been reported. The process of symbiosis has been established between *Astragalus sinicus* and *Mesorhizobium huakuii subsp. rengei* strain B3 by cloning phytochelatin synthase gene of *A. Sinicus* in *M. huakuii subsp. rengei* strain B3 to accumulate cadmium metals. A recombinant rhizosphere obtained by cloning *o*-monooxygenase genes of *Burkholderia cepacia* in strain *Pseudomonas fluorescens* 2-79 expressed a 63% of initial trichloroethylene degradation compared with unengineered host (9%).

The OPH gene encodes the enzyme organophosphorus hydrolase to metabolize the organophosphate pesticides contaminants in the soil. Due to intracellular enzyme secretion by the microbe and poor substrate diffusivity inside the cell, the metabolism is greatly affected and thereby complete removal of contaminant is not possible. The problem was solved by fusing the signal sequence with the OPH gene of *E. coli*, to support the secretion of OPH protein into the periplasm. The recombinant *E. coli* increases the OPH activity to 1.8-fold when matched with native strain producing cytosolic OPH (Kang et al. 2006).

Many organic pollutants require sufficient oxygen for mineralization or degradation. Bioremediation of such compounds can be a problem if the contaminant site lacks enough oxygen. Supply of oxygen to the sites are expensive. To overcome this problem, designing an aerobic bacteria which can be able to grow better under hypoxic condition is an alternate solution. This can be achieved by expressing the haemoglobin gene, *vgb* in aerobic bacteria to synthesize additional oxygenases, thus enhancing the oxygen supplement to degrade organic pollutants at less oxygen site.

4.2.1 Factors Influencing Genetically Engineered Microorganisms

The success of genetically engineered microorganisms depends on factors such as survivability, stability under field conditions and rate of gene transfer from engineered genotypes to native microbes.

(a) Survival of genetically modified microorganisms (GEMs)
The parameters considered to determine the survival of GEMs are growth rate, the presence of competing microbes, availability of nutrient source, size of the inoculum and environmental conditions. The specific growth rate of plasmid-bearing cells is a major factor influencing survivability and establishment of GEMs. A metabolic burden is added to plasmid-bearing cells, and

hence, the plasmid-bearing cells possess reduced growing capability when compared with plasmid-free cells. Application of GEMs in the bioaugmentation has certain limitation for its establishment and stable growth due to the competition with indigenous microorganisms growing under same resource (Dechesne et al. 2005). The parameter that affects the recombinant plasmid stability includes copy number, variance in copy number, type of insert, growth rate, composition of medium, oxygen availability and environmental conditions.

(b) Transmission of genetically engineered microorganism into native organisms
When GEMs are incorporated into the polluted sites to enhance the bioremediation, the survival of GEMs has been adversely affected by the horizontal transfer of recombinant DNA. It was observed that incorporation of GEMs on indigenous microbial community is not regularized and greater attention is required to analyse this issue on a case-by-case basis. It was demonstrated by several investigations about horizontal gene transfer of GEMs occurs between other microbes. Enhanced 2,4-D removal has been observed in indigenous bacteria due to horizontal gene transfer from the host *Pseudomonas putida* UWC3 (Dejonghe et al. 2000).

It was witnessed that recombinant plasmid of *E. coli* was mixed with indigenous bacterial community and observation revealed that DNA from recombinant *E. coli* was degraded rapidly, and in some cases, it retained its stability more than seven days, but there is no evidence of plasmid transfer to native bacteria. Another study also reported the transfer of a recombinant *P. putida* to local microbes on water sediment contaminated with PCB, showed a rapid disappearance of GEMs but with little transfer of PCB genes to other native microbes (Min et al. 1998).

Some of the GEMs also exhibit indirect effects on native flora. An interesting investigation reports that the recombinant *P. putida* possesses 80% reduction in 2, 4-Dichlorophenoxyacetic acid (2,4-D) levels than native flora. Simultaneously, it was observed that there is a drastic reduction in fungus population, due to the accumulation of 2,4-dichlorophenol, a metabolite of 2,4-D. Another example is a 14-fold increase in phytoplankton biomass was observed in alkaline phosphatase enzyme expressing recombinant *Achromobacter* sp. grown in sea water when compared with wild-type microbe (Sobecky et al. 1996). Many investigations reported that the rate of horizontal gene transfer is found to be maximum in laboratory experimental runs rather than natural occurrence. This is due to the fact that the native microbial population is large in number and their inherent selection advantages in natural environment. Based on this, it can be concluded that introduction of GEMs in indigenous sites is found to be applicable than laboratory studies. Some investigation demonstrated that the transfer of GEMs-bearing plasmid to native species may be useful in enhancing the potential of bioremediation in maintaining and sustaining the degradation capability in microbial communities (Newby et al. 2000).

4.2.2 Strategies to Control GEMs Transfer

It is an important concern to regulate the instability of plasmid and thereby loss of desired phenotype while using the GEMs-bearing recombinant genes.

4.2.2.1 Mini-transposon-mediated GEM Transfer Control

The problem of plasmid transfer can be rectified by using mini-transposons, which provide a stable integration of host chromosomes with recombinant gene. Fusion of the mini-transposons with non-antibiotic resistance selection systems eliminates the transfer of genes into environment. Utilizing this approach, *P. putida* was engineered for enhanced degradation of toluene, in which the mini-transposons integrated antibiotic resistance markers can be deleted after inclusion of target gene sequence in the host chromosome (De Lorenzo et al. 1998; Herrero et al. 1990).

4.2.2.2 Suicide Genes-mediated GEM Transfer Control

Another useful strategy, inclusion of suicide systems with mini-transposons, which provides for death of GEM after the bioremediation process gets completed. Such mechanism involves a controlled expression of suicide genes, which can be activated in near absence of pollutant at the contaminant site. In the absence of the pollutants, the suicide genes get activated and lethal to the engineered host-microbe (Pandey et al. 2005; Paul et al. 2005). For example, gene encoding streptavidin acts as suicide gene, this gene was repressed in the presence of substrate, when the substrate gets exhausted the streptavidin gene gets activated to produce streptavidin. The streptavidin binds with D-biotin and inhibits the production of thymine and consequently promotes the cell death.

4.2.2.3 *gef* Gene Expression and GEM Transfer Control

Expression of *gef* gene is the third method of controlling horizontal transfer of DNA. The strain *P. putida* capable to degrade the pollutant substrate, 3-methylbenzoate was engineered with *gef* gene expression. The expression system was constructed in such a way that induction of *gef* gene occurs in the absence of 3-MB. Hence, after exhaustion of 3-MB, not only *gef* gene but also *asd* gene encodes the production of different essential amino acids were inhibited. Thus, two different independent modes of suicide systems are involved in this system (Ronchel and Ramos 2001).

4.2.2.4 Composting and GEM Transfer Control

Another important strategy to control the horizontal gene transfer of GEM is composting. In this process, GEMs were subjected to elevated temperature above 90 °C and decreased pH to induce cell lysis and thereby the release of DNA to minimize the horizontal gene transfer. Thus, composting is the safest method for proper removal of GEMs after completion of required bioremediation process (Singh et al. 2006).

4.2.3 Techniques to Identify Genetically Modified Microbes

It is essential to detect and quantify the GEMs in mixture of microbial samples to detect the potential loss of gene segments and their possible horizontal gene transfer to other existing microbial community. The important parameters to be considered while tracking the GEMs are its survival, rate of dispersion, maintenance of activity, specific growth rate, etc. Several methods are applicable in this field, but a real time, simple, accurate and inexpensive technique should be chosen.

4.2.3.1 PCR-based Techniques

Counting the number of grown colonies on plates is a traditional method for tracking GEMs. This method is simple with limited accuracy and sensitivity. Application of molecular techniques offers solutions to these limitations. Southern hybridization-based technique to detect DNA of soil organisms also exhibits lesser sensitivity, and a PCR-based amplification of nucleic acids of particular strain quantifies both the dead and live cells. Metabolically active cells can be detected by amplifying only using reverse transcription-PCR (RT-PCR). Determination of total cell numbers in the soil samples can be done using most probable number-PCR (MPN-PCR) and competitive PCR (cPCR). In MPN-PCR, a dilution series of soil samples in triplicates was made and the presence or absence of organisms in each dilution was compared with the standard table. The presence of organism is displayed by the ability of target gene sequence amplification rather than the number of viable cells. The cPCR also provides the similar information as MPN-PCR, and here, the amount of product DNA of sample is compared with the standard templates (Widada et al. 2002).

4.2.3.2 Fluorescent-based DNA Hybridization Technique

Fluorescent-labelled-specific DNA probes of a particular strain are used to detect the presence of engineered microbe. Identification and quantification of *P. fluorescens* cells after introduction into microcosm was determined by the

fluorescent labelling of specific ribosomal RNA probe. The metabolic state of the cells at particular time can be easily detected as the ribosomal RNA increases with growth rate. The limitation to this technique is that it requires a considerable time to complete the process due to hybridization procedure (Boye et al. 1995).

4.2.3.3 Bioluminescence-mediated Technique

The selectable characteristics of recombinant organisms, for example, bioluminescent or production of coloured product are used to detect the specific phenotypic characteristics of GEM. Genes such as *xylE* (catechol 2,3-oxygenase), *lacA* (β-galactosidase) and gusA (β-glucuronidase) encode the corresponding enzymes to give coloured end product. Expression of uroporphyrinogen III methyl transferase by genetically engineered microbe cloned with *lux*, *luc* and *cob* A genes to produce bioluminescent product was reported elsewhere (Feliciano et al. 2006).

4.2.3.4 DNA Microarray Technique

DNA microarrays use both DNA and rRNA as probes to identify and enumerate the non-recombinant cells and GEMs. The degree of quantification is limited by the sensitivity and specificity of this technique (Cho and Tiedje 2002). Identification of GEMs from indigenous populations can be carried out using another detection method. For example, engineering of *E. coli* with 5S rRNA gene from *Vibrio proteolyticus* can be easily determined by the comparison of gene sequence of GEM and *V. proteolyticus* 5S rRNA (Hedenstierna et al. 1993). GEMs fused with novel surface protein genes *phoE-caa* can be detected by reaction of the single cells with specific monoclonal antibodies (Zaat et al. 1994).

The molecular method offers 90% accuracy in quantifying GEMs and its effects on surrounding environment, when combining the traditional and novel tracking tools. To achieve such accuracy, a case-by-case investigation involving appropriate or combination of detection techniques is required.

4.3 Molecular Tools for Construction of Genetic Engineering of Microbes for Bioremediation

4.3.1 Molecular Cloning

Cloning techniques are employed to make multiple copies of genes, expression and to study the specific function of genes. The molecular cloning requires the DNA

fragment possessing a specific function to be copied or expressed and also a vector, known as plasmid. Plasmid is a small circular DNA molecule that has capability to replicates independently of the chromosomal DNA in the host bacteria. Engineered plasmids are reinserted into host bacteria and allowed for replication. During bacterial division, the inserted DNA fragment gets copied along with the rest of the bacterial DNA. The vector consists of many short DNA sequences that can be digested with the help of restriction endonucleases. The restriction endonucleases recognize the specific site (restriction site) in the DNA sequences and cut them accordingly to create "sticky ends". Most of the restriction enzyme recognizes the four- to eight-nucleotide sequences in the plasmid DNA, which is also called as palindrome sequences. This means the forward nucleotide sequence is similar to the backward complementary sequence. The digested DNA fragments with sticky ends of both foreign DNA and host DNA are annealed together to form double-strand DNA with the help of enzyme known as DNA ligase. Thus, plasmids carrying the foreign gene are called as recombinant DNA, and generated proteins are called as recombinant proteins. The expression of protein can be stimulated or suppressed by certain environmental factors, so that the expression of particular protein can be controlled by scientists.

4.3.2 Electroporation

Electroporation is a simple and fast technique for introducing the foreign gene into host-microbe. This technique depends on the input of high-voltage electric pulses to induce temporary permeation of the plasma membranes to uptake the DNA (Fig. 4.1). Foreign DNA and the protoplast of the host cells are kept between two electrodes in tank containing suitable buffer. An alternating current of about 1 MHz is applied to align the protoplasts by di-electrophoresis. Electric field damages the membranes and creates pores, which makes the DNA to diffuse through these pores immediately. Once the DNA entered into the host cell, direct current pulses of 1–3 kV is applied to induce the fusion. The factors influencing optimum field strength involve: (i) The voltage, resistance and capacitance of electric pulse (ii) pH and temperature of buffer used (iii) size and density of protoplast (iv) host cell and genetic parameters (v) growth condition and post-pulse treatment effect of host cell. The molecular size of foreign DNA as big as 240 kb can be electroporated successfully. This property adds advantage to this technique as the sequencing of genomes demands large fragments of DNA.

4.3.3 Protoplast Transformation

Polyethylene glycol (PEG) induced DNA uptake in protoplast and subsequent cell wall regeneration is the principle behind the technique, protoplast transformation.

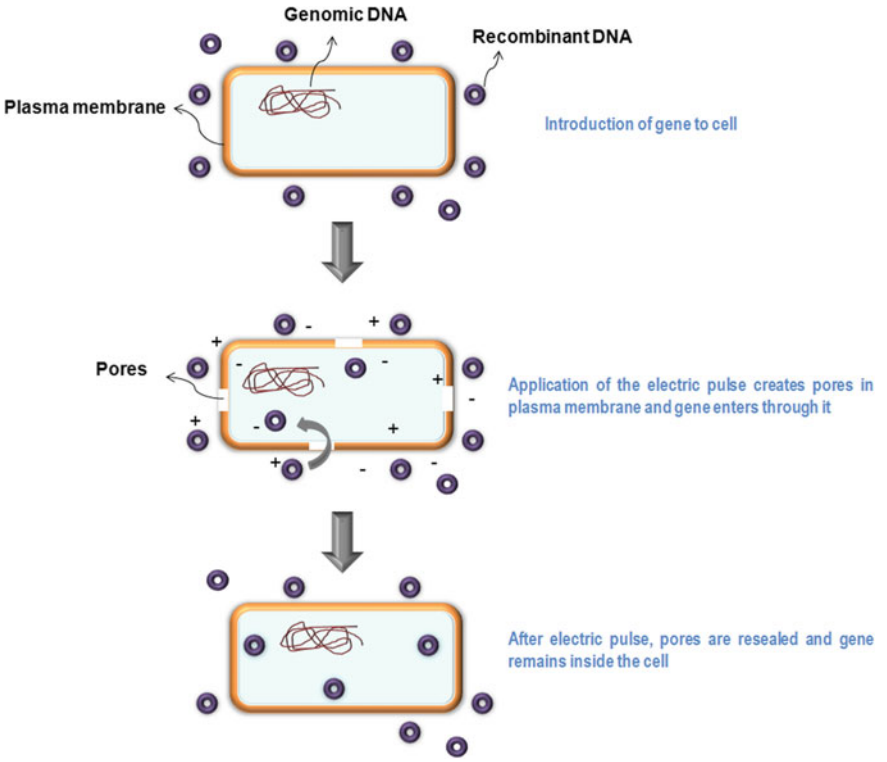


Fig. 4.1 Scheme of electroporation method

This method is more suitable and efficient for transformation of even cryptic plasmids and yields up to 80% of transformants. Protoplast transformation involves two steps: (i) Treatment of DNA under hypertonic conditions (ii) PEG treatment to complete transformation process. The important factors affecting PEG-mediated protoplast transformation are as follows: (a) Culture conditions and cell density—cells at late log phase are suitable for efficient transformation (b) DNA concentration—a concentration range between 0.1 and 1 μg yields high transformation (c) Effect of tonicity—it is observed that sorbitol, sucrose, NaCl, KCl, LiCl or NH_4Cl at certain concentration induce transformation (d) Effect of pH—pH between 3.5 and 5 found optimum for PEG mediation protoplast transformation (e) Effect of temperature and reaction time—a process time of 10 min and optimum temperature of 22 °C induce an efficient DNA uptake in protoplast.

4.3.4 Biolistic Transformation

In biolistic transformation, the gene of interest is coated in a chemically inert tungsten or gold beads of 0.36–6 μm and fired through a stopping screen into the host bacterial cells with the help of helium gas acceleration. The bead-associated DNA molecule passes through the bacterial cells and leaves the foreign DNA inside. As the helium pulse sweeps the microcarrier-coated DNA in the sample cartridge which then moves through barrel and maintains the target to hit accurately the host cell. The major advantages of this method include: no binary vector is required and transformation protocol is relatively simple. However, this technique has some drawbacks such as difficulty in obtaining single-copy transgenic events, high cost of the equipment and microcarriers, random intracellular targeting and transfer DNA is not protected.

4.4 Genetically Modified Microorganisms for Bioremediation Purposes

4.4.1 GMOs in Removal of Toxic Heavy Metals

Heavy metals are generally more stable and long shelf life when compared with the organic contaminants and their by-products. Release of heavy metals by anthropogenic activities enters into the environment and biological systems which adversely affects the activity of soil, plants and human health. Heavy metals have a strong ability to bind with different biological molecules, and due to this property, they accumulate in the biological systems leading to health hazards.

Mercury is one of the most hazardous heavy metal in the ecosystem. Expression of bacterial *mer* genes in mercury resistant bacteria is associated with the biodegradation of mercury. The *mer* A gene from *E. coli* BL308 was cloned and expressed in a well-studied radiation-resistant organism, *Deinococcus radiodurans* to remediate the mercury-contaminated site (Brim et al. 2000). Development of a broad-spectrum mercury resistant recombinant strain, *Cupriavidus metallidurans* MSR33 possesses a strong capacity to remove the mercury from polluted water (Rojas et al. 2011). The strain was constructed with two large plasmids, pMOL28 and pMOL30 contained a merRTPADE operon. The two plasmids have narrow-spectrum resistance to mercury individually, and when combined it exhibits a broad-spectrum mercury resistance.

Arsenic in its oxidized forms is more toxic. Traditional method involves reduction of arsenic (V) to arsenic (III), but it is not a potential remediation pathway as arsenic (III) is more toxic than arsenic (V). Alternatively, the arsenic in soluble bioavailable forms can be converted into volatile arsenic compounds such as mono-,

di-, and trimethylarsine. Many indigenous microbial floras have been reported to volatile arsenic at 2.2–4.5% only in 30 days of treatment. Hence, upcoming research focussed on the development of genetically engineered organisms is capable to bioremediate the arsenic at maximum level in short period. It has been reported that a tenfold increase in release of the volatile methylated arsenic gas by cloning *ars*M gene from *Sphingomonas desiccabilis* and *Bacillus idriensis* in *E. coli* compared with indigenous microbial flora (Chen et al. 2013). Another investigation reported a conversion of arsenic to non-toxic form by TTHB128 and TTHB127 genes encoding arsenite oxidase produced by *Thermus thermophilus* HB8 (Yang et al. 2010). The vector pBBR1MCS-5 is widely used to clone most of the microbial genes to oxidize a maximum of 80% of arsenite.

Different microorganisms have potential to active uptake and accumulation of lead (Pb) and convert it efficiently into non-toxic form. The genes encoding bacterial metallothioneins (*smt* A and *smt* AB) express the system to resist uptake and accumulate the lead in the chromosomal and extrachromosomal genetic material within the cell. The microorganisms such as *Pseudomonas aeruginosa* strain 4EA and *Salmonella choleraesuis* strain 4A along with *Proteus penneri* strain GM10 have been reported for lead resistance on plasmids and genomic DNA, respectively (Naik et al. 2012).

4.4.2 GMOs in Phytoremediation

Genes from microorganisms are introduced and expressed in plants (transgenic plants) to improve the phenotypes, disease resistance, drought resistance, etc. Bacteria are heterotrophs; hence, they occupy the enzymatic system need to complete remediation of bioorganic molecules which are toxic to the environment. The first-developed genetically modified plants for phytoremediation was attempted to increase the tolerance to heavy metals; for example, *Nicotiana tabacum* (tobacco plant) establishes a yeast metallothionein gene for maximum cadmium tolerance (Krystofova et al. 2012). Mercuric ion reductase gene of *Arabidopsis thaliana* in peanut was introduced and over-expressed to improve the tolerance to mercury (Yang et al. 2003). Similarly, phytoremediation of halogenated organic compounds has been reported in tobacco plants. Although the tobacco plants and *Arabidopsis thaliana* were proved as good models in laboratory scale, it is not applicable in field studies. The poplar trees, the fast-growing and high biomass yielding trait, gain interest in research investigation for phytoremediation nowadays. A plant pathogen cum natural genetic engineer, *Agrobacterium tumefaciens*, was used as an excellent vector for plant genetic transfer (Fig. 4.2). But *A. tumefaciens*-associated transformation was reported to be a challenging task to transfer gene to the forest trees.

The first transgenic poplar trees cloned and expressed with gamma-glutamyl cysteine synthase gene to produce glutathione, which involves in remediating the chloroacetanilide herbicides was reported.

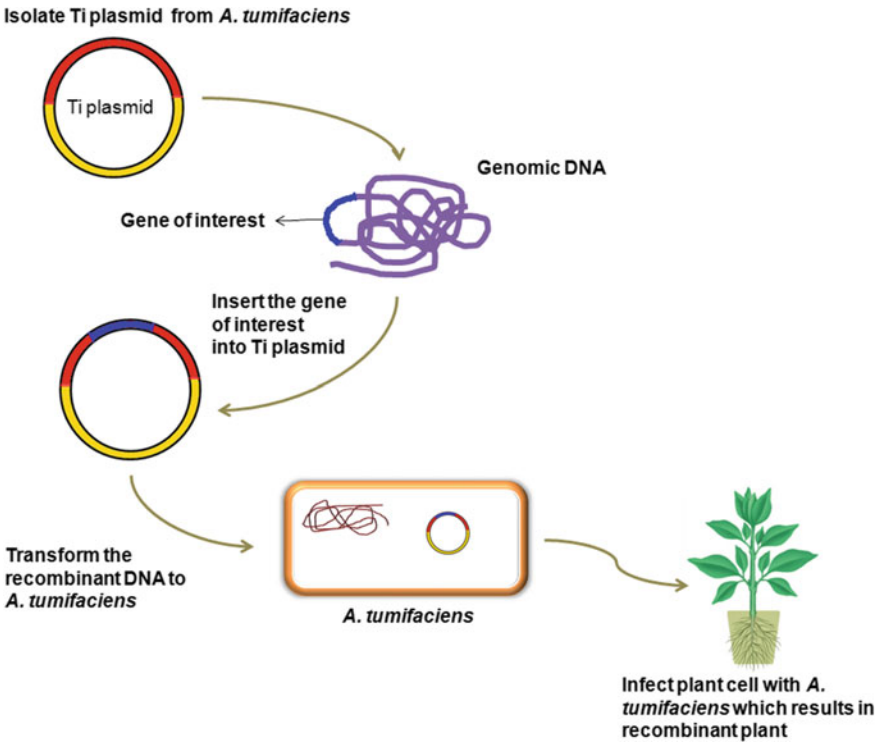


Fig. 4.2 *Agrobacterium tumifaciens*-mediated gene transfer

4.5 Pros and Cons of Genetically Engineering Organisms

In order to observe a clear view of the genetically modified microorganisms, it is important to (i) define them and explain the context, by which they are used (ii) possible risks of their uses and (iii) we shall ask questions to clarify the doubts about can we do without them or not? Based on this, several pros and cons about the usage of GMOs are as follows:

4.5.1 The Pros

- GMO technology can develop crops with faster evolution and production, higher yield, less fertilizer, less pesticides and more nutrients.
- GMO technology is more predictable than traditional breeding, because it is a random transfer of thousands of genes to the offspring, whereas the genetic engineering moves a block or specific set of genes at a particular time.

- Even though GMOs are not natural, but sometimes natural is also not good. For example, poisonous mushrooms are natural but we can modify it into edible form through genetic engineering aspects.
- Almost around 20 years, the GMOs were in research and practice in artificially implanting of DNA from one species to another. Rather than waiting for traditional breeding results, the goal can be reached instantaneously with GMO.

4.5.2 The Cons

- Most of the GMOs are not always tested thoroughly and minimum time required for a simple GMO testing is merely 90 days.
- The GMOs produced by transgenic modification procedures are not considered as natural existence, and also the effect of such transgenic is highly unpredictable.
- Though GMOs were developed with a view to reduce the amount of pesticides used, but it is not sure that the crop will be safe by using such recombinant microbes.
- There is no proper labelling of GMO-based products, and it is still difficult to decide whether the products of genetically engineered organism can be consumed.
- GMO testing often involves performing experiments upon animals, which some people feel is a breach of animal rights.

4.6 Ethical Issues and Risk Assessments in Usage of GMOs in Bioremediation

The creation and use of genetically modified microbes, especially in bioaugmentation, are primarily related to the ethical concerns. It is a general argument by the public that humans should not change the natural existence in the name of GMO. Due to possibilities of genetic crossover and mutation by changing the genetic of an organism may lead to potential risks rather than beneficial effect to human beings (Tiedje et al. 1989). There is a debate raised ethically that exposing GMOs for bioremediation without concerning about the proper field trial, testing and control will possibly reflect an unforeseen negative impacts on environment and thereby indirectly harms the living system (Stewart et al. 2000). The use of genetically improved microorganism for bioremediation purpose is created by a sensible and potential application known as recombinant DNA techniques. GMOs when exposed

to the ecosystem, they may produce unintentional consequences to the environment than the wild types. Sometimes, genetically engineered microorganisms are able to multiply at faster rate to establish them as dominant and persistent strain and induce its long-term effects on the living system. Of course, the results of DNA modification may impart the specific characteristics of tailored gene, but it is a major concern to ensure that the released recombinant organism in the environment must not harm the human health or environment. Hence, a cautious approach is necessary to study the risk assessment of GMOs release in the natural environment.

To implement successful risk assessment, the following information is required for GMO:

i. Detailed information of gene size, sequence and molecular characteristics of the GMO.
ii. Techniques employed to bring the genetic changes.
iii. Properties of introduced gene and its possible effects after transformation.
iv. Chromosomal analysis and automated karyotyping of gene of interest.
v. Comparison study on growth kinetics of the GMOs with the wild-type organisms,
vi. Conditions required for growth such as nutrient, soil, climatic and other requirements,
vii. Pattern of interaction with other microbes in the contamination site.
viii. Controlled field trials of the GMO under normal ecological conditions.

The first inter-governmental document to address the issues based on usage of GMOs in surrounding was published by Organization for Economic Cooperation and Development (OECD), known as "Recombinant DNA Safety Considerations", in the year 1986. This reports the environmental risk assessment on a case-by-case basis, and it was widely accepted by the public. A process-based risk assessment was adopted by countries like USA and Europe called as biosafety regulatory frameworks, deals with the safe use of GMOs-based products.

The protocol designed for risk management of GMO, called as Cartagena Protocol of Biosafety, provides the risk assessment measures to maintain, regulate and manage the risks identified. According to the European food safety authority, the appropriate points to be considered while using GMOs

(i) The effect of survival and persistence of GMOs in the host environment.
(ii) The effect of gene modifications.
(iii) The impact of negative consequences based on interactions of GMOs with native microorganisms.
(iv) Effects of GMOs on plants, animals and humans.
(v) Involvement of GMOs in irreversible biogeochemical reactions.

Identification of hazards and its level of exposure can be assessed by combining mesocosm experiments and small-scale field experiments. Earlier studies with small-scale trials are enough to provide valuable information regarding

above-mentioned points about GMOs. Alternatively, a large number of GMOs established in various site can reveal the impact on relationship and interactions between different species and ecosystem.

The risk management strategies can be mitigated to study the potential risk by the use of GMOs to make the proposed activities acceptable. A precautionary approach to premise the notion to reduce the risk of GMOs to biological system and environment is derived from Rio Declaration (United Nations Environment Programme 1992). This can be implemented when two or more undesirable risks encountered with unknown cause.

A long-term and effective perspective should be implemented as a precautionary approach to protect the environment. To execute this implementation, three components such as (1) available scientific data (2) risks occurs due to irreversible damage of ecosystem and (3) steps taken to control and regulate the effect of risk should be analyzed. The aims of precautionary principle are to avoid the uncertainty and ensure the safety measurements before the other solutions implemented. The precautionary principle shows a clear pathway to achieve indispensable and sustainable safety development over a long period. As the main role of the precautionary principle is to protect the environment beyond the present scientific knowledge, and hence, it cannot be questioned or justified based on the existing scientific data (Prakash et al. 2011).

Several countries have adopted precautionary principle as reference for well-drafted environmental and biodiversity laws. The countries like India, Africa and Pakistan cited the laws of precautionary principle, for example, Mozambique Environment Legislation (1997), South Africa's National Environmental Management Act and General Environmental Law of Cameroon (1996).

European Environment Agency (2002) reported a document, which summarizes the lesson learned about precautionary principle for environmental management. These lessons include:

(1) Avoidance of ignorance as well as uncertainty.
(2) Monitoring and managing the early warnings.
(3) Identification of gaps and blind spots in the scientific data.
(4) Learning and reducing the interdisciplinary obstacles involved.
(5) Estimating the real-world situation and justifying the claims based on its "pros" and "cons".
(6) Determination and evaluation of alternate ways to avoid uncertainty.
(7) Improving robust and adaptable solutions.
(8) Consulting and getting knowledge from relevant expertise person (Mayer and Stirling 2002).

Many developing countries adapted precautionary principles as an ultimate key in the risk management approaches to ensure a sustainable and healthy environment.

4.7 Conclusion

The efficiency of bioremediation can be improved by the use of genetic engineering techniques has become a productive way to develop the recombinant microorganisms to actively degrade specific contaminants. The main target involves the biodegradation of aromatic compounds through manipulating with genes, modification of pathway/alteration or construction of host gene sequences. The survivability and stability of GEMs are the critical issues in the field of bioremediation. Microcosm or laboratory scale studies suggest that this may not be an unsolvable problem, as many cases reported that gene transfer is less in GEMs when compared with the natural horizontal gene transfer in the same ecosystem. The present knowledge about the application of GEMs in the bioremediation process exists from the microcosm studies. There is a great promise by researchers that execution of the GEMs-based bioremediation of contaminant site will be harmless to biological system. But still, we are at an anxious junction where, on the one hand, we find the opportunities to change the genetic construction by recombinant DNA technology and make use of those modified organisms. Simultaneously, we faced with unacceptable hazards to human health and environment. A broad basis of decision is required to regulate the use of GMOs. Monitoring and detection methods are most important for assessment of the risk factors to control the environmental and health threats. The biosafety regulatory frameworks and the application of precautionary principles contribute the platform for the future development and application of genetically modified organism in the field of bioremediation.

References

Andreolli M, Lampis S, Brignoli P, Vallini G (2015) Bioaugmentation and biostimulation as strategies for the bioremediation of a burned woodland soil contaminated by toxic hydrocarbons: a comparative study. J Environ Manage 153:121–131

Barac T, Taghavi S, Borremans B, Provoost A et al (2004) Engineered endophytic bacteria improve phytoremediation of water-soluble, volatile, organic pollutants. Nat Biotechnol 22:583–588

Bathe S, Schwarzenbeck N, Hausner M (2009) Bioaugmentation of activated sludge towards 3-chloroaniline removal with a mixed bacterial population carrying a degradative plasmid. Bioresour Technol 100:2902–2909

Bento FM, Camargo FAO, Okeke BC, Frankenberger WT (2005) Comparative bioremediation of soils contaminated with diesel oil by natural attenuation, biostimulation and bioaugmentation. Bioresour Technol 96:1049–1055

Boldt TS, Sørensen J, Karlson U, Molin S, Ramos C (2004) Combined use of different Gfp reporters for monitoring single-cell activities of a genetically modified PCB degrader in the rhizosphere of alfalfa. FEMS Microbiol Ecol 48:139–148

Boye M, Ahl T, Molin S (1995) Application of strain-specific rRNA oligonucleotide probe targeting *Pseudomonas fluorescens* Ag1 in a mesocosm study of bacterial release into the environment. Appl Environ Microbiol 61:1384–1390

Brim H, McFarlan SC, Fredrickson JK et al (2000) Engineering *Deinococcus radiodurans* for metal remediation in radioactive mixed waste environments. Nat Biotechnol 18:85–90

Chen J, Qin J, Zhu Y-G, de Lorenzo V, Rosen BP (2013) Engineering the soil bacterium *Pseudomonas putida* for arsenic methylation. Appl Environ Microbiol 79:4493–4495

Cho J-C, Tiedje JM (2002) Quantitative detection of microbial genes by using DNA microarrays. Appl Environ Microbiol 68:1425–1430

Chuluun B, Shah SH, Rhee J (2014) Bioaugmented phytoremediation: a strategy for reclamation of diesel oil-contaminated soils. Int J Agric Biol 16:624–628

De Lorenzo V, Herrero M, Sanchez JM, Timmis KN (1998) Minitransposons in microbial ecology and environmental biotechnology. FEMS Microbiol Ecol 27:211–224

Dechesne A, Pallud C, Bertolla F, Grundmann GL (2005) Impact of the microscale distribution of *Pseudomonas* strain introduced into soil on potential contacts with indigenous bacteria. Appl Environ Microbiol 71:8123–8131

Dejonghe W, Goris J, Fantroussi SE et al (2000) Effect of dissemination of 2,4- dichlorophenoxyacetic acid (2,4-D) degradation plasmids on 2,4-D degradation and on bacterial community structure in two different soil horizons. Appl Environ Microbiol 66:3297–3304

Feliciano J, Liu Y, Daunert S (2006) Novel reporter gene in a fluorescent-based whole cell sensing system. Biotechnol Bioeng 93:989–997

Filonov AE, Akhmetov LI, Puntus IF et al (2005) The construction and monitoring of genetically tagged, plasmid-containing, naphthalene-degrading strains in soil. Microbiol 74:526–532

Hedenstierna KOF, Lee Y-H, Yang Y, Fox GE (1993) A prototype stable RNA identification cassette for monitoring plasmids of genetically engineered microorganisms. Syst Appl Microbiol 16:280–286

Herrero M, de Lorenzo V, Timmis KN (1990) Transposon vectors containing non-antibiotic resistance selection markers for cloning and stable chromosomal insertion of foreign genes in gram-negative bacteria. J Bacteriol 172:6557–6567

Jin R, Yang H, Zhang A, Wang J, Liu G (2009) Bioaugmentation on decolorization of C.I. direct blue 71 using genetically engineered strain Escherichia coli JM109 (pGEX-AZR). J Hazard Mater 163:1123–1128

Jussila MM, Zhao J, Suominen L, Lindström K (2007) TOL plasmid transfer during bacterial conjugation in vitro and rhizoremediation of oil compounds in vivo. Environ Pollut 146:510–524

Kang DG, Choi SS, Cha HJ (2006) Enhanced biodegradation of toxic organophosphate compounds using recombinant *Escherichia coli* with sec pathway driven periplasmic secretion of organophosphorous hydrolase. Biotechnol Prog 22:406–410

Krystofova O, Zitka O, Krizkova S et al (2012) Accumulation of cadmium by transgenic tobacco plants (*Nicotiana tabacum* L.) carrying yeast metallothionein gene revealed by electrochemistry. Int J Electrochem Sci 7:886–907

Massa V, Infantin OA, Radice F et al (2009) Efficiency of natural and engineered bacterial strains in the degradation of 4-chlorobenzoic acid in soil slurry. Int Biodeterior Biodegrad 63:112–115

Mayer S, Stirling A (2002) Finding a precautionary approach to technological developments—lessons for the evaluation of GM crops. J Agric Environ Ethics 15:57–71

Menn FM, Easter JP, Sayler GS (2000) Genetically engineered microorganisms and bioremediation. In: Rehm HJ, Reed G (eds) Biotechnology: environmental processes II, vol 11b, 2nd edn. Wiley, Germany

Min M-G, Kawabata Z, Ishii N, Takata R, Furukawa K (1998) Fate of a PCBs degrading recombinant *Pseudomonas putida* AC30 (PMFB2) and its effect on the densities of microbes in marine microcosms contaminated with PCBs. Int J Environ Stud 55:271–285

Monti MR, Smania AM, Fabro G, Alvarez ME, Argarana CE (2005) Engineering *Pseudomonas fluorescens* for biodegradation of 2,4-dinitrotoluene. Appl Environ Microbiol 71:8864–8872

Mrozik A, Piotrowska-Seget Z (2010) Bioaugmentation as a strategy for cleaning up of soils contaminated with aromatic compound. Microbiol Res 165:363–375

Naik MM, Shamim K, Dubey SK (2012) Biological characterization of lead resistant bacteria to explore role of bacterial metallothionein in lead resistance. Curr Sci 103:426–429

Newby DT, Josephson KL, Pepper IL (2000) Detection and characterization of plasmid pJP4 transfer to indigenous soil bacteria. Appl Environ Microbiol 66:290–296

Pandey G, Paul D, Jain RK (2005) Conceptualizing "suicidal genetically engineered microorganisms" for bioremediation applications. Biochem Biophys Res Commun 327:637–639

Paul D, Pandey G, Jain RK (2005) Suicidal genetically engineered microorganisms for bioremediation: need and perspectives. BioEssays 27:563–573

Prakash D, Verma S, Bhatia R, Tiwary BN (2011) Risks and precautions of genetically modified organisms. ISRN Ecol Article ID 369573, 13 p

Renninger N, Knopp R, Nitsche H, Clark DS, Keasling JD (2004) Uranyl precipitation by *Pseudomonas aeruginosa* via controlled polyphosphate metabolism. Appl Environ Microbiol 70:7404–7412

Rodrigues JLM, Kachel A, Aiello MR et al (2006) Degradation of Aroclor 1242 dechlorination products in sediments by *Burkholderia xenovorans* LB400 (ohb) and Rhodococcus sp. strain RHA1 (fcb). Appl Environ Microbiol 72:2476–2482

Rojas LA, Yanez C, Gonzalez M, Lobos S, Smalla K, Seeger M (2011) Characterization of the metabolically modified heavy metal-resistant *Cupriavidus metallidurans* strain MSR33 generated for mercury bioremediation. PLoS ONE 6:e17555

Ronchel MC, Ramos JL (2001) Dual system to reinforce biological containment of recombinant bacteria designed for rhizoremediation. Appl Environ Microbiol 67:2649–2656

Sayler GS, Ripp S (2000) Field applications of genetically engineered microorganisms for bioremediation processes. Curr Opin Biotechnol 11:286–289

Singh A, Billingsley K, Ward O (2006) Composting: a potential safe process for disposal of genetically modified organisms. Crit Rev Biotechnol 26:1–16

Sobecky PA, Schell MA, Moran MA, Hodson RE (1996) Impact of a genetically engineered bacterium with enhanced alkaline phosphatase activity on marine phytoplankton communities. Appl Environ Microbiol 62:6–12

Stewart CN Jr, Richards HA, Halfhill MD (2000) Transgenic plants and biosafety: science, misconceptions and public perceptions. Biotechniques 29:832–843

Strong LC, McTavish H, Sadowsky MJ, Wackett LP (2000) Field-scale remediation of atrazine-contaminated soil using recombinant *Escherichia coli* expressing atrazine chlorohydrolase. Environ Microbiol 2:91–98

Stroo HF (2010) Bioremediation of chlorinated solvent plumes. In: Stroo HF, Ward CH (eds) In situ remediation of chlorinated solvent plumes. Springer, New York, pp 309–324

Taghavi S, Barac T, Greenberg B et al (2005) Horizontal gene transfer to endogenous endophytic bacteria from poplar improves phytoremediation of toluene. Appl Environ Microbiol 71:8500–8505

Tiedje JM, Colwell RK, Grossman YL (1989) The planned introduction of genetically engineered organisms: ecological considerations and recommendations. Ecology 70:298–315

United Nations Environment Programme (1992) Rio declaration on environment and development. In: Proceedings of the United Nations conference on environment and development, Rio de Janeiro, Brazil, June 1992

Wasilkowski D, Swedziol Z, Mrozi A (2012) The applicability of genetically modified microorganisms in bioremediation of contaminated environments. Chemik 66:817–826

Widada J, Nojiri H, Omori T (2002) Recent developments in molecular techniques for identification and monitoring of xenobiotic degrading bacteria and their catabolic genes in bioremediation. Appl Microbiol Biotechnol 60:45–59

Wu J-F, Jiang C, Wang B, Liu Z, Liu S (2006) Novel partial reductive pathway for 4-chloronitrobenzene and nitrobenzene degradation in *Comamonas* sp. strain CNB-1. Appl Environ Microbiol 72:1759–1765

Yang H, Nairn J, Ozias-Akins P (2003) Transformation of peanut using a modified bacterial mercuric ion reductase gene driven by an actin promoter from *Arabidopsis thaliana*. J Plant Physiol 160:945–952

Yang C, Xu L, Yan L, Xu Y (2010) Construction of a genetically engineered microorganism with high tolerance to arsenite and strong arsenite oxidative ability. J Environ Sci Health A 45:740–745

Zaat SAJ, Slegtenhorst-Eegdeman K, Tommassen J et al (1994) Construction of *phoE-caa*, a novel PCR- and immunologically detectable marker gene for *Pseudomonas putida*. Appl Environ Microbiol 60:913–920

Chapter 5
Integration of Lignin Removal from Black Liquor and Biotransformation Process

Krishnamurthi Tamilarasan, Periyar Selvam Sellamuthu and Baskar Gurunathan

Abstract Industrial discharge has tremendously increased inorganic pollutants in water bodies all over the world. Paper and pulp mill effluent is included in one of the most pollution-generating discharges containing complex chemical compounds such as lignin. For clean and healthy water resources, the recovery of lignin from wastewater from the paper and pulp industry is of high importance. On the other hand, these pollutants can be carcinogenic, due to the chlorine lignin and chlorine phenols that are formed along the process. The main focus of this study on precipitation of lignin from the black liquor (influent) is one stage followed by dewatering/washing to improve purity of lignin. Lignin valorization is an essential process for an advanced, sustainable, and economical biomass-based industry. However, converting lignin into value-added products remains a challenge due to its heterogeneity and irregular structure. Complex nature of lignin depolymerized by aromatic-catabolizing organisms to create "biological funnels" that receive heterogeneous aromatic substrates and convert them to a few products. Microbes such as bacteria and fungi are involved in the lignin degradation. Degradation of lignin through white-rot fungi may be helpful for the biotechnical applications like biopulping, biobleaching and pulp mill effluents treatment, and soil bioremediation. White-rot fungi specifically *P. chrysosporium*, also known as model fungus, and *Coriolus versicolor* are potential degradation against recalcitrant chromophoric material in bleach plant effluents. The abundance and renewability of lignin potentially converted to valuable bioproduct may eventually replace existing technology on manufacturing industries.

K. Tamilarasan (✉)
Department of Chemical Engineering, SRM University, Chennai 603203, Tamilnadu, India
e-mail: tamilbio@gmail.com

P. S. Sellamuthu
Department of Food Process Engineering, SRM University, Chennai 603203, Tamilnadu, India

B. Gurunathan
Department of Biotechnology, St. Joseph's College of Engineering, Chennai 600119, Tamilnadu, India

S. J. Varjani et al. (eds.), *Bioremediation: Applications for Environmental Protection and Management*, Energy, Environment, and Sustainability,
https://doi.org/10.1007/978-981-10-7485-1_5

5.1 Introduction

Socio-environmental and supply–demand issues involving fossil fuels have driven researchers and technologists to investigate alternative nonfossil resources as probable source of petrochemicals. Lignocellulosic biomass is a relatively inexpensive renewable natural resource that may be processed to produce lignin. The lignin may be processed further to obtain value-added chemicals. Apart from cellulose, lignocellulosic biomass contains 10–35% lignin, making it the second most abundant natural biopolymer. Hence, lignin obtained from abundantly available lignocellulosic biomass has the potential to emerge as an alternative source for fossil fuel-derived chemicals (Lange et al. 2013; Lee et al. 2009).

Lignocellulosic wastes are produced in large amounts as economically viable rates are from many industries including those of forestry, pulp and paper, agriculture, and also municipal solid waste (Kalogo et al. 2007). Lignocellulosic wastes are made up of cellulose fibers, hemicellulose, lignin, small amount of pectin, nitrogenous compounds, and the secret ash. Current research discussed the development of biorefineries and integration of biomass transformation into biofuels, energy, and value-added chemical synthesis (Norgren and Edlund 2014). Paper and pulp industry's bleaching process generates enormous amount effluent and contains large amounts of lignin and lignin-derived compounds that cause discoloration and toxicity. Lignin is responsible to generate high color index, turbidity, and chemical oxygen demand (COD). Lignin is known as complex chemical structure which is formed by rearrangement of aromatic and aliphatic molecule due to radical-initiated reactions. The complexity of lignin molecule pyrolysis process is a challenging issue and needs the use of appropriate catalysts (Zakzeski et al. 2010). The many of physiochemical methods used for the lignin depolymerization approaches such as hydrolysis, pyrolysis, oxidation or reduction, and biochemical methods (Lange et al. 2013). The chemical methods of depolymerization challenge process for controlling the char formation and preventing repolymerization monomers. Biochemical approaches are high-selectivity process for fragments molecule with normal environmental conditions. Lignin degradation products could be hampered by their inherent toxicity to microorganisms. However, more research efforts are needed in applying microbial metabolic engineering to treat complex aromatic substrates such as lignin.

5.2 Structure of Lignin

Lignin is made up of complex polyphenol units with variable arrangement dependent on the type plant material. The heterogeneity of lignin molecules exists in many possible interconnecting patterns between different polyphenol monomer units (Fig. 5.1). Lignin classification is traditionally done according to the precursors of the polymer. However, lignin can be randomly polymerized into basic

polyphenol units, such as coumaryl alcohol, coniferyl alcohol, and sinapyl alcohols containing zero, one, and two methoxy groups, respectively (Norgren and Edlund 2014). Lignin includes three basic structural units: hydroxy-phenyl monomer (H type) derived from coumaryl alcohol, guaiacyl monomer (G type) derived from coniferyl alcohol, and syringyl monomer (S type) derived from sinapyl alcohol (Fig. 5.2) (Lange et al. 2013). Lignin structure including various functional groups, such as methoxyl, aliphatic hydroxyl, phenolic hydroxyl, benzyl alcohol, noncyclic benzyl ether, and carbonyl groups, which involve reactivity of the molecule in various chemical reactions (Azadi et al. 2013). However, these units differ in the degree of linkages together with carbon–carbon (C–C), predominantly β–5 and β–1 bonds, and carbon–oxygen (C–O–C), like β–O–4 bonds formed lignin macromolecules (Doherty et al. 2011; Vijay Kumar et al. 2014). The other major linkages include 5–5-biphenyl, 4–O–5-diaryl ether, and β–β resinol linkages also depicted in the structure. Carbon–carbon linkages are considered as condensed linkages, whereas ether linkages are noncondensed. The proportions of these linkages vary significantly in various types of hardwood and softwood. The majority of linkages is approximately two-thirds of bonds between phenylpropane units C–O–C ether, β–O–4, while remaining are such as C–C bonds between these units. In general, the content of methoxy groups found in softwood and hardwood is 0.92 and 0.94 per 1 phenyl propane unit, respectively.

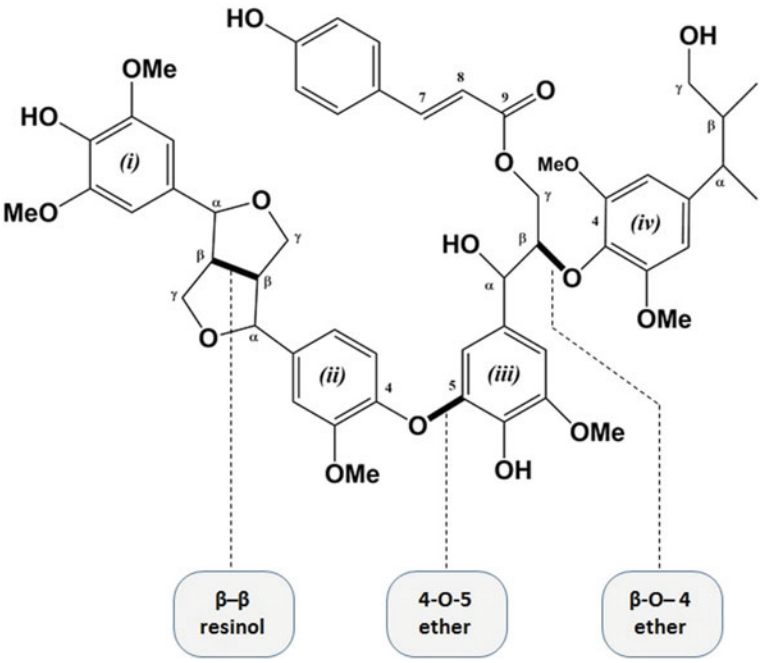


Fig. 5.1 Typical lignin structure units and the linkages (Joffres et al. 2014)

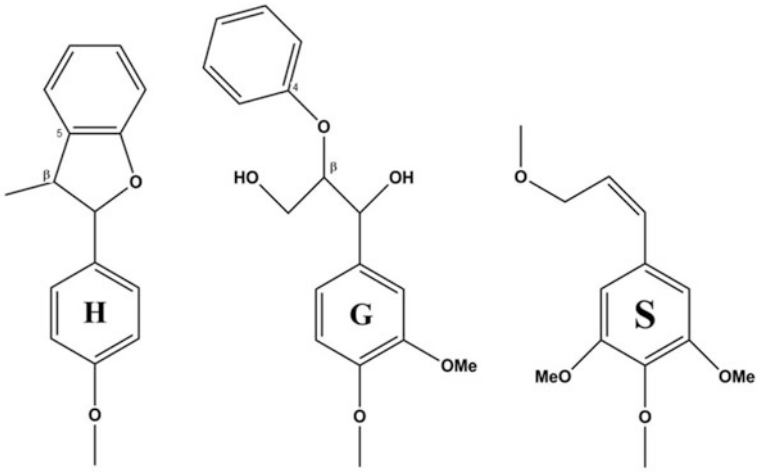


Fig. 5.2 Basic structural monomer unit of lignin (H: Hydroxy-phenyl monomer, G: Guaiacyl monomer, S: Syringyl monomer) (Vijay Kumar et al. 2014)

Lignin molecules contain primary aliphatic hydroxyl groups (bonded to the γ–C-atom), secondary aliphatic hydroxyl groups (bonded to the α–C-atom), and phenolic hydroxyl groups (bonded to the 4–C-atom of the aromatic ring) in their structure (Fig. 5.3). The average of hydroxyl group presents in lignin structure contains ~0.2, ~0.84, and ~0.30 primary aliphatic, secondary aliphatic, and phenolic hydroxyl groups per 1 phenyl unit, respectively. Naturally, lignin contains a low amount of COOH groups, about 0.05 per 1 phenyl unit, which may increase

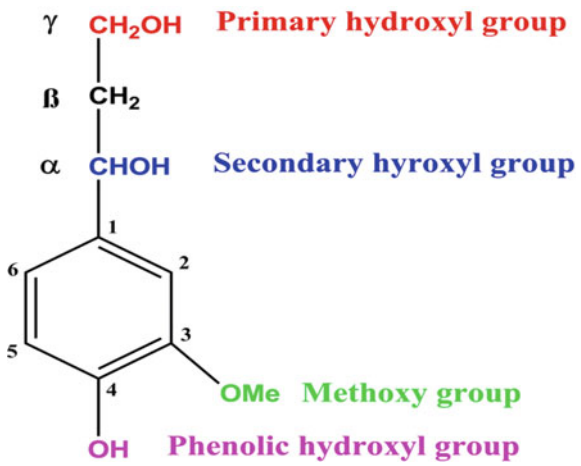


Fig. 5.3 Lignin nomenclature

result in increased hydrophilicity of lignin molecule. Carboxyl groups are connected to other functional groups via H-bonds which may result in increased molecular structure of lignin. The total amount of carbonyl groups in lignin is about 0.21 per 1 phenyl propane unit. However, these can be of four different kinds; there are a few aldehyde 5 groups at the γ–C-atom (0.04 per 1 phenyl propane unit). The rest of the carbonyl groups (about 0.17 per 1 phenyl propane unit) are ketonic groups. 0.07 groups per 1 phenyl propane unit are located at α–C-atom, and 0.1 groups per 1 phenyl propane unit are located at β–C-atom. In addition, lignin contains also a few quinonic groups.

5.3 Lignin Isolation Processes

There are several different methods used to recover lignin from lignocellulose material. The present isolation methods are currently used in research and industry process. The isolation methods might affect especially the structure of the lignin in the raw material, since isolated lignin represents the only source for obtaining chemical information.

5.3.1 Kraft Lignin

The kraft (sulfate) pulping is the most commonly used pulping methods of wood today with about 90% share of the total lignin isolation (Feng and Catherine 2017). In this process, lignin is isolated through dissolution of lignocellulose structure on wood chips in a solution of sulfide, sulfhydryl, and polysulfide. In this process, lignin dissolution occurs owing to the reaction between biomass with sodium hydroxide and sodium sulfide to cleave ether linkage by sulfide and bisulfide ions which consequently results in the formation of alkali-soluble lignin fragments (Sjostrom 1993). Sulfide pulping process, lignin structure hydroxyl groups is sulfonated in to lignosulfonates molecule. This pulping process, black liquor contains cellulose pulp, lignin, pulping chemicals, and other residues. After pulping process, kraft lignin can be precipitated by acidification of the black liquor with mineral acids (Fig. 5.4).

In general, black liquors from digestion of softwood contain more lignin than from hardwood. Kraft lignin is hydrophobic at neutral pH and contains around 1% sulfur in the form of aliphatic thiol groups, and other functional groups are methoxy group (14 w/w%), aliphatic hydroxyl group (10 w/w%), carboxylic acid group (4–7 w/w%), and phenolic hydroxyl group (2–5 w/w%) compositions that can vary depending on the plant source and processing conditions (EI Mansouri and Salvado 2007; Doherty et al. 2011).

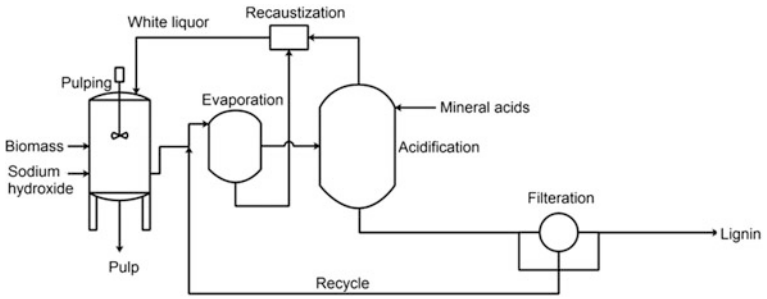


Fig. 5.4 Schematic diagram of the kraft pulping process

5.3.2 Lignosulfonate Lignin

Lignosulfonate is the sulfonated lignin that is recovered from lignocellulose material by sulfite pulping process. In this pulping process, the sulfur dioxide reacts to lignocellulosic biomass with water to form sulfonic acid at high temperatures. During pulping process, sulfonate groups are introduced in the lignin structure at the α-position of the propane side chain to form lignosulfonates. The resulting lignin has functional groups of lignosulfonic acid, lignosulfonate, carboxylic group together with phenolic/aliphatic hydroxyl groups (Saake and Lehnen 2000; Vishtal and Kraslawski 2011). The exhibit average molecular weight of sulfonated lignin is higher than that of kraft lignin because of sulfonated group linkages to arenes in lignin fragments (Feng and Catherine 2017). The sulfonate groups bind with most lignosulfonate lignin, so always water-soluble form makes different from other lignin types.

5.3.3 Soda Lignin

The soda process is the oldest pulping method for separation of lignin that uses sodium hydroxides as the chemical reagents. In this process, wood treated with concentrated sodium hydroxide along with oxidant under milder temperature. In the process, an α-ether bond can be hydrolytically cleaved by this method, resulting in lower molecular weight fragments produced. The advantage of sodium hydroxide pretreatments is easily recycled, thus lowering production costs and environmental impacts (Carvajal et al. 2016). The amount of lignin separation by soda pulping process was almost five times more than acid pulping process. Furthermore, soda lignin has been used as a raw material for synthesis of new fine chemical and polymers because of the absence of sulfur function group (Saake and Lehnen 2000)

5.3.4 Organosolv Lignin

Organosolv pretreatment method separated the lignin from lignocellulosic biomass using aqueous alcohol and organic acid. A wide variety of alcohols (methanol and ethanol (usually with around 50% water)), acids (formic and acetic acid), and inorganic alkali chemicals have been used for organosolv pulping process. Organic acid and organic solvent are efficiently cleave the α-ether linkages of lignin and dissolve the degraded fragment products (Azadi et al. 2013). The main advantage of the organosolv process is that carried out under mild conditions (~100 °C under atmospheric pressure) and thus requires less energy. Organosolv lignin can be separated from the pulping liquor with distilled water to precipitation of lignin molecule.

Most of organosolv lignin is insoluble in acidic aqueous solutions, but will dissolve in basic solutions and polar organic solvents. Organosolv lignin is generally low molecular weight lignin around 5 kDa and sulfur-free molecule, although this will obtained depends on types of carboxylic acids and organic solvent used for treatment methods (Chung and Washburn 2016). It is important that organosolv lignin is sulfur-free and less-condensed structure, easy to further valorization. In spite of these advantages, none of the pulping method has been yet widely used in a production process (Fig. 5.5).

5.3.5 Steam Explosion Lignin

The steam explosion process consists in biomass digestion with high temperature ~250 °C under high pressures ~25–35 bar at short contact times (1–20 min) followed by sudden reduction of pressure, which cleaves the linkages between lignin and biomass cell walls by explosive decompression (Duff and Murray 1996). Hydrolysis, aryl ether cleavage and homolytic cleavage of C–C bond, alkylation, demethoxylation, and condensation reactions occur during the steam process (Wang et al. 2009; Kang et al. 2011). While the aromatic groups in the lignin molecule are

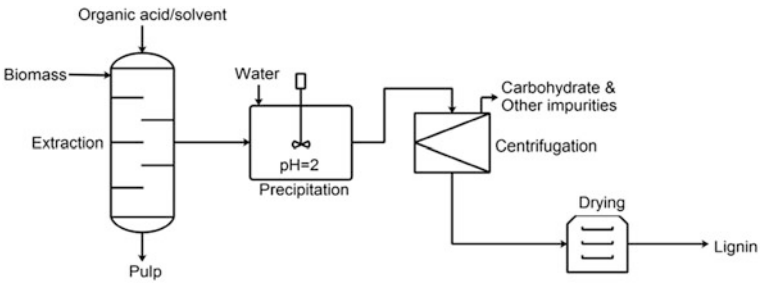


Fig. 5.5 Simplified process diagram of organosolv pulping process

not degraded, β–O–4 ether linkages and C_α–C_β bonds are being cleaved during steam treatment process. The content of phenolic hydroxyl functional groups was relatively high due to cleaving the main intermonomer lignin linkages, β–O–4 linkages, in the process. The resulting lignin has a low molecular weight as well as sulfur-free and good solubility in organic solvents (Dahlmann and Schroeter 1990).

5.3.6 Ionic Liquid Lignin

Ionic liquids (ILs) were recently introduced as promising green solvents for selectively dissolve lignin or cellulose and assist in the deconstruction of lignocellulosic biomass. Ionic liquids are composed of cations and anions that exist in the liquid state at atmospheric temperature and pressure. Ionic liquids are often viewed as green solvents due to their low vapor pressures, low toxicity, high thermal stability, and nonflammability. In addition, recovery of ionic liquids is relatively easy and so they can often be reutilized (Feng and Catherine 2017). Many different ILs have been used for lignin extraction, taking advantage of the various combinations of anions and cations. Anions can be divided into organic parts and inorganic parts, such as trifluoromethylsulfonate, hexafluorophosphate, tetrafluoroborate, trifluoroethanoate, halide, ethanoate, and nitrate. Organic cations include tetraalkylammonium, 1-alkyl-3methylimidazoliym, N-alkylpyridinium, and tetraalkyl phosphonium (Jinhuo et al. 2016). However, the drawback of the ionic liquid is its high cost for production and handling of a high viscosity solution. Such drawbacks can be overcome by using an aqueous ionic liquid and recycling it after each extraction process. The biomass is dissolved suitable ionic liquid typically at 80–130 °C; lignin can be further separated using precipitation agents such as water and acetone–water mixture. However, rapid development of biorefineries may lead to a significant increase in the removal of lignin from biomass using different ionic liquids.

5.4 Physicochemical Properties of Lignin

The overall physicochemical properties of lignin depend on the source of lignin extracted (soft wood/hard wood/grass) and the process of extraction methods (He et al. 2013; Lora and Glasser 2002). The difficulty rises in accurately determining the physicochemical properties of lignin molecular weight distribution due to varying functionality and three-dimensional net structure. The average molecular weight of lignins is ~0.5–3 kDa for the organosolv lignin, ~1–5 kDa for the kraft lignin, and ~5–400 kDa for lignosulfonate polymers. The polydispersity for lignosulfonate and kraft lignin is high compared to the organosolv lignin (Norgren and Edlund 2014). The molecular weight of lignin determines the osmometric method, super-centrifugation, light-scattering method, high-pressure liquid chromatography, and gel permeation chromatography (Gao and Tang 1996). The hydroxyl functional

groups in the structure of lignin, resulting in strong intermolecular hydrogen bonds, and making the intrinsic lignin insoluble in any solvent. The presence of other group's phenolic hydroxyl and carboxyl makes the lignin able to be dissolved in alkaline solution. The amorphous thermoplastic polymer of lignin softening temperature ranges from 127 to 129 °C, which remarkably decreased with increased water content, indicating that water acts as a plasticizer in lignin polymer (Jiang 2001). The lignin color can be varied with separation and preparation processes. There are various functional groups in the structure of lignin including methoxyl, aliphatic hydroxyl, benzyl alcohol, phenolic hydroxyl, noncyclic benzyl ether, and carbonyl groups which result in reactivity of the lignin in various chemical reactions (Azadi et al. 2013).

5.5 Lignin: Recent Advances Applications

The large industrial process requires a renewable feedstock that can meet the needs of value-added products with novel properties. Lignin is available in large quantities in the byproduct of biorefinery, paper and pulp industry. Lignin is converted in to potential environmental-friendly renewable material for multifunctional applications. The abundant functional groups available in lignin molecule susceptible to chemical modification leads to new feedstock material (Vijay Kumar and Manju Kumari 2015). Here, we briefly discuss about the potential applications of lignin classified in different groups as (i) renewable energy, (ii) macromolecule synthesis, and (iii) fine chemical production.

5.5.1 Lignin for Power-Fuel Gas Production

Lignocellulosic biomass contains enormous quantities of lignin, and the development of sustainable and efficient refinery processes should aim at the valorization of lignin to produce biofuels. The thermochemical conversion technology, lignin pyrolysis, undergoes the formation of primary volatiles, small-molecule gases, fuel additives, and high value-added chemical forms in the absence of oxygen (Wang et al. 2016). Lignin gasification processes to produce syngas (CO_2:H_2), the addition of a second-step employing production of a "pure" hydrogen stream with carbon dioxide.

5.5.2 Lignin for Macromolecule Synthesis

Lignin can be used as renewable material for the production of end products such as plastic, resins, and polymer materials. Lignin-based polymers are low cost and

highly biodegradable with novel properties. Lignin-based carbon fibers are mainly used for the fabrication of strong and light materials in defense and aerospace and general material in the industrial markets. Increasing demand for carbon fibers, relatively limited quantities are produced in the current market due to the high costs and lack of material availability. Currently, lignin-based fiber mats are produced from kraft lignin with higher strength, smooth surfaces, and without defects. The flexible carbon fiber mats synthesis from lignin would be novel and sustainable materials for high-performance super capacitors (Norgren and Edlund 2014). Organosolv lignin also used for the synthesis of phenolic resins that exhibited good curing and other properties compared to nondegradable resin. Phenol formaldehyde (PF) resins are replaced by lignin-based resins, which are cross linked under alkaline conditions and dark colored represent a higher market value. Lignin is used to synthesis of epoxies resins for fabrication of printed circuit boards with good mechanical and electrical properties, and high toughness (Kosbar et al. 2001; Simionescu et al. 1993). It also used for low-cost formaldehyde adhesives synthesis in fiber board production. The lignin-blended ethylene and propylene polymers are higher strength, elongation, and other mechanical properties (Stewart 2008). Lignin recently used for synthesis of hydrogels has interesting applications due to bioadhesive characteristics, making them novel candidates in enhancing the drug residence time and tissue permeability (Vijay Kumar and Manju Kumari 2015). Thermoplastic compounds based on renewable resources are compostable and biodegradable and thus offer environment-friendly alternatives to synthetic petrochemical plastics.

5.5.3 Fine Chemical Synthesis

Low molecular weight aromatic compounds benzene, toluene, and xylene (BTX) are commonly used as a solvent and basic monomer unit for value-added chemical, which are easy to incorporate into the existing industrial processes and products. Phenols constitute are basic platform chemicals and used to synthesis new materials and applied in a wide range of products such as automotive and textiles. The lignin-based solvent to replace the petroleum-derived aromatics in the existing industries can be used as high-boiling solvents, particularly in the context of future biorefineries. Lignin produces aromatic chemicals used in a lot of consumer products such as high-performance polymers, resins, fuel additives, flavors, agrochemicals, fragrances, coatings, and bottles. Lignin derived water dispersed nanoparticle can be used in the application of antimicrobial, antiviral, antioxidant and drug delivery system (Table 5.1). Lignin nanoparticle is naturally biodegradable, biocompatible, good stability, and environmental-friendly nanomaterial compared to other nanoparticles used for cancer therapy (Richter et al. 2015).

Table 5.1 Potential application of lignin

Power-fuel gas	Fine chemical	Macromolecules
Syngas (CO/H_2)	BTX (benzene, toluene, xylene)	Carbon fibers
Water–gas	Phenols and phenolic acids	Phenolic resins
C1–C7 gases	Vanillin and vanillic acid	Additives (paints, coatings, surfactants)
	Catechols	Binders
	Cinnamic and benzoic acids	Composites
	Catechols	Polyolefins
	Vanillin and vanillic acid	Low cost fillers
		Thermoplastics
		Polyurethanes

5.6 Lignin Removal in Paper and Pulp Industry

Paper and pulp industry's wastewater contains highly contaminated organic complex substances. Pulp and paper mills generate varieties of pollutants depending upon the type of the pulping process. The sources of the contamination of wastewater are fibrous materials, additives, wax, lignin, and their derivates and organic chlorides. These effluents are characterized of high coloration, suspended solids, unstable pH, high COD and BOD, besides chlorides, sulfate, etc. On the other hand, these pollutants can be carcinogenic, due to the chlorine lignin and chlorine phenols that are formed along the process (Ramos et al. 2009). Removal of effluents containing lignin and lignin-related compounds from black liquor would offer several advantages like recovery of valuable chemicals and decreasing the load on environment.

5.6.1 Lignin Removal by Coagulation Process

Coagulation is the process of the removal of suspended organic substance from black liquor by making flocs for easy precipitated out. The coagulation process involves interactive forces between organic substance and type of coagulant and other environmental factors. Several coagulants, aluminum chloride, and aluminum sulfate polyaluminum chloride are used for removal of lignin from waste black liquor. When an aluminum and ferric ion are used as coagulant, the aluminum and iron cations promote the formation of functional group and ligands bridging enhancement of organic solid forming large amorphous flocs (Nawaz et al. 2014; Licsko 1993).

5.6.2 Lignin Precipitation by Acidification Process

Acid precipitation by acidification of black liquor was most potentially promising method for removal of lignin. A number of studies related to lignin precipitation by acidification can be found in the literature. The precipitation was carried out using different organic and inorganic acids at pH 2, 4, 7, 9, and 10 with the quantifying lignin separation from black liquor. The lignin precipitation from block liquor was obtained by introducing CO_2 and SO_2 gases and acids including waste generator acids from chlorine plant (Kamble and Bhattacharyulu 2015). The precipitated solution was filtered at high temperature in order to improve the dead-end filtration. The black liquor sample was send to closed tank for mixing around 1 h in order to reach the desired temperature around 70–75 °C. After obtaining the target temperature, concentrated acid was slowly added according to the desired addition rate with the high speed of agitation, and the pH of the solution was monitored continuously with an electronic pH meter. The charged groups of lignin molecule are more associated with higher temperature to form larger colloidal suspension in the black liquor (Kouisni et al. 2012). The tank was mixing at fixed rate for 1 h to obtain a lignin-rich precipitation. When precipitation was complete, the black liquor sample was slowly dispensed through the filtering apparatus to remove lignin content. After the filtration process, precipitated lignin is crushed and dried to form lignin in powder form (Fig. 5.6).

5.6.3 Electrocoagulation Process

Electrocoagulation process is consisting of generating floc metallic hydroxides from effluent, to be removed, by electro dissolution of soluble anodes. Electrolysis process is continuous separating lignin from effluent via electrocoagulation methods. The advantage of removing the smaller particles is due to their greater probability of being coagulated in the electric field (Ugurlu et al. 2008). In this, experiments were carried out with two electrodes in order to separate membrane compartments. In the anode compartments, oxygen is generated, pH drops, and

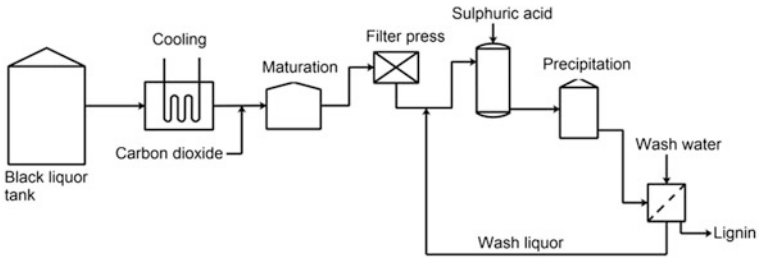


Fig. 5.6 Schematic diagram of lignin precipitation by acidification process

lignin precipitates via oxidation, while metallic hydroxide is generated on the cathode compartment (Loutfi et al. 1991). The anodes are oxidized to produce metal ions, soluble monomeric and polymeric metal complexes formed in the electrolysis process as shown in the following equation (Ravi et al. 2013). These hydroxo-metal complexes act as coagulant for the removal of lignin macromolecules and its derivatives by electrocoagulation.

Oxidation of anode

$$M_{(S)} \rightarrow M^{n+}_{(aq)} + ne^-$$

Water reduction at cathode

$$nH_2O + ne^- \rightarrow n/H_2O + nOH^-$$

At alkaline condition

$$M^{n+} + nOH^- \rightarrow M(OH)_n$$

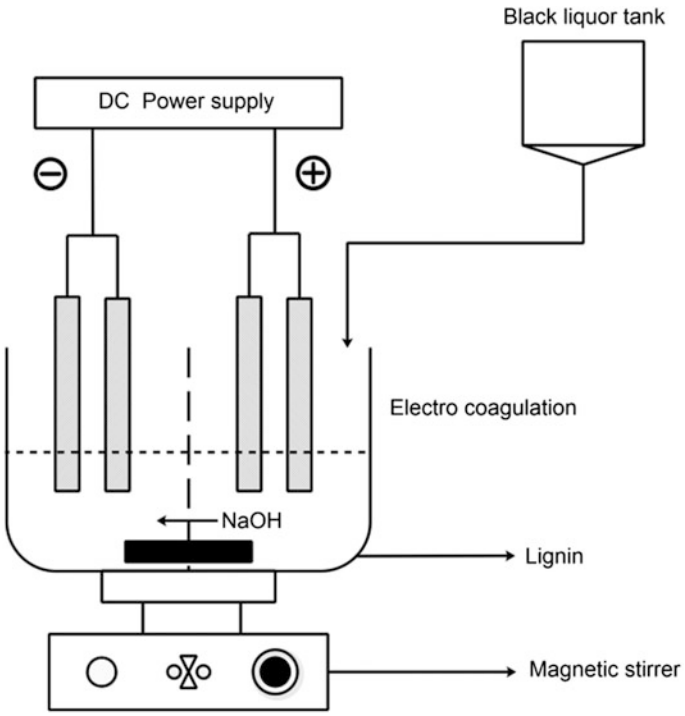


Fig. 5.7 Experimental setup of electrocoagulation process

At acidic condition

$$M^{n+} + nH_2O \rightarrow M(OH)_n + nH^+$$

The main challenges of this electrocoagulation process are membrane foaling, power requirement, and removal of lignin from compartment (Fig. 5.7).

5.7 Biodegradation and Bioremediation of Lignin

Microbes such as bacteria and fungi are involved in the lignin degradation. Lignase (EC number 1.14.99) or lignin peroxidase is a hemoprotein and isolated initially from *Phanerochaete chrysosporium* (white-rot fungus) and using hydrogen peroxide as an oxygen source. Microbial enzymes such as manganese peroxidase and copper-based laccase are carried out the lignin degradation. Lignin reduces the accessibility of cellulose and hemicellulose to the microbial enzymes (cellobiose dehydrogenase) and minimized overall plant part digestibility which leads to protect against pathogens and pests.

Bioremediation is a transformation or degradation of contaminants into nonhazardous or less hazardous substances obtained through microbes. The bacteria, fungi, algae, and plants are employed in the bioremediation technology to reduce the pollutants. It mainly depends on the favorable environmental conditions for the microbial growth and action. The efficiency of microbes may be improved through the modification of environmental conditions to permit the microbial growth and degradation. Bioremediation mainly based on exact microbes and specific environmental factors. Microorganisms present in the specific contaminated environments are more readily adjusted to persist in the existing contaminants and environmental conditions such as temperature, pH, and oxidation/reduction potential. Bioremediation application comprises biotransformation and biodegradation through converting hazardous to nonhazardous or minimum hazardous chemicals. Frequently, microbes degrade the chemicals and release CO_2 or methane, H_2O, and biomass. Biotransformation is any modification of the compound or arrangement of molecules through microbes. Biodegradation is the breaking down of organic or bioaccumulation and biotransformation of inorganic molecules into environmental-friendly molecules.

Lignin is main pollutant from paper–pulp mill effluent due to its strong unesthetic brown color, hydrophobicity, and low mechanical properties. Textile companies discharged containing more quantity of colored dyes. Extracellular lignin peroxidase enzymes from microbes have a capability to degrade lignin as well as wide range of complex aromatic dyestuffs.

The effective natural lignin degraders are wood-rotting basidiomycetous fungi which causes white rot in wood (Kirk and Farrell 1987). They have specific character to depolymerize and mineralize lignin by extracellular ligninolytic enzymes. Degradation of lignin through white-rot fungi may be helpful for the

biotechnical applications like biopulping, biobleaching and pulp mill effluents treatment, and soil bioremediation (Lamar and Dietrich 1992; Messner and Srebotnik 1994). Initially, only laccase had been reported during 1980s, and later two more enzymes such as lignin peroxidases (LiPs) and manganese peroxidases (MnPs) also reported for the lignin degradation. Mixed cultures such as fungi, bacteria, and actinomycetes are exhibiting lignin mineralization in the soil and compost (Tuomela et al. 2000). The *P. chrysosporium* naturally produces LiPs beside through MnPs and LiP is concluded that highly significant lignin degrading enzyme. Even though, certain fungi does not even yield LiP, e.g., *Ceriporiopsis subvermispora*, only produces MnP and laccase and important fungus for biopulping.

Chemically and morphologically different kinds of decay concluded the occurrence of diverse microbes. Wood decay fungi are generally divided into three major divisions such as white rot, soft rot, or brown rot. Wood-degrading bacteria stated that inclusion of erosion, tunneling, and formation of cavity (Eriksson et al. 1990; Blanchette 1995; Daniel and Nilsson 1998). A biological treatment method includes employment of microbes such as fungi, bacteria, algae, and enzymes, as a sole step treatment or in combination with other physical and/or chemical techniques (Singhal and Thakur 2009). The wastewater treatments through biological methods are concluded to be cost-benefit, eco-friendly, and appropriate reduction in BOD and COD from the effluents when compared to physicochemical methods.

5.7.1 Bacteria

Lignified cell walls of wood can be degrading by certain bacteria. The genus *Streptomyces* is recognized degraders of lignin. Usually, *Streptomyces spp*. solubilizes the portion of lignin, and final product is water-soluble, acid-precipitable polymeric lignin. Actinomycetes produce extracellular peroxidases, e.g., lignin peroxidase-type enzyme (Ramachandra et al. 1988; Adhi et al. 1989). *Pseudomonas spp.* is highly potent degraders of lignin (Vicuæa 1988; Zimmermann 1989).

The two bacteria isolates such as *Pseudomonas aeruginosa* and *Serratia marcescens* had potential to decolorize from 44 to 49% of lignin. The research reported that biobleaching of paper–pulp mill effluent provided 60–75% color reduction, whereas in the textile dye-based effluent only 50–58% decolorization attained. The heterogeneous combination of lignin peroxidases from mixed groups contributed 80–85% color reduction in paper–pulp mill effluent treatment and 70–75% decolorization in textile dye-based effluent treatment. The lignin peroxidase may be efficiently employed in biobleaching and biodegradation of effluents from paper–pulp and textile industries. The possible applications of lignin degrading microbes and their enzymes have become attractive, because they may provide environmental-friendly technologies for the paper–pulp and various other industries.

The bacteria such as *P. aeruginosa, S. marcescens, Nocardia, Arthrobacter, Flavobacterium, Micrococcus,* and *Xanthomonas* have recognized as lignocellulosic degrading microbes (Kalyani et al. 2008). Different bacterial species have been evaluated for their decolorization capabilities. The more potential microbes such as *Bacillus subtilis* and *Micrococcus luteus* were found to be 87.2% of BOD reduction and up to 94.7% COD reduction and 97% of lignin reduction after nine days in shaking conditions and also reduced the pH of raw pulp and paper mills effluent to neutral and increase the dissolved oxygen in effluent (Tyagi et al. 2014). Only a few strains have the ability to degrade the lignin derivatives attained from various pulping processes (Hao et al. 2000; Chandra et al. 2011; Chandra and Bharagava 2013). *Pseudomonas aeruginosa* had the potential to reduce up to 26–54% kraft mill effluent color and higher under the aerobic conditions (Ramsay and Nguyen 2002). The two strains of *P. aeruginosa* and *B. cereus* reported for the decolorization of bleach kraft effluent (Tiku et al. 2010 and Raj et al. 2007). *Streptomycetes badius* and *S. viridosporous* were capable to utilize a commercial kraft lignin for their carbon source (Abd El-Rahim and Zaki 2005; Chandra et al. 2011). *Pseudomonas putida* and *Acienetobacter calcoaceticus* are reported for degradation of black liquor from a kraft pulp and paper mill in a continuous reactor which has the capacity to remove 70–80% of COD and lignin, and color removal potential was found to be 80% in eight days (Murugesan 2003; Abd El-Rahim and Zaki 2005). *Pseudomonas, Ancylobacter,* and *Methylubaclerium* are evaluated for the removal of organochlorine from bleached kraft pulp and paper mill effluents (Keharia and Madamwar 2003). Various soil isolates of *Actinomycetes* are evaluated for their ability to utilize spent sulfite bleach effluents from a paper mill. Extracellular peroxidase and cell wall-bound catalase activities were produced during microbial growth on bleach effluents (Raj et al. 2007; Chandra et al. 2012). The mixed population of bacteria and protozoa obtained in lake's bottom sediment near to the effluent kraft paper mill had potential to degrade lignin sulfonate (Raj et al. 2007).

5.7.2 Algae

The decolorization of algae causes metabolic transformation of colored compounds to colorless compounds or compounds degradation (Chandra and Singh 2012). *Microcystis* spp. has been reported for the decolorization of diluted effluents from bleach kraft mill (Iyovo et al. 2010; Sharma et al. 2014) and also concluded that pure and mixed algal cultures degraded the color up to 70% in 2-month incubation. The mixed algal culture such as *Chlorella, Chlamydomonas, and Microcystis* is employed for the removal of adsorbable organic halides (AOX) and color and concluded that 70% AOX reduction and 80% color reduced in 30 days under continuous lighting conditions (Sharma et al. 2014; Chandra and Singh 2012).

5.7.3 Soft-Rot Fungi

Ascomycetes and deuteromycetes (fungi imperfecti) commonly cause soft-rot decay in wood (Blanchette 1995; Daniel and Nilsson 1998). The wood decay has appeared as brown, soft, and cracked when dry. Two types of soft rot have been called, type I containing of biconical or cylindrical cavities which are formed within secondary walls, whereas type II states to erosion form of degradation (Blanchette 1995). In contrast to nonselective white-rot fungi, the middle lamella is not attacked by type II soft-rot fungi.

5.7.4 Microfungi or Molds

Microfungi or molds are deuteromycetes. The ligninolytic microbes were isolated from forest soil for ligninolytic activity and identified as *Penicillium chrysogenum, Fusarium oxysporum, and Fusarium solani* (Rodriguez et al. 1996).

5.7.4.1 Brown-Rot and White-Rot Basidiomycetes

The major wood-depredating fungi are basidiomycetes. Wood-rotting basidiomycetous fungi are commonly separated into white-rot and brown-rot fungi. They are taxonomically narrowly associated, and white-rot and brown-rot fungi can be found in the same genera. Brown-rot fungi primarily decompose the cellulose and hemicellulose components; however, they can also modify the lignin to a limited extent. Brown-rotted wood is dark, shrink, and typically broken into brick-shaped or cubical fragments that simply break down into brown powder (Blanchette 1995). The brown color specifies the occurrence of modified lignin in wood. Many brown-rot fungi such as *Serpula lacrymans, Coniophora puteana, Meruliporia incrassata,* and *Gloeophyllum trabeum* are damaging the wood used in buildings and other structures (Blanchette 1995).

Biotechnical applications of brown-rot fungi have been reported that solid-state fermentation of pine sawdust for the cattle feed production, or the usage of brown-rotted lignin for adhesives, to substitute phenol formaldehyde flake board resin. Brown-rotted lignin is highly potential than native lignin due to the increased phenolic hydroxyl groups (Jin et al. 1990). The *G. trabeum* produced a significant release of alkali-soluble lignin particularly in pine sawdust in the first week of the growth (Agosin et al. 1989).

The basidiomycetous white-rot fungi and related litter-decomposing fungi had the potential to mineralizing lignin (Kirk and Cullen 1998). Various white-rot fungi differ significantly in the comparative rates lignin and carbohydrates degradation in woody tissues. Certain fungi have the capability to degrade lignin than carbohydrates (Blanchette 1995). Several white-rot fungi cause cell wall erosion. Eroded

zones merge as decay progresses and large voids filled with mycelium are designed. This type of rot is stated as nonselective or simultaneous rot (Blanchette 1995). *Trametes* (syn. Coriolus, Polyporus) *versicolor* is a typical simultaneous-rot fungus (Eriksson et al. 1990).

Certain white-rot fungi favorably eliminate the lignin without a substantial loss of cellulose and cause white-pocket/white mottled type of rot, e.g., *Phellinus nigrolimitatus* (Blanchette 1995). Some fungi produced both types of degradation in the same wood (Eriksson et al. 1990) such as *Ganoderma applanatum* and *Heterobasidion annosum.*

The fungi specifically removing lignin are concluded the most promising fungi for applications in the pulp and paper industry. Though, the ratio lignin, hemicellulose, and cellulose degraded through particular fungus can vary extremely, and even different strains of the similar species, e.g., *P. chrysosporium* and *Ceriporiopsis subvermispora*, may act inversely on the similar type of wood (Eriksson et al. 1990; Blanchette et al. 1992). Several screening studies reported to find the appropriate fungi for biopulping of wood or straw have revealed fungi that, under certain conditions, degrade lignin especially to cellulose. Such lignin-selective fungi are *P. chrysosporium, C. subvermispora* (Eriksson et al. 1990), *Pycnoporus cinnabarinus* (Ander and Eriksson 1977), *Pleurotus ostreatus* (Martínez et al. 1994), *Pleurotus eryngii* (Martínez et al. 1994), *Phlebia radiata* (Ander and Eriksson 1977), *Phlebia tremellosus* (syn. *Merulius tremellosa*) (Ander and Eriksson 1977; Eriksson et al. 1990), *Phlebia subserialis* (Akhtar et al. 1997), *Phellinus pini* (Eriksson et al. 1990), and *Dichomitus squalens* (Eriksson et al. 1990).

White-rot fungi specifically *P. chrysosporium*, also known as model fungus and *Coriolus versicolor,* are potential degradation against recalcitrant chromophoric material in bleach plant effluents.

Bioremediation of pulp and paper mill effluent using microbes to decolorizing the effluents has been reported effectively. Further research is needed to develop fast biodegradation processes as well as found out commercial application strains that are possible to provide an economically feasible of process.

References

Abd El-Rahim WM, Zaki EA (2005) Functional and molecular characterization of native Egyptian fungi capable of removing textile dyes. Arab J Biotech 8:189–200

Adhi TP, Korus RA, Crawford DL (1989) Production of major extracellular enzymes during lignocellulose degradation by two *Streptomycetes* in agitated submerged culture. Appl Environ Microbiol 55:1165–1168

Agosin E, Jarpa S, Rojas E, Espejo E (1989) Solidstate fermentation of pine sawdust by selected brown-rot fungi. Enzyme Microb Technol 11:511–517

Akhtar M, Blanchette RA, Kirk TK (1997) Fungal delignification and biomechanical pulping of wood. Adv Biochem Eng Biotechnol 57:159–195

Ander P, Eriksson KE (1977) Selective degradation of wood components by white-rot fungi. Physiol Plant 41:239–248
Azadi P, Inderwildi OR, Farnood R, King DA (2013) Liquid fuels, hydrogen and chemicals from lignin: a critical review. Renew Sust Energy Rev 21:506–523
Blanchette RA, Burnes TA, Eerdmans MM, Akhtar M (1992) Evaluating isolates of Phanerochaete chrysosporium and Ceriporiopsis subvermispora for use in biological pulping processes. Holzforschung 46:109–115
Blanchette RA (1995) Degradation of lignocellulose complex in wood. Can J Bot 73:S999–S1010
Carvajal JC, Gómez Á, Cardona CA (2016) Comparison of lignin extraction processes: economic and environmental assessment. Bioresour Technol 214:468–476
Chandra R, Abhishek A, Sankhwar M (2011) Bacterial decolorization and detoxification of black liquor from rayon grade pulp manufacturing paper industry and detection of their metabolic products. Bioresour Technol 102:6429–6436
Chandra R, Bharagava RN (2013) Bacterial degradation of synthetic and kraft lignin by axenic and mixed culture and their metabolic products. J Environ Biol 34:991–999
Chandra R, Singh R (2012) Decolourisation and detoxification of rayon grade pulp paper mill effluent by mixed bacterial culture isolated from pulp paper mill effluent polluted site. Biochem Eng J 61:49–58
Chung H, Washburn NR (2016) Extraction and types of lignin. Lignin in polymer composites. Copyright © 2016 Elsevier Inc, pp 13–25
Dahlmann G, Schroeter MC (1990) The Organocell process: pulping with the environment in mind. Tappi J 73:237–240
Daniel G, Nilsson T (1998) Developments in the study of soft rot and bacterial decay. In: Bruce A, Palfreyman JW (eds) Forest products biotechnology. Taylor and Francis, London, p 326
Doherty WOS, Mousavioun P, Fellows CM (2011) Value-adding to cellulosic ethanol: lignin polymers. Ind Crops Prod 33:259–276
Duff SJ, Murray WD (1996) Bioconversion of forest products industry waste cellulosic to fuel ethanol: a review. Bioresour Technol 55:1–33
El Mansouri NE, Salvado J (2007) Analytical methods for determining functional groups in various technical lignins. Ind Crops Prod 26:116–124
Eriksson KEL, Blanchette RA, Ander P (1990) Microbial and enzymatic degradation of wood and wood components. Springer, Berlin, Germany
Feng C, Catherine EB (2017) Producing jet fuel from biomass lignin: potential pathways to alkylbenzenes and cycloalkanes. Renew Sustain Energy Rev 72:673–722
Gao J, Tang LG (1996) Cellulose science. Science Press, Beijing
Hao OJ, Kim H, Chiang PC (2000) Decolorization of wastewater. Crit Rev Environ Sci Technol 30:449–505
He ZW, Yang J, Lu QF, Lin Q (2013) Effect of structure on the electrochemical performance of nitrogen- and oxygen-containing carbon micro/nanospheres prepared from lignin-based composites. ACS Sustain Chem Eng 1:334–340
Iyovo GD, Du G, Chen J (2010) Sustainable bioenergy bioprocessing: biomethane production, digestate as biofertilizer and as supplemental feed in algae cultivation to promote algae biofuel commercialization. J Microb Biochem Technol 2:100–106
Jiang TD (2001) Lignin. Chemical Industry Press, Beijing
Jin L, Sellers T, Schutz TP, Nicholas NN (1990) Utilization of lignin modified by brown-rot fungi. Holzforschung 44:132–138
Jinhuo D, Antonio FP, Kei S (2016) Recent developments in chemical degradation of lignin: catalytic oxidation and ionic liquids. Tetrahedron Lett 57:4945–4951
Joffres B, Lorentz C, Vidalie M, Laurenti D, Quoineaud AA, Charon N, Daudin A, Quignard A, Geantet C (2014) Catalytic hydro conversion of a wheat straw soda lignin: characterization of the products and the lignin residue. Appl Catal B 145:167–176
Kalogo Y, Habibi S, MacLean HL, Joshi SV (2007) Environmental implications of municipal solid waste-derived ethanol. Environ Sci Technol 41:35–41

Kalyani DC, Patil PS, Jadhav JP, Govindwar SP (2008) Biodegradation of reactive textile dye Red BL1 by an isolated bacterium *Pseudomonas sp*. SUK1. Bioresource Technol 99:4635–4641

Kamble SV, Bhattacharyulu YC (2015) Selective separation of biomass from black liquor waste by inorganic and organic acids. Int J Adv Res 3:684–692

Kang SM, Li XL, Fan J, Chang J (2011) Classified separation of lignin hydrothermal liquefied products. Ind Eng Chem Res 50:11288–11296

Keharia H, Madamwar D (2003) Bioremediation concepts for treatment of dye containing wastewater: a review. Indian J Exp Biol 41:1068–1075

Kirk K, Cullen D (1998) Enzymology and molecular genetics of wood degradation by white rot fungi. In: Young RA, Akhtar M (eds) Environmental friendly technologies for pulp and paper industry. Wiley, New York, pp 273–307

Kirk TK, Farrell RL (1987) Enzymatic "combustion": The MICROBIAL degradation of lignin. Annu Rev Microbiol 41:465–505

Kosbar LL, Gelorme J, Japp RM, Fotorny WT (2001) Introducing biobased materials into the electronics industry. J Ind Ecol 4:93–98

Kouisni L, Holt-Hindle P, Maki K, Paleologou M (2012) The Lignoforce system: a new process for the production of high quality lignin from black liquor. J Sci Technol For Prod 2:6–10

Lamar RT, Dietrich DM (1992) Use of lignin-degrading fungi in the disposal of pentachlorophenol-treated wood. J Ind Microbiol 9:181–191

Lange H, Decina S, Crestini C (2013) Oxidative upgrade of lignin—recent routes. Eur Polym J 49:1151–1173

Lee SH, Doherty TV, Linhardt RJ, Dordick JS (2009) Ionic liquid-meditated selective extraction of lignin from wood leading to enhanced enzymatic cellulose hydrolysis. Biotechnol Bioeng 102:1368–1376

Licsko I (1993) Dissolved organics removal by solid-liquid phase separation (adsorption and coagulation). Water Sci Technol 27:245–248

Lora JH, Glasser WG (2002) Recent industrial applications of lignin: a sustainable alternative to nonrenewable materials. J Polym Environ 10:39–48

Loutfi H, Blackwell B, Uloth V (1991) Lignin recovery from Kraft black liquor: preliminary process design. Tappi 74:203–210

Martínez AT, Camarero S, Guillén F, Gutiérrez A, Munoz C, Varela E (1994) Progress in biopulping of non-woody materials: chemical, enzymatic and ultrastructural aspects of wheat-straw delignification with ligninolytic fungi from the genus Pleurotus. FEMS Microbiol Rev 13:265–274

Messner K, Srebotnik E (1994) Biopulping: an overview of developments in an environmentally safe paper-making technology. FEMS Microbiol Rev 13:351–362

Murugesan K (2003) Bioremediation of paper and pulp mill effluents. Indian J Exp Biol 441:1239–1248

Nawaz A, Ahmed Z, Shahbaz A, Khan Z, Javed M (2014) Coagulation–flocculation for lignin removal from wastewater—a review. Water Sci Technol 69:1589–1597

Norgren M, Edlund H (2014) Lignin: recent advances and emerging applications. Curr Opin Colloid Interface Sci 19:409–416

Raj A, Reddy MMK, Chandra R, Purohit HJ, Kapley A (2007) Biodegradation of kraft-lignin by *Bacillus* sp. Isolated from sludge of pulp and paper mill. Biodegradation 18:783–792

Ramachandra M, Crawford DL, Hertel G (1988) Characterization of an extracellular lignin peroxidase of the lignocellulolytic actinomycete *Streptomyces viridosporus*. Appl Environ Microbiol 54:3057–3063

Ramosa WDlS, Poznyaka T, Chairezb I, Córdova RI (2009) Remediation of lignin and its derivatives from pulp and paper industry wastewater by the combination of chemical precipitation and ozonation. J Hazard Mater 169:428–434

Ramsay JA, Nguyen T (2002) Decoloration of textile dyes by *Trametes versicolor* and its effect on dye toxicity. Biotech Lett 24:1757–1761

Ravi S, Lovjeet S, Prasenjit M, Shri C (2013) Removal of lignin from wastewater through electro coagulation. World J Environ Eng 1:16–20

Richter AP, Brown JS, Bharti B, Wang A, Gangwal S, Houck K, Cohen Hubal EA, Paunov VN, Stoyanov SD, Velev OD (2015) An environmentally benign antimicrobial nanoparticle based on a silver-infused lignin core. Nanotechnology 10:817–823

Rodriguez A, Perestelo F, Carnicero A, Regalado V, Perez R, De la Fuente G, Falcon MA (1996) Degradation of natural lignins and lignocellulosic substrates by soil-inhabiting fungi imperfecti. FEMS Microbiol Ecol 21:213–219

Saake B, Lehnen R (2000) Lignin. Ullmanns encyclopedia of industrial chemistry. Wiley. KGaA

Sharma R, Chandra S, Singh A, Singh K (2014) Degradation of pulp and paper mill effluents. IIOAB J 5:6–12

Simionescu CI, Rusan V, Macoveanu MM, Cazacu G, Lipsa R, Vasile C, Stoleriu A, Ioanid A (1993) Lignin/epoxy composites. Compos Sci Technol 48:317–323

Singhal A, Thakur IS (2009) Decolourization and detoxification of pulp and paper mill effluent by Emericella nidulans var. nidulans. J Hazard Mater 171:619–625

Sjostrom E (1993) Wood chemistry: fundamentals and applications. Academic Press, San Diego

Stewart D (2008) Lignin as a base material for materials applications: chemistry, application and economics. Ind Crops Prod 27:202–207

Tiku DK, Kumar A, Chaturvedi R, Makhijani SD, Manoharan A, Kumar R (2010) Holistic bioremediation of pulp mill effluents using autochthonous bacteria. Int Biodeterior Biodegrad 64:173–183

Tuomela M, Vikman M, Hatakka A, Itavaara M (2000) Biodegradation of lignin in a compost environment: a review. Biores Technol 72:169–183

Tyagi S, Kumar V, Singh J, Teotia P, Bisht S, Sharma S (2014) Bioremediation of pulp and paper mill effluent by dominant aboriginal microbes and their consortium. Int J Environ Res 8:561–568

Ugurlu M, Gurses A, Dogarc C, Yalcın M (2008) The removal of lignin and phenol from paper mill effluents by electrocoagulation. J Environ Manage 87:420–428

VicuTaa R (1988) Bacterial degradation of lignin. Enzyme Microb Technol 10:645–655

Vijay Kumar T, Manju Kumari T (2015) Recent advances in green hydrogels from lignin: a review. Int J Biol Macromolec 72:834–847

Vijay Kumar T, Manju Kumari T, Prasanth R, Michael RK (2014) Progress in green polymer composites from lignin for multifunctional applications: a review. ACS Sustain Chem Eng 2:1072–1092

Vishtal AG, Kraslawski A (2011) Challenges in Industrial applications of technical lignins. BioResources 6:3547–3568

Wang HJ, Zhao Y, Wang C, Fu Y, Guo QX (2009) Theoretical study on the pyrolysis process of lignin dimer model compounds. Acta Chim Sinica 67:893–900

Wang Y, Dai L, Fan L, Shan S, Liu Y, Ruan R (2016) Review of microwave-assisted lignin conversion for renewable fuels and chemicals. J Anal Appl Pyrol

Zakzeski J, Bruijnincx PC, Jongerius AL, Weckhuysen BM (2010) The catalytic valorization of lignin for the production of renewable chemicals. Chem Rev 110:3552–3599

Zimmermann W, Broda P (1989) Utilization of lignocellulose from barley straw by *actinomycetes*. Appl Microbiol Biotechnol 30:103–109

Chapter 6
Role of Nanofibers in Bioremediation

Sekar Aiswarya Devi, Muthukumar Harshiny and Manickam Matheswaran

Abstract Scarcity of pure water is a threatening issue worldwide; water is an essential need for human survival and all activities on earth. Effluent water from industries containing recalcitrant pollutants causes dangerous impacts to the environment and human health. In the current epoch, bioremediation is an alternative technology for decontamination of water systems by use of specific microorganisms and it can provide green, efficient, cost-effective, and sustainable remediation of water contaminants. Immobilized nanofibers possess enhanced catalytic activity, high stability, and very good reusability of novel nano-biocomposites which has remarkable potential for the treatment of water and wastewater. It also plays a major role in safe preservation of bioremediating bacteria for potential wastewater treatment applications. Nanofibers have become a popular carrier matrix for immobilization of specific microorganisms. Simple, versatile, and cost-effective properties of nanofibers made them a promising tool for microbial integration which enhances the bioremediation by efficient removal of contaminants such as dyes and heavy metals from wastewater. This chapter describes the immobilization of specific bacteria on electrospinning nanofibers and its application in bioremediation process.

Keywords Nanofibers · Bioremediation · Contaminants · Immobilization Nano-biocomposites

6.1 Introduction

Water is one of the most plentiful natural resources on earth; however, only about 1% is available for human consumption. It is estimated that over 1.1 billion people lacking supply of adequate drinking water and may affect up to 4 billion people by 2050 (Anjum et al. 2016; Suja et al. 2017). Currently, water demand has become a

S. Aiswarya Devi · M. Harshiny · M. Matheswaran (✉)
Department of Chemical Engineering, National Institute of Technology, Tiruchirappalli 620015, India
e-mail: math.chem95@gmail.com

S. J. Varjani et al. (eds.), *Bioremediation: Applications for Environmental Protection and Management*, Energy, Environment, and Sustainability,
https://doi.org/10.1007/978-981-10-7485-1_6

critical issue worldwide owing to rapid industrialization, increase in population, depletion of natural water resources, and changes in climate. The foremost cause for water demand is contamination of water resources by continuous discharge of various toxic pollutants such as recalcitrant hydrocarbons, pesticides (DDT, Triazine and atrazine), heavy metals (arsenic, mercury, and chromium), pharmaceuticals drugs, dyes (Tan et al. 2015), microbial pathogens, and parasites (Rawat et al. 2013; Plakas et al. 2012; Abigail and Das 2012). Hence, the significant consideration has been drawn to develop an effective treatment technology for removing the persisting contaminants from the aqueous systems. Various existing technologies such as physicochemical methods, membrane filtration, ion exchange, ozonation, and electrochemical destruction require high-energy, incomplete pollutant removal, high treatment cost, and not environmental friendly (Ferroudj et al. 2013). Other conventional water treatment processes were not able to address adequately the removal of a wide spectrum of toxic chemicals from the ecosystem (Thavasi et al. 2008; Anjum et al. 2016).

In this perspective, there is a genuine requirement for more powerful method to decontaminate water and wastewater for safe pursue. Eco-friendly, great proficiency, reduction of sludge in chemical and biological system, metals specific, no additional nutrient supplies, renewal of biosorbent, probability of recovering metal, and cost-effective nature of bioremediation make them suitable for effective removal of contaminants (Rizwan et al. 2014). The method of productive utilization of living organisms such as bacteria, virus, fungi, algae, and some plants to degrade, detoxify, transform, immobilize, or stabilize toxic environmental contaminants into an innocuous state or to levels below the standard acceptable limits is known as "Bioremediation." *Escherichia, Citrobacteria, Klebsiella, Rhodococcus, Staphylococcus, Alcaligenes, Bacillus, and Pseudomonas* are the organisms that are commonly used in bioremediation (Kumar and Gopinath 2016). These microorganisms can remediate water pollutants either by biosorption or bioaccumulation. Although dead cell biomasses can only be used for biosorption, living cells can possess both bioaccumulation and biosorption (Sarioglu et al. 2017a). Maintaining high biomass of bacterial population is the important key factor for bioremediation. Bacterial cells must be immobilized to increase the survival and retention of the bioremediation agents in the contaminated sites (Bayat et al. 2015). It also improves microorganism reusability, lower space, and growth medium and higher resistance to unfavorable environmental extremes (Sarioglu et al. 2017b; Seow et al. 2016).

Hence, bacterial cells can be immobilized on polymeric matrix to make them more unaffected to severe environmental conditions, such as excesses of salinity, temperature, pH, and metal toxicity (Sarioglu et al. 2013). Electrospun polymer nanofibers are promising platform for immobilization systems. They can be used as the popular carrier matrix for encapsulation of nanoparticles, enzymes, proteins, and whole cells (Zussman 2011). A simple, versatile, and cost-effective electrospinning technology fabricates nanofibers with unique properties like large surface area, surface-to-volume, porosity, and diameters lower than 100 nm (Lu et al. 2016). These biohybrid materials possess great attention due to safe preservation and enhanced activity of bioremediating bacteria for water and wastewater treatment

applications. Nanotechnology development has enhanced the scope for fabricating microorganism immobilized nanofibers. Therefore, this chapter summarizes the currently available approaches for the fabrication of nanofibers, biohybrid nanofibers and discusses their application in bioremediation.

6.2 Nanofibers

Fibers with diameters less than 50–500 nm are defined as nanofibers (Bharat et al. 2012). When the diameter reduces to nanometer range, several interesting properties appear such as high porosity, surface area-to-volume ratio, aspect ratio (length to diameter), low density, unique physicochemical properties, possess flexible chemical/physical surface modifications (Panthi et al. 2015), and greater mechanical performance than conventional materials. These outstanding properties made polymer nanofibers promising material for extensive environmental uses (Huang et al. 2003; Malwal and Gopinath 2016; Ramakrishna et al. 2006). At present, nanofibers are synthesized by three techniques such as self-assembly, phase separation, and electrospinning. Among these, electrospinning is the most used technique (Vasita and Katti 2006) and it yields flexible nanofibers at ease of production with low cost and better control over fiber arrangement and diameter (Pal et al. 2017).

6.3 Electrospinning

Electrospinning is a versatile technology for the fabricating polymeric materials into nanofibers with diameters of nanometer range (Pal et al. 2017; Zussman 2011). The electrospinning technique consists of three basic units: a power supply, a collector, and syringe pump with a needle were shown in Fig. 6.1. In this method, steady high voltage is provided to the tip of the needle in the syringe containing polymer solution. Repulsion between charges in the polymer solution and surface tension of the polymer solution was overcome by attraction toward oppositely charged collector and appearance of Taylor cone on the tip of the needle (Panthi et al. 2015).

From the Taylor cone, a charged jet of fluid is ejected out with high voltage. The released jet undergoes stretching followed by solvent loss resulting in randomly oriented nanosized fibers.

System parameters and process parameters are needed to be maintained properly to obtain nanofibers with good quality. Molecular weight, viscosity, conductivity, surface tension, and dielectric constant are the system parameters; electric potential, flow rate, and working distance are the process parameters. High specific surface area, porosity, and easily tunable surface functionalities make electrospun nanofibrous membrane a promising material for the adsorption of organic impurities and toxic metal ions from aqueous solution (Suja et al. 2017; Huang et al. 2012)

Polymer solution shows a major role in fabrication of nanofibers. The quality and morphology of nanofibers depend on the type of polymer solution,

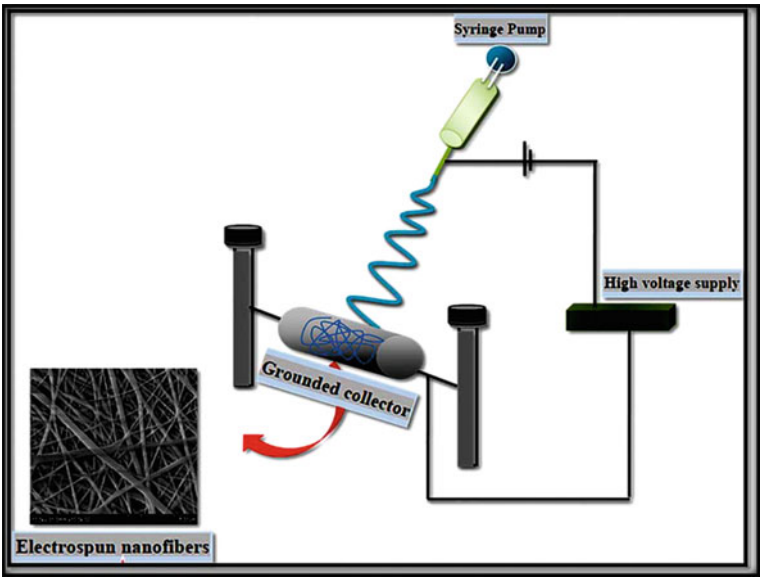


Fig. 6.1 Electrospinning setup and the SEM morphology of the nanofiber (Suja et al. 2017)

electrospinning parameters, and conditions. For electrospinning of nanofibers, different types of polymers have been used; natural polymers such as hyaluronic acid, collagen, gelatin, chitosan, and silk fibroin (Vasita and Katti 2006). Synthetic polymers such as poly (vinyl alcohol) (PVA), PEO poly(ethylene oxide), poly (acrylic acid) (PAA), polyurethane (PUR), and fluoro polymers were used in nanofiber fabrication. Polyvinylidene fluoride, polysulfone, polyacrylonitrile, polyvinyl alcohol, cellulose acetate, and polyurethane are most commonly used polymers in water treatment system (Botes and Eugene 2010; Lim 2017).

Live cell membranes prepared through nanofibers containing biological matters such as enzymes, bacteria, and viruses were nonwovens composed of polymer cleverly without total loss of biological functionality are called as biohybrid nanofibers (Lee and Belcher 2004; Agarwal et al. 2009). These biohybrid nanofibers possess safe preservation and enhanced activity of bioremediating bacteria for various bioremedial applications.

6.4 Biohybrid Nanofibers

Biohybrid nanofibers are composite materials containing entire microbial cells encapsulated in polymer solution. Ghasemzadeh et al. (2014) have developed biohybrid fibers by simultaneously electrospinning of living organisms such as *Micrococcus luteus* and poly (ethylene oxide) (Ghasemzadeh et al. 2014). Liu et al.

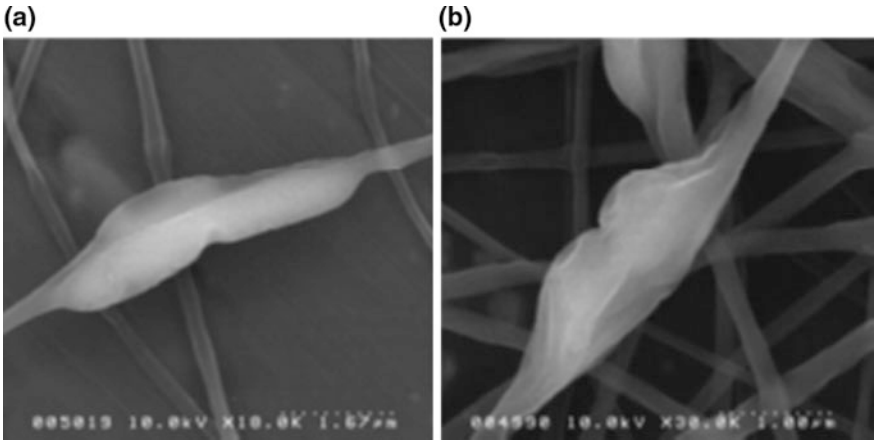


Fig. 6.2 Electrospun PEO nanofibers containing the bacteria **a** *E. coli* and **b** *M. luteus.* (Agarwal et al. 2009)

(2009) described the use of poly (ethylene oxide) poly (propylene oxide)—poly (ethylene oxide) triblock polymers to entrap *Pseudomonas, Zymomonas, and Escherichia* via electrospinning. Biohybrid materials are produced through immobilization techniques such as physical entrapment, encapsulation, and covalent binding are commonly used to immobilize bacteria (Liu et al. 2009; Lopez et al. 2009; Homaeigohar and Elbahri 2014). Figure 6.2 shows the safe encapsulation of bacteria in PEO nanofiber. Bacteria have been encapsulated in matrices varied from silica gels to alginate microcapsules or grafted on substrates varied from glass beads to polymer microparticles, depending on the bacteria properties, process parameters, and ultimate uses (Agarwal et al. 2009; Xie et al. 2016). For the purpose of using bacteria safely, bacteria immobilization has been explored aiming at limiting the active area of bacteria.

6.5 Immobilization

The physical process of restricting the free movement of microbial cells in confined region and protecting them from surrounding environment to enhance their catalytic property for continuous and repeated use is known as immobilization. (Bayat et al. 2015; Martins et al. 2013). There are different types of immobilization such as entrapment, adsorption, covalent coupling/cross-linking, and capture behind semipermeable membrane or encapsulation. Hence, these types of immobilization can be congregated as "passive" (naturally microorganisms attached to surfaces natural or synthetic, and grow on them) and "active" (flocculant agents, chemical attachment, and gel encapsulation) (Martins et al. 2013).

6.6 Types of Microorganism Immobilizations

6.6.1 Adsorption

Adsorption is the process of physical interaction amid the surface of water-insoluble carriers and microorganism (Xie et al. 2016). In Figs. 6.3 and 6.4, adherence of cell due to adsorption process was shown. Adsorption is economically strategic due to free, quick, and simple method without chemicals. Weak forces like hydrogen bonds, ionic bonds, hydrophobic bonds, and van der Waals forces were used in the interaction between microorganism and the surface of the matrix (Ahmad and Sardar 2015). Leakage from immobilized cell matrix was the disadvantage of

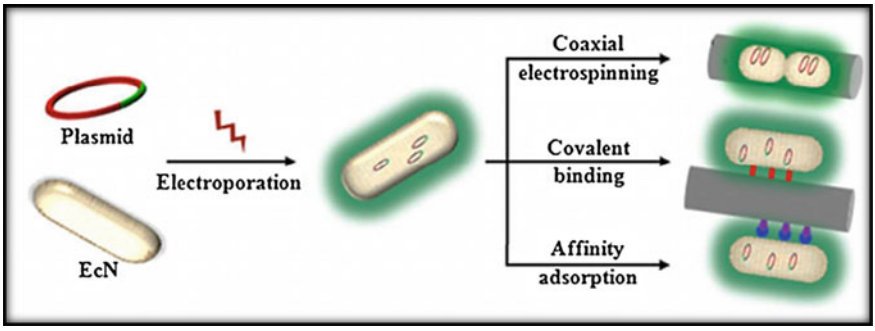


Fig. 6.3 Types of immobilizations (Xie et al. 2016)

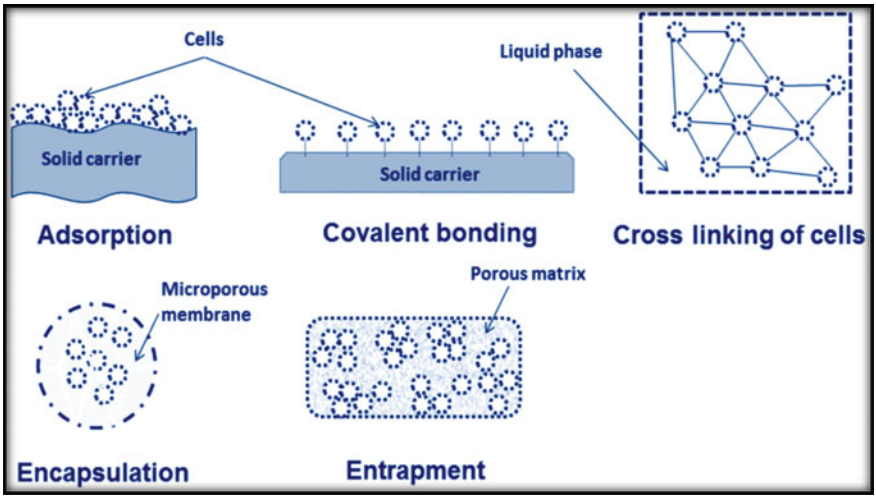


Fig. 6.4 Methods of immobilizing microbial cells (Das and Adholeya 2015)

adsorption technique that is due to the weak interactions. Also, reproducibility is low due to uncontrollable loading (Bayat et al. 2015).

6.6.2 Covalent Binding/Cross-Linking

In the presence of binding agent, the formation of covalent bond in amid microbes and activated inorganic carrier and cell is known as covalent binding or cross-linking (Martins et al. 2013; Ahmad and Sardar 2015). FDMA fibrous hydrogel material was described by cross-linking method of immobilization of microbes through electrospinning (Liu et al. 2009). Mostly all enzyme immobilization were covalent method. However, because of toxic coupling agents, reduced cell viability or enzyme activity, it is hardly applied in whole cell immobilization. Figures 6.3 and 6.4 demonstrate the covalent binding (Bayat et al. 2015; Xie et al. 2016).

6.6.3 Entrapment

Capturing of particles or cells surrounded by a support matrix or within a hollow fiber is known as entrapment, and it is an irreversible method. Cell leakage from the polymers into surrounding medium during mass transfer of nutrients and metabolites was prevented by defensive around the immobilized microbes as shown in Fig. 6.4. Entrapment is most extensively applied in cell immobilization (Bayat et al. 2015). The polymeric matrices used are agar, alginate, cellulose, and its derivatives are collagen, gelatin, epoxy resin, photo-cross-linkable resins, polyacrylamide, polyester, polystyrene, and polyurethane (Martins et al. 2013). Fast, inexpensive, and mild conditions are the merits of entrapment required for the reaction process.

Charges of immobilization, damage of support material, deactivation during immobilization, and biocatalysts small loading capacity were the major demerits of this technique (Bayat et al. 2015; Ahmad and Sardar 2015).

6.6.4 Encapsulation

In this method, biological components are enveloped inside several forms of spherical semipermeable membranes with a selectively controlled permeability were shown in Figs. 6.3 and 6.4 (Bayat et al. 2015). Encapsulation is also an irreversible process similar to entrapment. The significant factor in this method is ratio of membrane pore size to size of core material. One of the main advantages of microencapsulation is inadequate accessibility to the microcapsule inside; due to this biocatalyst got protected from the extreme conditions (Martins et al. 2013). It

prevents biocatalyst leakage and increases the efficiency similar to other immobilization process (Xie et al. 2016; Ahmad and Sardar 2015). Likewise, Salalha et al. have successfully encapsulated viruses and bacteria by electrospinning (Salalha et al. 2006). Recently, Sarioglu et al. have developed functional biocomposite materials that were produced by encapsulation of a MB remediating *Pseudomonas aeruginosa* strain within electrospun PVA and PEO nanofibrous webs (Sarioglu et al. 2017b).

Hence, from the above literature, it is confirmed that for the immobilization of microorganisms to nanofiber materials methods like covalent coupling, cross-linking, physical entrapment, and the natural bacterial adhesion can be used. Among these methods, natural adhesion is the most strategic as it facilitates the formation of biofilms for surface attachment, biochemical activity, and utmost cell viability (Sarioglu et al. 2013, 2015).

6.7 Fabrication of Biohybrid Nanofibers

Active bacterial cells were encapsulated within polymer fibers through electrospun (single polymer type–monolithic fibers) and co-electrospun fabricating process (dual-polymer fibers core–shell) (Zussman 2011). Single polymer requires a water-soluble polymer (PEO, PVA, silica). Core–shell allows the creation of hydrophilic core (PVA/PVP) and a hydrophobic porous shell (PEG/PVDF) in one step (Letnik et al. 2015).

6.7.1 Monolithic Nanofibers

Fibers fabricated from a single droplet are known as monolithic nanofibers. Salaha et al. have illustrated the bacterial cell (*Escherichia coli and Staphylococcus albus*) and bacterial viruses (T7, T4, λ) encapsulation in nanofibers fabricated via electrospinning with polymer polyvinyl alcohol (PVA) (Salaha et al. 2006). Similarly, Gensheimer et al. reported the significant unevenness in bacterial viability between encapsulated live *Micrococcus luteus* and *E. coli* cells produced by electrospun polyethylene oxide (PEO) nanofibers (Gensheimer et al. 2007). Further Liu et al. studied the encapsulation of several microorganisms: *Pseudomonas fluorescens*, *Zymomonas mobilis*, and *E. coli* in Pluronic F127 dimethacrylate (FDMA) fibrous hydrogel combined with PEO via electrospinning (Liu et al. 2009).

Above results have concluded that monolithic nanofibers have its disadvantage due to the liquid environment required for the application of such composites with inevitable breakdown of polymer structure, and to overcome this problem, cross-linking of organic solvents such as acetone or glutaraldehyde was done. Even though some microorganisms could survive these processes in a fair percentage of viable cells, their lifespan and activity were limited (Letnik et al. 2015).

6.7.2 *Core–Shell Nanofibers*

Core–sheath fibers were fabricated by coaxial electrospinning/co-electrospinning of dual dissimilar solutions at the same time to entrap bacteria inside fiber cores were shown in Fig. 6.5 (Xie et al. 2016).

In this method, the microorganisms are dispersed in the inner polymer which is immobilized within a solid outer matrix was shown in Fig. 6.5.

Shell is the solid matrix surrounding the "fluid" section, i.e., the core that provides chemical stability, mechanical strength, and aids in transportation Fig. 6.6a and b shows the shell and core of concurrently fabricated core–shell nanofibers. Miscibility of solutions, ratio of flow rate, viscoelastic relaxation time, viscosity, conductivity ratio, relative permittivity, electrical strength interfacial tension, and the projection of the core outside the shell nozzle affect the stability of the co-electrospinning.

Ying Liu and coworkers have described the FDMA fibrous hydrogel material with encapsulated microbes via coaxial electrospinning (Liu et al. 2009). Similarly, degradation of atrazine was demonstrated by Ho-Wang Tong using *E. coli* encapsulated via novel coaxial electrospinning of reactive nanofibers with PVA and a silica/PVA composite shell (Tong et al. 2014). In another study, Klein et al. have demonstrated the encapsulation of *Pseudomonas sp. ADP* in core–shell nanofibers for atrazine degradation (Klein et al. 2009). Further, Xie et al. have reported the engineered bacteria that were entrapped inside core–sheath fibers by coaxial electrospinning (Xie et al. 2016). Ilya Letnik and his team have demonstrated the coaxial electrospinning of nanofibers with yeast cells for bioremediation and ethanol production (Letnik et al. 2015).

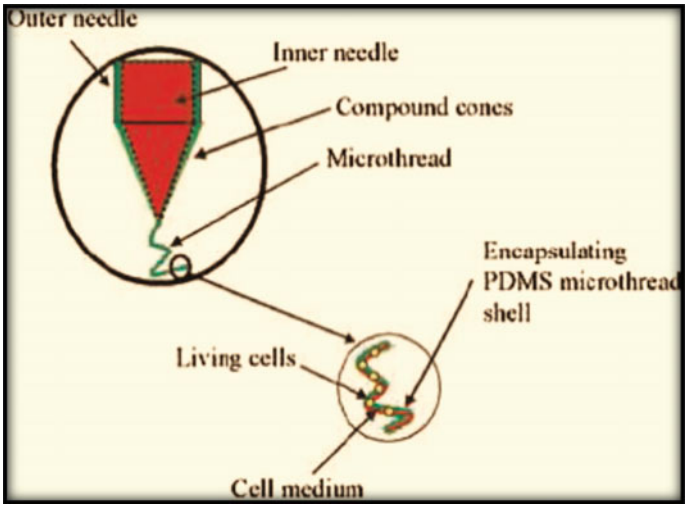


Fig. 6.5 Coaxial cell electrospinning (Zussman 2011)

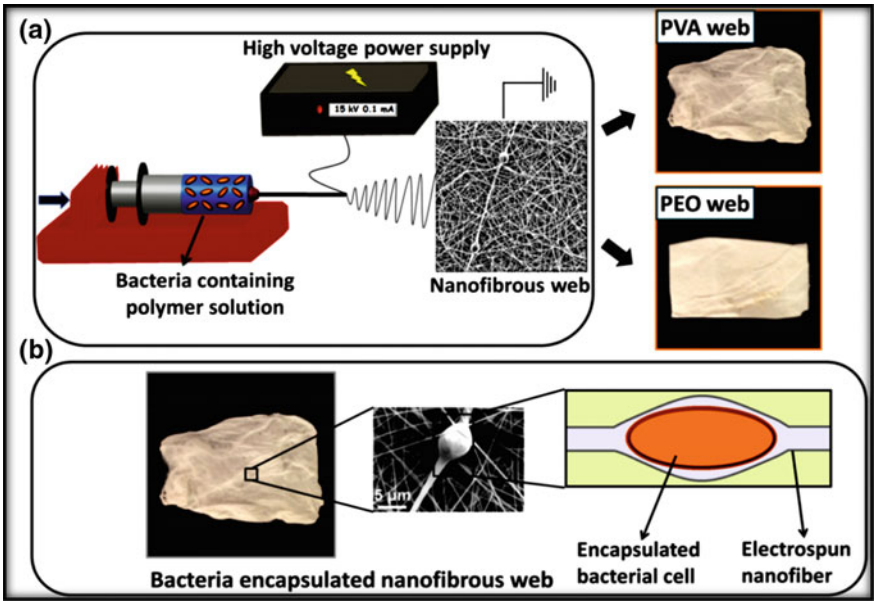


Fig. 6.6 **a** Schematic representation of electrospinning process for bacteria encapsulation and **b** representative images for bacteria encapsulated webs including a SEM micrograph (Sarioglu et al. 2017b)

These findings demonstrated that the co-electrospun biocomposite can be an impartially easy, promising platform, and harmless way to encapsulate cells within polymer nanofibers for water and wastewater treatments.

6.8 Biohybrid Nanofibers for Bioremedial Applications

As one of the safest nanomaterials, the long and highly porous nanofibers serve as a promising platform for numerous emerging environmental applications, in particular in liquid filtration and particulate separation for water treatment and environmental remediation, respectively (Kenry and Chwee 2017).

6.8.1 Nanofibers on Dye Removal

The distinctive properties such as lively loading sites, large surface area, and mass transfer resistance made nanofiber an ideal immobilization matrix for microorganism. At present, several studies have reported development of nanofibers encapsulated with microorganism for treatment of dye from wastewater (Cai et al.

2017). Reusability and decolorization property of electrospun nanofiber in methylene blue wastewater were reported by OyaaSan. Before 24 h, 95% of dye decolorization was attained by bacteria immobilized NFW and its reusability study showed 45% of dye decolorization efficiency after four cycles. From these results, OyaaSan has reported that bacteria immobilized CA-NFW can be repeatedly used for removal of dyes (OyaaSan 2014). In addition, San et al. have demonstrated that the decolorization capacity of microalgae/PSU-NFW for RB5 and RB221 dyes was even better than the free microalgae cells and it was much better than the pristine PSU-NFW. The decolorization increased during the first 14 days, the dye decolorization of RB5 for microalgae/PSU-NFW was 73.0 ± 0.3% within 14 days when compared to 68.8 ± 0.3% and 12.4 ± 0.3% for free microalgae cells and pristine PSU-NFW, respectively (San et al. 2015a). Again removal of reactive dyes from wastewater using microalgae immobilized nanofibrous web was studied by San et al. Further he compared the RB5 removal efficiency of pristine PSU-NFW after immersion of bacteria and PSU-NFW nano-biocomposite into the liquid media after 24 h incubation was 35.8 ± 0.4% and 99.7 ± 0.9% dyes, respectively (San et al. 2015b). Recently, Sarioglu et al. have studied remediation of methylene blue dye in water, the removal capacities of bacteria/PVA, and bacteria/PEO webs are very close to each other and both of them are higher than the free-bacteria sample. His study revealed that the bacteria encapsulated web samples can improve the MB removal by increasing the encapsulation with more capable bacterial cell viabilities (Mohamed et al. 2016; Sarioglu et al. 2017b).The above studies concluded that microorganism encapsulated nanofibers are suitable material than free microbial cells for removal of harmful dyes from aqueous system.

6.8.2 Nanofibers on Atrazine Removal

The developments in nanotechnology and microbiology have introduced a novel technique to immobilize active microbes of selected dominant microbial culture in nanofiber for effective remediation of pesticides. Vancov has demonstrated the atrazine degradation using alginate beads containing *Rhodococcus erythropolis* NI86/21 cells. His results kept breakthrough for suitable encapsulation of microorganism for herbicide removal (Vancov et al. 2007). Followed the above research, Sumana Siripattanakul and his coworkers have studied the atrazine degradation by immobilized pure and mixed cultures on PVA. The immobilized J14a and MC were shown approximately 50 and 40% of atrazine biodegradation within 120 h, respectively, at 3.5 mg/ml cell to matrix ratio (Siripattanakul et al. 2008). Lately, Shiri Klein studied the atrazine bioremediation *using Pseudomonas sp*. ADP cells encapsulated in electrospun microtubes. The encapsulated cells performed significant atrazine removal even under non-growth conditions bringing potential savings in process operation (Klein et al. 2012). Thus, the above reports

delivered biohybrid nanofiber are appropriate materials for removal of harmful atrazine from water system.

6.8.3 Nanofibers on Chromium Removal

Immobilization of active microbes has increased the stability, resistance of damage to the outside world. Hence, the removal of recalcitrance metal ions could be treated from organic wastewater. Pang et al. have studied the chromium tolerance of *P. aeruginosa using* MCNTs, PVA, and sodium alginate for excluding Cr (VI). About 50% of 80 mg/L Cr (VI) was reduced by the immobilized bacteria in 84 h, conversely deactivation of the free cells was occurred at this concentration. Hence, chromium tolerance capacity of the bacteria was increased by immobilization (Pang et al. 2011). In other study, Monica Rawat and his team reported the free and *P. xylanilyticus* MR12 is a Cr(VI)-tolerant bacteria bacterial cells immobilized in PVA–alginate to study the removal of Cr(VI). About 25 mg/L of Cr (VI) was completely removed by immobilized cells in 9 h, whereas free cells removed in 18 h (Rawat et al. 2013). Likewise, San and his coworkers have studied the Cr (VI) removal efficiency using bacteria/PSU-NFW and pristine PSU-NFW materials were 98.2 ± 0.6% and 32 ± 0.6%, respectively. Approximately at 60 min, Cr (VI) removal process started and at 24 h reached maximum yield. Further decolorization of RB5 was examined using bacteria/PSU-NFW and achieved 99.7 ± 0.9% of dye decolorization efficiency (San et al. 2015a). More recently, removal of Cr(VI) at three different initial concentrations (10, 15, and 25 mg/L) was studied using STB5/PS and STB5/PSU biocomposite webs that have shown efficient within 72 h. Sarioglu et al. proposed that biocomposites nanofiber webs can remediate Cr(VI) as effectively as freely floating cells Cr(VI) (Sarioglu et al. 2016). The above results revealed the importance of encapsulating microorganism in nanofibers and their advantages over free microbial cells in bioremediation.

6.8.4 Nanofibers on Nitrate and Ammonium Removal

Evidently, for the initial removal of nitrate in the liquid was triggered by the presence of biohybrid material. While the remaining nitrate in the liquid was utilized by algae in further stages with slower growth rate. Eroglu et al. studied the cross-linked chitosan nanofiber mat as a water-insoluble and non-toxic support for algal growth, and its nitrate removal rates were 32 ± 3%, and 87 ± 4%, for the "without" and "with" microalgae chitosan mats, respectively (Eroglu et al. 2012). In recent day study, electrospun nanofibrous webs immobilized with specific bacterial or algal strains for ammonium removal. Around 48 h complete removal of 100 mg

L^{-1} ammonium was accomplished by STB/CA (Sarioglu et al. 2013). Thus, the above researches elucidate microorganism encapsulated electrospun nanofibers as a promising material for nitrate removal in bioremediation process.

6.8.5 Nanofibers on Heavy Metal Removal

Zahabi and his team members have been studied the maximum removal efficiency of heavy metal using Nylon 66 nanofiber membranes functionalized with TMPTMS from aqueous solution using multistage filters with mechanism of the membrane is the blocking pores. The removal efficiencies were 93.0% for cadmium and for nickel 97.6% (Zahabi et al. 2016). Likewise in another study, removal of toxic heavy metals such as nickel, cadmium, copper, and chromium were reported using electrospun membranes (Anjum et al. 2016). Electrospun polyacrylonitrile nanofiber mats and carbon nanofibers are grown on iron used for heavy metal ion removal because of their tremendous potential as a heterogeneous adsorbent for removal of arsenic (V) in wastewater (Kumar and Gopinath 2016).

The studies related to microorganism impregnated nanofibers for heavy metal removal are less, whereas nanoparticles impregnated nanofibers and nanomembranes are abandoned.

6.9 Analysis of Advantages–Disadvantages

Large surface area along with nanoscale porosity makes electrospun nanofibrous webs capable for membranes and filters. Moreover, immobilization of microorganism in electrospun nanofibers brings several advantages over conventional fiber such as flexibility to design photobioreactors with maximum efficiency, improved catalytic activity, enhanced survival rates of immobilized microbes in harsh environments, less space occupancy, less quantity of growth medium, and easier handling makes electrospun nanofibers suitable for bioremediation.

However, technical issues need to be addressed for effective scale-up of nanofibers for real-time and industrial applications. Cost of immobilization matrix, difficulty in release rate, and contact of nanomaterials, sometimes decreased viability and lower growth rate of microbes due to insufficient nutrients and inadequate mechanical strength were the difficulties need to be addressed. Overcoming the drawbacks of the existing methodology will improve the use of nanofibers in bioremediation, biodegradation, biodeterioration, and biocatalysts.

6.10 Conclusion

Electrospun nanofibers are promising nano-biocomposite material for immobilization of microorganism since they can afford large surface area-to-volume ratios, pore, and surface functionalization and several active sites for contact or attachment. It was inferred from the above reports that increase in initial cell viability numbers increases the removal performances. Due to the lack of internal and external mass transfer resistance, better biodegradation percentage was obtained by immobilized microbial cells. Therefore, biohybrid materials are potentially an attractive, reusable, modest, highly robust, and suitable for water and wastewater treatment with storable and improvable properties. In this chapter, finally, we conclude that the incessant research and enhancement in nanofiber technology will become an effective and practical technology for wastewater treatment of contaminant removal in bioremediation process.

References

Abigail MEA, Das N (2012) Microbial degradation of atrazine, commonly used herbicide. Int J Adv Biol Res 2(1):16–23

Agarwal S, Greiner A, Wendorff JH (2009) Electrospinning of manmade and biopolymer nanofibers-progress in techniques, materials, and applications. Adv Funct Mater 19(18):2863–2879

Ahmad R, Sardar M (2015) Enzyme immobilization: an overview on nanoparticles as immobilization matrix. Biochem Anal Biochem 4(2). https://doi.org/10.4172/2161-1009.1000178

Anjum M, Miandad R, Waqas M et al (2016) Remediation of wastewater using various nano-materials. Arabian J Chem. https://doi.org/10.1016/j.arabjc.2016.10.004

Bayat Z, Hassanshahian M, Cappell S (2015) Immobilization of microbes for bioremediation of crude oil polluted environments: a mini review. Open Microbiol J 9:48–54

Bharat Kumar SK, Lakshmanarao P, Anjaneyulu V et al (2012) Nanofibers in pharmaceuticals—a review. Am J Pharm Tech Res 2(6) ISSN: 2249–3387

Botes M, Eugene Cloete T (2010) The potential of nanofibers and nanobiocides in water purification. Crit Rev Microbiol 36(1):68–81

Cai Z, Sun Y, Liu W et al (2017) An overview of nanomaterials applied for removing dyes from wastewater. Environ Sci Pollut Res Int 1–23. https://doi.org/10.1007/S11356-017-9003-8

Das M, Adholeya A (2015) Potential Uses of immobilized bacteria, fungi, algae, and their aggregates for treatment of organic and inorganic pollutants in wastewater. In: Water challenges and solutions on a global scale. ACS, pp 319–337

Eroglu E, Agarwal V, Bradshaw M et al (2012) Nitrate removal from liquid effluents using microalgae immobilized on chitosan nanofiber mats. Green Chem 14(10):2682–2685

Ferroudj N, Nzimoto J, Davidson A et al (2013) Maghemite nanoparticles and maghemite/silica nanocomposite microspheres as magnetic Fenton catalysts for the removal of water pollutants. Appl Catal B 136:9–18

Gensheimer M, Becker M, Brandis-Heep A et al (2007) Novel biohybrid materials by electrospinning: nanofibers of poly (ethylene oxide) and living bacteria. Adv Mater 19 (18):2480–2482

Ghasemzadeh G, Momenpour M, Omidi F et al (2014) Applications of nanomaterials in water treatment and environmental remediation. Front Environ Sci Eng 8(4):471–482
Homaeigohar S, Elbahri M (2014) Nanocomposite electrospun nanofiber membranes for environmental remediation. Materials 7(2):1017–1045
Huang Y, Ma H, Wang S (2012) Efficient catalytic reduction of hexavalent chromium using palladium nanoparticle-immobilized electrospun polymer nanofibers. ACS Appl Mater Interfaces 4(6):3054–3061
Huang ZM, Zhang YZ, Kotaki M et al (2003) A review on polymer nanofibers by electrospinning and their applications in nanocomposites. Compos Sci Technol 63(15):2223–2253
Kenry, Chwee TL (2017) Nanofiber technology: current status and emerging developments. https://doi.org/10.1016/j.progpolymsci.2017.03.002
Klein S, Kuhn J, Avrahami R (2009) Encapsulation of bacterial cells in electrospun microtubes. Biomacromol 10(7):1751–1756
Klein S, Avrahami R, Zussman E et al (2012) Encapsulation of pseudomonas sp. ADP cells in electrospun microtubes for atrazine bioremediation. J Ind Microbiol Biotechnol 39(11):1605–1613
Kumar SR, Gopinath P (2016) Chapter 2 nano-bioremediation applications of nanotechnology for bioremediation. Remediation of heavy metals in the environment. CRC Press, pp 27–48
Lee SW, Belcher AM (2004) Virus-based fabrication of micro-and nanofibers using electrospinning. Nano Lett 4(3):387–390
Letnik I, Avrahami R, Rokem JS (2015) Living composites of electrospun yeast cells for bioremediation and ethanol production. Biomacromol 16(10):3322–3328
Lim CT (2017) Beyond the current state of the syntheses and applications of nanofiber technology. Prog Polym Sci. https://doi.org/10.1016/j.progpolymsci.2017.03.002
Liu Y, Rafailovich, MH, Malal R et al (2009) Engineering of bio-hybrid materials by electrospinning polymer-microbe fibers. Proc Natl Acad Sci 106(34):14201–14206
Lopez-Rubio A, Sanchez E, Sanz Y et al (2009) Encapsulation of living bifidobacteria in ultrathin PVOH electrospun fibers. Biomacromol 10(10):2823–2829
Lu H, Wang J, Stoller M et al (2016) An overview of nanomaterials for water and wastewater treatment. Adv Mater Sci Eng. https://doi.org/10.1155/2016/4964828
Malwal D, Gopinath P (2016) Fabrication and applications of ceramic nanofibers in water remediation: a review. Crit Rev Environ Sci Technol 46(5):500–534
Martins SCS, Martins CM, Fiúza LMCG et al (2013) Immobilization of microbial cells: a promising tool for treatment of toxic pollutants in industrial wastewater. Afr J Biotechnol 12 (28):4412–4418
Mohamed A, El-Sayed R, Osman TA et al (2016) Composite nanofibers for highly efficient photocatalytic degradation of organic dyes from contaminated water. Environ Res 145:18–25
OyaaSan N (2014) Reusable bacteria immobilized electrospun nanofibrous webs for decolorization of methylene blue dye in wastewater treatment. RSC Adv 4(61):32249–32255
Pal DB, Singh P, Mishra PK (2017) Composite ceria nanofiber with different copper loading using electrospinning method. J Alloy Compd 694:10–16
Pang Y, Zeng GM, Tang L et al (2011) Cr (VI) reduction by Pseudomonas aeruginosa immobilized in a polyvinyl alcohol/sodium alginate matrix containing multi-walled carbon nanotubes. Bioresour Technol 102(22):10733–10736
Panthi G, Park M, Kim HY et al (2015) Electrospun polymeric nanofibers encapsulated with nanostructured materials and their applications: a review. J Ind Eng Chem 24:1–13
Plakas KV, Karabelas AJ (2012) Removal of pesticides from water by NF and RO membranes—a review. Desalination 287:255–265
Ramakrishna S, Fujihara K, Teo WE et al (2006) Electrospun nanofibers: solving global issues. Mater Today 9(3):40–50
Rawat M, Rawat AP, Giri K et al (2013) Cr (VI) sorption by free and immobilised chromate-reducing bacterial cells in PVA–alginate matrix: equilibrium isotherms and kinetic studies. Environ Sci Pollut Res 20(8):5198–5211

Rizwan M, Singh M, Mitra CK et al (2014) Ecofriendly application of nanomaterials: nanobioremediation. J Nanopart Res. https://doi.org/10.1155/2014/431787

Salalha W, Kuhn J, Dror Y et al (2006) Encapsulation of bacteria and viruses in electrospun nanofibres. Nanotechnology 17(18):4675–4681

San Keskin NO, Celebioglu A, Sarioglu OF et al (2015a) Removal of a reactive dye and hexavalent chromium by a reusable bacteria attached electrospun nanofibrous web. RSC Adv 5 (106):86867–86874

San Keskin NO, Celebioglu A, Uyar T et al (2015b) Microalgae immobilized by nanofibrous web for removal of reactive dyes from wastewater. Ind Eng Chem Res 54(21):5802–5809

Sarioglu OF, Yasa O, Celebioglu A et al (2013) Efficient ammonium removal from aquatic environments by *Acinetobacter calcoaceticus* STB1 immobilized on an electrospun cellulose acetate nanofibrous web. Green Chem 15(9):2566–2572

Sarioglu OF, Celebioglu A, Tekinay T et al (2015) Evaluation of contact time and fiber morphology on bacterial immobilization for development of novel surfactant degrading nanofibrous webs. RSC Adv 5(124):102750–102758

Sarioglu OF, Celebioglu A, Tekinay T et al (2016) Bacteria-immobilized electrospun fibrous polymeric webs for hexavalent chromium remediation in water. Int J Environ Sci Technol 13 (8):2057–2066

Sarioglu OF, Celebioglu A, Tekinay T et al (2017a) Evaluation of fiber diameter and morphology differences for electrospun fibers on bacterial immobilization and bioremediation performance. Int Biodeterior Biodegrad 120:66–70

Sarioglu OF, San Keskin NO, Celebioglu A et al (2017b) Bacteria encapsulated electrospun nanofibrous webs for remediation of methylene blue dye in water. Colloids Surf B Biointerface 152:245–251

Seow TW, Lim CK, Anif MNM (2016) Review on wastewater treatment technologies, IJAES 11 (1):111–126 ISSN 0973-6077

Siripattanakul S, Wirojanagud W, McEvoy J et al (2008) Effect of cell-to-matrix ratio in polyvinyl alcohol immobilized pure and mixed cultures on atrazine degradation. Water Air Soil Pollut 8 (3):257–266

Suja PS, Reshmi CR, Sagitha P et al (2017) Electrospun nanofibrous membranes for water purification. Polym Rev 57(3):467–504

Tan KB, Vakili M, Horri BA et al (2015) Adsorption of dyes by nanomaterials: recent developments and adsorption mechanisms. Sep Purif Technol 150:229–242

Thavasi V, Singh G, Ramakrishna S (2008) Electrospun nanofibers in energy and environmental applications. Energy Environ Sci 1(2):205–221

Tong HW, Mutlu BR, Wackett LP et al (2014) Manufacturing of bioreactive nanofibers for bioremediation. Biotechnol Bioeng 111(8):1483–1493

Vancov T, Jury K, Rice N, Van Zwieten L et al (2007) Enhancing cell survival of atrazine degrading Rhodococcus erythropolis NI86/21 cells encapsulated in alginate beads. J Appl Microbiol 102(1):212–220

Vasita R, Katti DS (2006) Nanofibers and their applications in tissue engineering. Int J Nanomedicine 1(1):15

Xie S, Tai S, Song H et al (2016) Genetically engineering of Escherichia coli and immobilization on electrospun fibers for drug delivery purposes. J Mater Chem B 4(42):6820–6829

Zahabi SR, Ravandi SAH et al (2016) Removal of nickel and cadmium heavy metals using nanofiber membranes functionalized with (3-mercaptopropyl) trimethoxysilane (TMPTMS). J Water Health 14(4):630–639

Zussman E (2011) Encapsulation of cells within electrospun fibers. Polym Adv Technol 22 (3):366–371

Chapter 7
Bioremediation of Industrial Wastewater Using Bioelectrochemical Treatment

N. Samsudeen and Manickam Matheswaran

Abstract Industrial waste/wastewater possesses a severe environmental threat to aquatic life. But, it also provides good potential sources for the removal and recovery of precious metal using various treatment processes. Several conventional treatment technologies such as physical, chemical, and biological (aerobic/anaerobic) methods used in the industries are an energy-consuming process and also expensive. Bioelectrochemical treatment system provides a novel platform to remove organic matter as well as recovery of heavy metal ions from various wastewater process streams. In bioelectrochemical treatment, organic matter is bioremediated by electroactive biofilms and coupled to cathode reduction to remove metal ions from the wastewater. This review summarizes the research on bioelectrochemical systems for bioremediation of organic matter as well as recovery of heavy metal ions from the wastewater. There are two different methods being discussed: firstly, removal of organic matters using different bioelectrochemical treatment systems; secondly, recovery of metal ions using abiotic and biotic cathodes in the system.

7.1 Introduction

Energy from fossil fuel and environment is required for process evolution, economic growth of all nations. But, the depletion of fossil fuels over a year and environmental damages due to greenhouse emission are the reasons to find an alternative energy technology (Du et al. 2007). The energy production from several sources such as nuclear, thermal, hydro, and wind power are not reliable sources, and these cannot completely meet out an increasing energy demand. Industries are utilizing the major energy resources for the manufacturing process as well as treating the wastewater. Industries are not consuming energy alone but discharge

N. Samsudeen · M. Matheswaran (✉)
Department of Chemical Engineering, National Institute of Technology, Tiruchirappalli, India
e-mail: matheswaran@nitt.edu

S. J. Varjani et al. (eds.), *Bioremediation: Applications for Environmental Protection and Management*, Energy, Environment, and Sustainability,
https://doi.org/10.1007/978-981-10-7485-1_7

the wastewater that threatens the environment. For example, the dairy, pulp, and paper, leather and distillery industries released a large quantity of wastewater into aquatic environment that causes eutrophication of water bodies (Pant and Adholeya 2007). The metal-contaminated wastewater possesses environmental issues, because most of the metals are non-biodegradable and can be harmful to human bodies causing severe diseases. Industrial wastewater contains several metal ions such as silver, gold, copper, zinc, cadmium, nickel, uranium, selenium, and vanadium, depending on the type of industries used. Generally, physical, chemical, and biological (aerobic and anaerobic) treatment methods have been employed to remove metal ions from the waste stream. Fu and Wang (2011) have reported review articles on the removal of heavy metals from wastewater and discussed various methods such as adsorption, chemical precipitation, electrocoagulation, electrochemical, membrane filtration, and ion exchange for the removal of heavy metals from wastewater (Fu and Wang 2011). They reported that ion exchange, adsorption, and membrane filtration are the most studied for the treatment of metals from wastewater. Though these processes are cost-effective, which need to develop alternative methods to remove and recover the metal in order to make the treatment as cost-effective and sustainable.

In this context, bioelectrochemical systems (BES) referred as microbial fuel cell (MFC) is a promising technology, which produces electricity and removes simultaneously the pollutants in terms of COD, color, and total dissolved solids from the wastewater (Logan and Regan 2006; Logan 2009). In MFC, the microorganism oxidized the organic matter into adenosine triphosphate (ATP) by the sequential reaction and releases protons (H^+) and electrons (e^-). These are transferred via membrane and external circuit to cathode chamber and combined with oxygen/air to generate energy in the form of energy or other value-added products (Nimje et al. 2012). Various types of wastewater including dairy, paper recycling, molasses, chemical, domestic, and brewery wastewater have been investigated as a substrate in the MFC (Pant et al. 2010). In recent years, BES offers a potential solution for integrated wastewater treatment through oxidation and reduction reaction for recovering energy, water, and metal ions. In this study, we have reviewed the organic matter removal using different BES and removal and recovery of inorganic metals using abiotic and biotic cathodes in MFC and microbial electrolysis cell (MEC) system.

7.2 Organic Matter Removal Using Different System

BES uses low-cost and self-sustaining microorganism (either in mixed or individual) to oxidize organic and inorganic matter from waste/wastewater and transfer electrons to the electrode surface. These electrons are captured through an external circuit for electricity generation or hydrogen or other value-added products. Various types of organic matter in pure (glucose, acetate, lactate, etc.) and wastewater are used for the production of electricity as well as treatment efficiency (COD, BOD,

TDS, etc.) from wastewater (Pant et al. 2010). Several types of BES system have been developed to produce electricity as well as treatment of industrial wastewater. In general, Venkat Mohan et al. (2008) constructed dual-chamber MFC and discussed the feasibility of the system for bioelectricity generation from chemical wastewater treatment without using a mediator in anode chamber (Venkata Mohan et al. 2008). Rabaey et al. (2005) demonstrated a tubular, single-chambered MFC that generates high power outputs. They used granular graphite matrix and acetate and glucose as an electron donor in the anode and ferricyanide solution as the cathode. When wastewater was used in the anode side, the organic matter up to 96% was converted to electricity on a Coulombic basis (Rabaey et al. 2005). Karra et al. (2013) reported that the two flow patterns (plug and mixed) of MFC with multiple anodes and cathodes were compared in the continuous flow mode for wastewater treatment and power generation. They demonstrated that reactor configuration is a significant factor to enhance power generation and wastewater treatment (Karra et al. 2013). Microbial electrolysis cell (MEC) is another promising technology since it converts organic wastes into hydrogen and other value-added products and used for the treatment of wastewater. MEC is analogous to MFC, but the cathode of MEC was also in an anaerobic environment. Several studies have been carried out in MEC based on various operating and design conditions (Kadier et al. 2016). In another study, microbial desalination cell (MDC) is another design of MFC, which consists of three chambers, namely anode, cathode, and desalination. Anode and cathode are separated from the desalination chamber by anion exchange membrane (AEM) and cation exchange membrane (CEM), respectively. This type of design achieved wastewater treatment efficiency along with desalination of wastewater simultaneously. Saeed et al. (2015) reviewed the MDC technology in general and discussed the various configurations of this technology (Saeed et al. 2015). Similarly, Wang and Ren reported review articles of the microbial electrochemical system. They have discussed comprehensive and quantitative of various function and system construction with different acronyms developed on the microbial electrochemical system (Wang and Ren 2013).

7.3 Metal Removal Using Bioelectrochemical System

Bioelectrochemical system (BES) is a novel technology that uses microorganism as a catalyst to convert the chemical energy stored in the biodegradable materials into electricity and other products such as alcohol and hydrogen. This offers a new solution for integrated wastewater treatment, energy, and resource recovery because it offers a flexible platform for both oxidation and reduction. The BES consists of anode and cathode chambers separated by a membrane called proton exchange membrane (Liu et al. 2004, 2005; Kim et al. 2007). The configuration of the system mainly depends on the target functions to be accomplished (Janicek et al. 2014). In general, microorganism oxidized the organic matter from the wastewater in the anode chamber and generates electron flow (current) to the cathode, where in the

cathode chamber, the electrons can be used for electricity production (microbial fuel cell, and MFC) (Rabaey et al. 2003), or the electrons are used for the reduction in water (microbial electrolysis cell, MEC) (Kadier et al. 2016) or oxidized chemicals such as metal ions (microbial electrosynthesis), CO_2, or organic chemicals (microbial electrochemical cell) (Wang and Ren 2013). In recent years, the BES has been intensely investigated for wastewater treatment, and several reviews are discussed the suitability for different wastewater conditions (Pant et al. 2010; Li et al. 2014). The cathodic Half-cell reactions of the microbial fuel cell or microbial electrolysis cell are used for removal and recovery of various metal ions as shown in Table 7.1.

The BES system is showing effective platform in the reduction and recovery of different metals by numerous feasibility studies. However, no review on different fundamental mechanism and engineering feasibilities of the system for metal removal and recovery has been reported yet. The main aim of this article reports the removal and recovery of metal under biocathode and abiotic cathode in bioelectrochemical system with and without external power supply.

Table 7.1 Cathode half-cell in BES for removal and recovery of metal ions

Cathodic reaction		Standard potential (V)	References
Cobalt(III)	$LiCoO_2 + 4H^+ \rightarrow Co^{2+} + Li + 2H2O$	+1.61	Huang et al. (2014)
Chromium (VI)	$Cr_2O_7^{2-} + 6e^- + 14H^+ \rightarrow 2Cr^{3+} + 7H_2O$	+1.33	Li et al. (2009)
Gold(II)	$AuCl_4^- + 3e^- \rightarrow Au + 4Cl^-$	+1.00	Choi and Hu (2013)
Mercury(II)	$Hg^{2+}(aq) + 2e^- \rightarrow Hg^0(s)$	+0.99	Wang et al. (2011)
Zinc(II)	$Zn^{2+}(aq) + 2e^- \rightarrow Zn^0(s)$	−0.764	Fradler et al. (2014)
Cadmium (II)	$Cd^{2+}(aq) + 2e^- \rightarrow Cd^0(s)$	−0.403	Modin et al. (2012)
Nickel(II)	$Ni^{2+}(aq) + 2e^- \rightarrow Ni^0(s)$	−0.25	Qin B et al. (2012)
Cobalt(II)	$Co^{2+}(s) + 2e^- \rightarrow Co^0(s)$	−0.232	Jiang et al. (2014)
Vanadium (II)	$VO^{2+} + 2H^+ + e^- \rightarrow VO^{2+} + H_2O$	+0.991	Zhang et al. (2012)
Selenium (IV)	$Se(IV)(aq) + 4e^- \rightarrow Se^0(aq)$	+0.41	Catal et al. (2009)
Copper(II)	$Cu^{2+}(aq) + 2e^- \rightarrow Cu^0(s)$	+0.286	Tao et al. (2011)

7.3.1 Metal Ions Using Abiotic Cathode System in MFC

In single- and/or dual-chamber MFCs, heavy metals are removed in the anoxic cathode chamber through cathodic metal reduction (Mathuriya and Yakhmi 2014). In MFC, the direct reduction in metal involves an abiotic cathode for those with redox potential higher than the anode potential. The metals include Au(III), V(V), Cr(VI), Ag(I), Cu(II), Fe(III), and Hg(II), and their respective reduced into an element (Ter et al. 2010). This reduction is thermodynamically favorable, and the metals can be directly used as the electron acceptor without any external power consumption. For example, Cr is a common element used in electroplating, leather tanning, metallurgy, and dye-manufacturing process. Chromium exists in hexavalent (Cr(VI)) and trivalent (Cr(III)) forms, and these are stable, which are mostly found in the environment. Cr(VI) is water soluble at all pHs and extremely toxic. But the Cr(III) is less soluble in water and can form $Cr(OH)_3$. The biological treatment method of Cr removal is still challenging because Cr(VI) toxic to microbes, which affects the metabolic reaction. In BES, Cr(VI) can act as an efficient terminal electron acceptor (TEA) since the half-cell Cr(VI) reduction reaction has a redox potential of +1.33 V (vs. standard hydrogen electrode), much higher than O_2. Wang et al. (2008) demonstrated the Cr reduction coupled with energy generation in dual-chamber MFC using acetate and potassium dichromate acts as electron donors and acceptors, respectively (Wang et al. 2008). They reported that at lower pH, higher Cr(VI) removal rate was observed because of dependency of the chromate reduction reaction. At pH 2, a complete removal of Cr (VI) was observed at a concentration of 100 mg/L of catholyte solution. Li et al. reported that the modification of the cathode with rutile (natural form of TiO2) coating for improving Cr(VI) reduction through light-induced photocatalysis at the cathode (Li et al. 2009). Zhang et al. reported the simultaneous reduction of chromium and vanadium at the cathode using acetate as an electron donor in dual-chamber MFC. The redox potential of Cr(VI)/Cr(III) and V(V)/(IV) couples are 1.33 V and 0.9 V, respectively. They observed the higher reduction of Cr(V) than V(V) because of the higher redox potential of chromium. At the cathode, the deposits were observed which are mainly composed of Cr(III) with some amount of vanadium (Zhang et al. 2012). Similarly, Choi and Hu (2013) reported the removal of precious metal such as gold (Au(III)) from wastewater using MFC. The removal efficiency of 97.8% was achieved for an initial concentration of 1000 mg/L Au(III) in 12 h. The maximum power output of 0.89 W/m^2 was achieved for 100 mg/l of Au(III) (Choi and Hu 2013).

Copper is another element present in the wastewater arising from copper polishing industry, metallurgy, electroplating, etc. The copper reduction (from Cu(II) to Cu(0)) occurs spontaneously in the catholyte solution of MFC. Tao et al. studied that the copper recovery from copper sulfate solution in dual-chamber MFC at different initial Cu(II) concentrations from 50 to 6412 mg/L. With glucose as a substrate, the maximum power density generated was 339 mW/m^3 at an initial concentration of 6412.5 mg Cu^{2+}/L. High Cu(II) removal efficiency (>99%) was

achieved from a 196 mg/L Cu(II) solutions. They also successfully recovered Cu in a 16 L pilot scale membrane less MFC (Tao et al. 2011). Zhang et al. (2009) reported that sulfide-containing wastewater and vanadium-containing wastewater can also be treated in MFC based on their chemical conditions. A novel process for MFC system was investigated by employing sulfide, organics, and V(V) as electron donors and acceptor. Sulfide and total organics removal approached 84.7 ± 2.8% and 20.7 ± 2.1%, with a V(V) reduction rate of 25.3% (Zhang et al. 2009). Wang et al. (2011) investigated the cathodic reduction of Hg(II) in a dual-chamber MFC using acetate as an electron donor. The cathode chamber was loaded with an $HgCl_2$ solution made anaerobic by purging with N_2 gas. A removal efficiency of more than 98% Hg(II) was observed in 10 h (Wang et al. 2011). Catal et al. (2009) investigated the selenite removal in air cathode MFC using glucose as the electron donor. The selenium reduction in 75 and 200 mg/L of selenite was achieved in 78 and 72 h (Catal et al. 2009), respectively. One of their studies showed that the three-chamber system including a dual-chamber MFC and strip chamber adjacent to the cathode chamber is to recover Zn(III) ions. The design enhanced the power production as compared to control experiment, and the deposited Zn(II) was extracted through the supported liquid membrane.

7.3.2 Metal Removal Using Abiotic Cathode in MEC

Some metal ions possess lower redox potential than the BES anode potential. This metal ions cannot accept electrons spontaneously from the cathode, due to thermodynamic limits that prevent the electron flows from anode to the cathode. However, these metal ions can be reduced by accepting the electrons from the cathode when external power is used to forcibly drive the electrons from the high-potential anode to the low-potential cathode. For example, the reduction potential of nickel (Ni(II)/Ni(0)) is −0.25 V, which cannot be naturally reduced into nickel metal under standard MFC conditions. When the applied external voltage of 0.5–1.1 V, Ni(II) reduction in 67% was observed at an initial concentration of 500 mg/L (Qin B et al. 2012). Luo et al. sequentially removed Cu^{2+}, $Ni^{2+,}$ and Fe^{2+} from artificial mine drainage (AMD) with simultaneous H_2 production when an external voltage of 1.0 V is being applied. They obtained the maximum energy recovery efficiency, which suggested that the produced H_2 gas was sufficient to offset the energy consumed during the metal recovery (Luo et al. 2014).

Cobalt is also another element that is recovered when external load is supplied in the MFC. The standard redox potential of the Co(II)/Co(0) couple is negative (−0.232 V vs. SHE), and an input energy is required to drive the reduction of Co (II) at the cathode. A self-driven MFC and MEC can complete the conversion of these processes in which Co(II) is firstly released from the particles on the cathodes of MFCs and then reduced to Co(0) on the cathodes of MECs that was powered by Co(II) leaching MFC (Huang et al. 2014). Jiang et al. reported that cobalt was successfully recovered as elemental Co(0) deposits on the cathode along with

simultaneous H_2 production in a MEC with an applied voltage of 0.2–0.5 V. A removal of 92% from 847 μM of Co(II) solution was observed within the first 6 h (Jiang et al. 2014). Cadmium is an another metal, in which redox potential of the Cd (II)/Cd(0) couple is −0.4 V. Choi et al. used a Cr(VI)-reducing MEC to power the operation of a MEC for Cd(II) reductive precipitation. They have successfully demonstrated Cd(II) reduction in an MFC Cr(VI)–MEC Cd(II) system without any extra energy input (Choi et al. 2014). Luo et al. (2014) reported that aqueous nickel was recovered on the cathode at an applied voltage of 0.7 V in dual-chamber MFC. The maximum removal efficiency of 94% was recovered in 40 h (Luo et al. 2014). During the MEC operation, Ni was recovered and deposited on the cathode electrode surface. They reported that the MEC performance was also affected by various conditions such as Ni(II) concentration, applied voltages, and initial pH conditions. Similarly, several metal ions such as Ur(VI), Zn(II), and Pb(II) can be made possible by applying external power sources (Modin et al. 2012). Theoretically, all the metal ions may be reduced into metals using such an approach; if required, potential can be applied to BES system. However, the cost-benefit and chemical recovery rates need to be studied before potential applications for such processes.

7.3.3 Metal Ion Removal and Recovery Using Biocathode MFC System

Microorganism plays an important role in metal distribution and circulation in nature; the interaction between microbes and metal mainly includes metal assimilation and metal dissimilation. Limited studies have been reported on the bioelectrochemical recovery of metals employing biocathodes. In the biocathode MFC system, the microorganism oxidizes the organic matter and releases electrons and protons, which are travelled via an external circuit and membrane to the cathode chamber. The dissimilarity metal-reducing bacteria (DMRB) species used the metal ions as the terminal electron acceptors (TEA) in the cathode side to generate energy. DMRBs respire using metals or metalloids outside the cell membrane for cellular energetics, and they have been widely used in bioremediation of metal-contaminated sites. For example, DMRBs can reduce Cr(VI) to Cr(III), which are less toxic (Daulton et al. 2007). There are several bacteria commonly used to reduce the metal ions in MFC and MEC systems as shown in Table 7.2. Xafenias et al. (2013) investigated that the biocathodes for the reduction of Cr(VI) using *Shewanella oneidensis* MR-1 as a biocatalyst. The Cr(VI)-reducing bacteria receive electrons from the poised cathode and catalyze the reduction of Cr(VI) to Cr(III). The MFC with MR-1 and lactate present in both anode and cathode produced a maximum current density of 32.5 mA/m^2 (1000 Ω) after receiving a 10 mg/L Cr (VI) addition in the cathode, and cathodic efficiency increased steadily over an 8-day operation period with successive Cr(VI) additions (Xafenias et al. 2013). Wu

Table 7.2 Microbial reduction and precipitation of metal ions

Microbes	Metal ions	References
Shewanella oneidensis, Trichococcus pasteurii and Pseudomonas aeruginosa,	Cr (VI)	Daulton et al. (2007), Tandukar et al. (2009)
Bacillus Sp.	Se (VI)	Dungan et al. (2003)
Geobacillus sp.	Au (III)	Correa-Llantén et al. (2013)
Shewanella Oneidensis	V(V)	Carpentier et al. (2003)
Plectonema boryanum	Ag(I)	Lengke et al. (2007)
Shewanella putrefucians, Geobacter psychrophilus	Fe(III)	Kanso and Greene (2002)
Bacillus subterraneus	Mn (IV)	Kanso and Greene (2002)

et al. (2015) developed an electroactive biofilm on the anode in the MFC and subsequently used it as the cathode for chromium reduction. They reported that efficient reduction in Cr(VI) was observed when an anode with a functional electrode biofilm as the biocathodes MFC. They further reported that the biocathode acclimatization period was shortened by 19 days, and the Cr(VI) reduction rate was increased by a factor of 2.9 (Wu et al. 2015). He and Yao (2011) reported that an *Anaeromyxobacter dehalogenans* reduced Se(IV) to Se(0) as a detoxification mechanism. The potential role of these microorganisms in the biogeochemical cycling of selenium is to control the selenium contamination in the wastewater (He and Yao 2011). DMRBs have also been used for metallic nanoparticles production, which carries superior catalytic functions compared to the ones prepared using chemical methods. Yates et al. reported that the DMRB Geobacter sulfurreducens showed to reduce soluble Pd(II) to Pd(0) nanoparticles primarily outside the cell, reducing the toxicity of metal ions, and allowing nanoparticle recovery without cell destruction (Yates et al. 2013). Several reviews are discussed on the metallic reduction mechanism of microorganism and its application (Liu et al. 2002). DMRBs have also been found and used for MFC anode reduction associated with organic oxidation in the anode chamber.

7.3.4 Metal Ion Removal and Recovery Using Biocathode MEC System

Some of the metals may have lower redox potential. External power sources can be used on biocathode to facilitate the reduction. When poising the cathode potential at a certain level, although metals are not reduced electrochemically, metal ions can be extracted from solution and adsorbed into the biofilms of the electrodes, and then microorganisms on the electrode reduce metal during microbial respiration.

Gregory and lovely 2005 reported that U(VI) was reduced to U(IV) by *Geobacter sulferreducens* with a poised cathode potential of −500 mV, which is much lower than the electrochemical reduction of U(VI) at −900 mV (Gregory and Lovley 2005). Similarly, Haung et al. (2011) showed that the reduction rate and power generation were improved by applying reduction potential of −300 mV as compared to control reaction with set potential (Huang et al. 2011). The poised potential attracts the migration and adsorption of the charged metal ions onto the cathode, while the biofilm on the cathode help retain the ions from desorption when the potential is removed. Metal conversion using biocathodes requires less set potential that can quickly remove metals from contaminated environment due to electrical and biological adsorptions, and as a result, it requires less energy compared with traditional electrochemical reduction methods.

7.4 Conclusion

Bioelectrochemical systems (BES) offer versatile platform and great potential for recovering metals from wastewater and aqueous streams. This review discussed the organic matter removal in various BES systems. This review also summarized the concept of cathodic reduction reaction coupled with oxidation of organic matter using biotic and abiotic cathodes in MFC and MEC systems. Overall, the BES platform shows great potential in recovering different metals such as Ni, Co, Cu, Ag, Cd, Se, and V with redox potentials due to its great flexibility, but more attention is needed for further understanding the mechanism and for the investigation of the feasibility of engineering applications.

References

Carpentier W, Sandra K, De Smet I, Brigé A, De Smet L, Van Beeumen J (2003) Microbial reduction and precipitation of vanadium by Shewanella oneidensis. Appl Environ Microbiol 69:3636–3639. https://doi.org/10.1128/AEM.69.6.3636-3639.2003

Catal T, Bermek H, Liu H (2009) Removal of selenite from wastewater using microbial fuel cells. Biotechnol Lett 31:1211–1216. https://doi.org/10.1007/s10529-009-9990-8

Choi C, Hu N (2013) The modeling of gold recovery from tetrachloroaurate wastewater using a microbial fuel cell. Bioresour Technol 133:589–598. https://doi.org/10.1016/j.biortech.2013.01.143

Choi C, Hu N, Lim B (2014) Cadmium recovery by coupling double microbial fuel cells. Bioresour Technol 170:361–369. https://doi.org/10.1016/j.biortech.2014.07.087

Correa-Llantén DN, Muñoz-Ibacache SA, Castro ME, Muñoz PA, Blamey JM (2013) Gold nanoparticles synthesized by Geobacillus sp. strain ID17 a thermophilic bacterium isolated from Deception Island, Antarctica. Microb Cell Fact 12:75. https://doi.org/10.1186/1475-2859-12-75

Daulton TL, Little BJ, Jones-Meehan J, Blom DA, Allard LF (2007) Microbial reduction of chromium from the hexavalent to divalent state. Geochim Cosmochim Acta 71:556–565. https://doi.org/10.1016/j.gca.2006.10.007

Du Z, Li H, Gu T (2007) A state of the art review on microbial fuel cells: a promising technology for wastewater treatment and bioenergy. Biotechnol Adv 25:464–482. https://doi.org/10.1016/j.biotechadv.2007.05.004

Dungan RS, Yates SR, Frankenberger WT (2003) Transformations of selenate and selenite by Stenotrophomonas maltophilia isolated from a seleniferous agricultural drainage pond sediment. Environ Microbiol 5:287–295. https://doi.org/10.1046/j.1462-2920.2003.00410.x

Fradler KR, Michie I, Dinsdale RM, Guwy AJ, Premier GC (2014) Augmenting microbial fuel cell power by coupling with supported liquid membrane permeation for zinc recovery. Water Res 55:115–125. https://doi.org/10.1016/j.watres.2014.02.026

Fu F, Wang Q (2011) Removal of heavy metal ions from wastewaters: a review. J Environ Manag 92:407–418. https://doi.org/10.1016/j.jenvman.2010.11.011

Gregory KB, Lovley DR (2005) Remediation and recovery of uranium from contaminated subsurface environments with electrodes. Environ Sci Technol 39:8943–8947. https://doi.org/10.1021/es050457e

He Q, Yao K (2011) Impact of alternative electron acceptors on selenium(IV) reduction by Anaeromyxobacter dehalogenans. Bioresour Technol 102:3578–3580. https://doi.org/10.1016/j.biortech.2010.10.046

Huang L, Chai X, Chen G, Logan BE (2011) Effect of set potential on hexavalent chromium reduction and electricity generation from biocathode microbial fuel cells. Environ Sci Technol 45:5025–5031. https://doi.org/10.1021/es103875d

Huang L, Yao B, Wu D, Quan X (2014) Complete cobalt recovery from lithium cobalt oxide in self-driven microbial fuel cell—microbial electrolysis cell systems. J Power Sources 259:54–64. https://doi.org/10.1016/j.jpowsour.2014.02.061

Janicek A, Fan Y, Liu H (2014) Design of microbial fuel cells for practical application: a review and analysis of scale-up studies. Biofuels 5:79–92. https://doi.org/10.4155/bfs.13.69

Jiang L, Huang L, Sun Y (2014) Recovery of flakey cobalt from aqueous Co(II) with simultaneous hydrogen production in microbial electrolysis cells. Int J Hydrogen Energy 39:654–663. https://doi.org/10.1016/j.ijhydene.2013.10.112

Kadier A, Kalil MS, Abdeshahian P, Chandrasekhar K, Mohamed A, Azman NF et al (2016a) Recent advances and emerging challenges in microbial electrolysis cells (MECs) for microbial production of hydrogen and value-added chemicals. Renew Sustain Energy Rev 61:501–525. https://doi.org/10.1016/j.rser.2016.04.017

Kadier A, Simayi Y, Abdeshahian P, Azman NF, Chandrasekhar K, Kalil MS (2016b) A comprehensive review of microbial electrolysis cells (MEC) reactor designs and configurations for sustainable hydrogen gas production. Alexandria Eng J 55:427–443. https://doi.org/10.1016/j.aej.2015.10.008

Kanso S, Greene AC, Patel BKC (2002) Bacillus subterraneus sp. nov., an iron- and manganese-reducing bacterium from a deep subsurface Australian thermal aquifer. Int J Syst Evol Microbiol 52:869–874. https://doi.org/10.1099/00207713-52-3-869

Karra U, Troop E, Curtis M, Scheible K, Tenaglier C, Patel N et al (2013) Performance of plug flow microbial fuel cell (PF-MFC) and complete mixing microbial fuel cell (CM-MFC) for wastewater treatment and power generation. Int J Hydrogen Energy 38:5383–5388. https://doi.org/10.1016/j.ijhydene.2013.02.092

Kim BH, Chang IS, Gadd GM (2007) Challenges in microbial fuel cell development and operation. Appl Microbiol Biotechnol 76:485–494. https://doi.org/10.1007/s00253-007-1027-4

Lengke MF, Fleet ME, Southam G (2007) Biosynthesis of silver nanoparticles by filamentous cyanobacteria from a silver (I) nitrate complex. Langmuir 23:2694–2699. https://doi.org/10.1021/la0613124

Li Y, Lu A, Ding H, Jin S, Yan Y, Wang C et al (2009) Cr(VI) reduction at rutile-catalyzed cathode in microbial fuel cells. Electrochem Commun 11:1496–1499. https://doi.org/10.1016/j.elecom.2009.05.039

Li W-W, Yu H-Q, He Z (2014) Towards sustainable wastewater treatment by using microbial fuel cells-centered technologies. Energy Environ Sci 7:911–924. https://doi.org/10.1039/C3EE43106A

Liu C, Gorby YA, Zachara JM, Fredrickson JK, Brown CF (2002) Reduction kinetics of Fe(III), Co(III), U(VI), Cr(VI), and Tc(VII) in cultures of dissimilatory metal-reducing bacteria. Biotechnol Bioeng 80:637–649. https://doi.org/10.1002/bit.10430

Liu H, Liu H, Ramnarayanan R, Ramnarayanan R, Logan BE, Logan BE (2004) Production of electricity during wastewater treatment using a single chamber microbial fuel cell. Environ Sci Technol 38:2281–2285. https://doi.org/10.1021/es034923g

Liu H, Cheng S, Logan BE (2005) Production of electricity from acetate or butyrate using a single-chamber microbial fuel cell. Environ Sci Technol 39:658–662. https://doi.org/10.1021/es048927c

Logan BE (2009) Exoelectrogenic bacteria that power microbial fuel cells. Nat Rev Microbiol 7:375–381. https://doi.org/10.1038/nrmicro2113

Logan BE, Regan JM (2006) Microbial fuel cells—challenges and applications. Environ Sci Technol 40:5172–5180. https://doi.org/10.1021/es0627592

Luo H, Liu G, Zhang R, Bai Y, Fu S, Hou Y (2014) Heavy metal recovery combined with H_2 production from artificial acid mine drainage using the microbial electrolysis cell. J Hazard Mater 270:153–159. https://doi.org/10.1016/j.jhazmat.2014.01.050

Mathuriya AS, Yakhmi JV (2014) Microbial fuel cells to recover heavy metals. Environ Chem Lett 12:483–494. https://doi.org/10.1007/s10311-014-0474-2

Modin O, Wang X, Wu X, Rauch S, Fedje KK (2012) Bioelectrochemical recovery of Cu, Pb, Cd, and Zn from dilute solutions. J Hazard Mater 235–236:291–297. https://doi.org/10.1016/j.jhazmat.2012.07.058

Nimje VR, Chen C-Y, Chen H-R, Chen C-C, Huang YM, Tseng M-J et al (2012) Comparative bioelectricity production from various wastewaters in microbial fuel cells using mixed cultures and a pure strain of Shewanella oneidensis. Bioresour Technol 104:315–323. https://doi.org/10.1016/j.biortech.2011.09.129

Pant D, Adholeya A (2007) Biological approaches for treatment of distillery wastewater: a review. Bioresour Technol 98:2321–2334. https://doi.org/10.1016/j.biortech.2006.09.027

Pant D, Van Bogaert G, Diels L, Vanbroekhoven K (2010) A review of the substrates used in microbial fuel cells (MFCs) for sustainable energy production. Bioresour Technol 101:1533–1543. https://doi.org/10.1016/j.biortech.2009.10.017

Qin B, Luo H, Liu G, Zhang R, Chen S, Hou Y et al (2012) Nickel ion removal from wastewater using the microbial electrolysis cell, vol. 121. https://doi.org/10.1016/j.biortech.2012.06.068

Rabaey K, Lissens G, Siciliano SD, Verstraete W (2003) A microbial fuel cell capable of converting glucose to electricity at high rate and efficiency. Biotechnol Lett 25:1531–1535. https://doi.org/10.1023/A:1025484009367

Rabaey K, Clauwaert P, Aelterman P, Verstraete W (2005) Tubular microbial fuel cells for efficient electricity generation. Environ Sci Technol 39:8077–8082. https://doi.org/10.1021/es050986i

Saeed HM, Husseini GA, Yousef S, Saif J, Al-Asheh S, Abu Fara A et al (2015) Microbial desalination cell technology: a review and a case study. Desalination 359:1–13. https://doi.org/10.1016/j.desal.2014.12.024

Tandukar M, Huber SJ, Onodera T, Pavlostathis SG (2009) Biological chromium (VI) reduction in the cathode of a microbial fuel cell. Environ Sci Technol 43:8159–8165. https://doi.org/10.1021/es9014184

Tao H-C, Liang M, Li W, Zhang L-J, Ni J-R, Wu W-M (2011) Removal of copper from aqueous solution by electrodeposition in cathode chamber of microbial fuel cell. J Hazard Mater 189:186–192. https://doi.org/10.1016/j.jhazmat.2011.02.018

Ter Heijne A, Liu F, van der Weijden R, Weijma J, Buisman CJN, Hamelers HVM (2010) Copper recovery combined with electricity production in a microbial fuel cell. Environ Sci Technol 44:4376–4381. https://doi.org/10.1021/es100526g

Venkata Mohan S, Mohanakrishna G, Reddy BP, Saravanan R, Sarma PN (2008) Bioelectricity generation from chemical wastewater treatment in mediatorless (anode) microbial fuel cell (MFC) using selectively enriched hydrogen producing mixed culture under acidophilic microenvironment. Biochem Eng J 39:121–130. https://doi.org/10.1016/j.bej.2007.08.023

Wang H, Ren ZJ (2013) A comprehensive review of microbial electrochemical systems as a platform technology. Biotechnol Adv 31:1796–1807. https://doi.org/10.1016/j.biotechadv.2013.10.001

Wang G, Huang L, Zhang Y (2008) Cathodic reduction of hexavalent chromium [Cr(VI)] coupled with electricity generation in microbial fuel cells. Biotechnol Lett 30:1959. https://doi.org/10.1007/s10529-008-9792-4

Wang Z, Lim B, Choi C (2011) Removal of Hg^{2+} as an electron acceptor coupled with power generation using a microbial fuel cell. Bioresour Technol 102:6304–6307. https://doi.org/10.1016/j.biortech.2011.02.027

Wu X, Zhu X, Song T, Zhang L, Jia H, Wei P (2015) Effect of acclimatization on hexavalent chromium reduction in a biocathode microbial fuel cell. Bioresour Technol 180:185–191. https://doi.org/10.1016/j.biortech.2014.12.105

Xafenias N, Zhang Y, Banks CJ (2013) Enhanced performance of hexavalent chromium reducing cathodes in the presence of Shewanella oneidensis MR-1 and Lactate. Environ Sci Technol 47:4512–4520. https://doi.org/10.1021/es304606u

Yates MD, Cusick RD, Logan BE (2013) Extracellular palladium nanoparticle production using Geobacter sulfurreducens. ACS Sustain Chem Eng 1:1165–1171. https://doi.org/10.1021/sc4000785

Zhang B, Zhao H, Shi C, Zhou S, Ni J (2009) Simultaneous removal of sulfide and organics with vanadium (V) reduction in microbial fuel cells. J Chem Technol Biotechnol 84:1780–1786. https://doi.org/10.1002/jctb.2244

Zhang B, Feng C, Ni J, Zhang J, Huang W (2012) Simultaneous reduction of vanadium (V) and chromium (VI) with enhanced energy recovery based on microbial fuel cell technology. J Power Sources 204:34–39. https://doi.org/10.1016/j.jpowsour.2012.01.013

Chapter 8
Biosorption Strategies in the Remediation of Toxic Pollutants from Contaminated Water Bodies

P. Senthil Kumar and K. Grace Pavithra

Abstract Heavy metals, radioactive waste, hydrocarbon pollutant, and pesticides are some of the leading toxic pollutants in our environment. Challenges are faced in decontamination of these types of pollutants to soil and water for a long period of time. A number of methods such as membrane technology, electro-Fenton reaction, advanced oxidation process, and nanotechnology played a major role in removing toxic pollutants but difficulties are seen in degradation of toxic sludge, additional side reactions, high cost in initial installment and in maintenance, etc. Biosorption is a physiochemical metaprocess involving solid and liquid phases in which dissolved species to be sorbed. Low cost, high efficiency, and reusability of biosorbent are some of the advantages in biosorption. Biosorption involves removal of toxic pollutants by biomass. Some microorganisms are targeted for the removal of single pollutant alone. Algae, bacteria, fungi, yeast, waste materials from agricultural and food industries, etc., are used as biosorbent. Different mechanisms such as precipitation, absorption, adsorption, and ion exchange are combined with biosorption in order to treat toxic pollutants. This chapter provides collective ideas of various removal techniques in combination with biosorption and their applications to remediate water streams. This chapter also illustrates some of the problems faced during the biosorption activity and highlights the importance of improving the process for bioremediation in toxic pollutants.

Keywords Toxic pollutants · Degradation · Biosorption · Physiochemical Biosorbent · Bioremediation

P. Senthil Kumar (✉) · K. Grace Pavithra
Department of Chemical Engineering, SSN College of Engineering,
Chennai 603110, India
e-mail: senthilchem8582@gmail.com

K. Grace Pavithra
e-mail: kirubanandampavithra@gmail.com

S. J. Varjani et al. (eds.), *Bioremediation: Applications for Environmental Protection and Management*, Energy, Environment, and Sustainability,
https://doi.org/10.1007/978-981-10-7485-1_8

8.1 Introduction

An ecosystem consists of all living beings in our environment, and due to anthropogenic activities in our environment such as industrialization, scientific advancement, and rapid urbanization, many changes occurred. Modifications are in the form of physical, chemical, and also in biological terms. Environmental contaminations will affect soil, water as well as the atmosphere in the due course of time. It is like a cycle where the pollutant compositions which are made by a human from the earth components whether knowingly or unknowingly affect the living beings and the consequences are seen to be worse when the time is prolonged.

Life on the earth is mainly seen above the soil and some in water. Water is essential for all living beings in earth, and it is not evenly distributed in our earth surface. Major amount of water are seen in ocean, and only 2.5% of freshwater are only available for food production, recreational use, drinking water supply which are seen in the form of lake, pond, river, and streams. The esthetic value of surface water is affected due to the intervention of toxic pollutants which includes pharmaceutical, industrial waste, fertilizers, radioactive waste. The emission of these types of waste affects the water quality, and the living organisms in the ecosystem are also disturbed. Due to continuous emission, the soil under the water gets affected. The basic food substances for the food chain are plants and the soil microbes, and it is also considered as an energy source for a higher level of organisms. The work of soil microbes is incredible as they get energy from dead and decomposed matter and provide energy in the form of nitrogen, carbon, oxygen, potassium, hydrogen, phosphorus to plants for their growth. Because of smaller in size by nature, they have lesser biomass but the impact of microbes in the nutrient cycle was found to be effective. Microbes in the terrestrial environment play major role in carbon and nitrogen cycles. Soil microbes are bonded with toxic substance due to exposure of toxic pollutants.

The intervention of toxic pollutants such as heavy metals, radioactive waste, pesticides, herbicides, weedicide, hazardous waste, waste from paint, dye, battery, metal plating, manufacturing industries to our environment leads to a lot of impacts on the ecosystem. Some of the toxic pollutants that affect soil microbes are pesticides, herbicides which are found to be seen in excessive during agricultural practices. Compounds such as azoxystrobin, maneb, sulfur, atrazine, mecoprop, paraquat, endosulfan, cypermethrin are used in large scale for agricultural practices. Leaching and accumulation on soil make constant threat to human health as well as to the environment. Atrazine is used as weedicide in the farms of sugarcane and corn to enhance the production when used in suggested amount. Due to its low price and availability, farmers use atrazine in large amount, as a result of this not only plants and animals get affected, the aquatic life also affected due to leaching and runoff. Around 5 kg of agrotoxins is consumed annually per person in Brazil (Andleeb et al. 2016; Zhang et al. 2014; Xie et al. 2013; Chrisman et al. 2009). Polycyclic aromatic hydrocarbons will be coming under organic pollutants. Benzene, toluene, ethylbenzene, xylene, Polychlorinated biphenyls (PCB), and the

pharmaceutical components such as carbamazepine, caffeine, hormonal drugs are released from various sources like hospital municipal waste, human as well as animal excreta, wastage discharge from pharmaceutical manufacturing industries, and excess drug utilizing. Depends upon the intake and the individual human metabolisms, drugs are excreted from our body. The wastewater from households and from hospitals is treated with common effluent treatment plants, and these treatment plants are not specially designed for removing those drugs (Zhang et al. 2012; Hooper et al. 2009). Heavy metals such as lead, mercury, cadmium, chromium, arsenic, and beryllium are in terms of individual metals and metal compounds which affect people's health adversely. In mere amount, some of the metal compounds are necessary to lead our life and if it consumed in a large amount, they become toxic. Heavy metals are generally derived from industries like plastic manufacturing, electroplating, fertilizers, pigment, and mining.

Algae, bacteria, and some aquatic plants are best-known indicators in terms of species count and in densities; it also acts as biological monitoring system in assessment of water quality. Some of the indications on the top of water bodies such as eutrophication and algal blooming prove that the water is affected by toxic pollutants. As a result of it, the life of the species inside as well as on the surface of the water is interrupted from their routine life. Due to prolonged emission of toxic pollutants, photosynthesis does not occur, and in due course of time, living organisms never exist in water bodies. In addition, when these toxic pollutants mixed with water found on the terrestrial level, the differences in characteristics such as pH, COD, BOD, electrical conductivity, turbidity, odor, the taste of water are affected, and the changes depend upon the pollutants in which the water interacts. The soil which is beneath the water gets affected in the due course of time and during the runoff; water acts as a natural carrier and settles the toxic pollutants in various zones of the environment. Finally, the soil gets affected and the microbes in the soil are accumulated with toxicity; this process may lead to biomagnification, defined as an increase in the composition of a particular substance in living organisms at successively higher levels in the food chain and finally affect human's health.

As they come under the classification of toxic substance, they require special handling and special disposal sites. Municipal wastewater is not designed for the removal of heavy metals and for toxic pollutants. Pretreatment has to be done at the source itself, and the treatment provided should be cheaper because it often deals with a lot of effluents.

Many treatment technologies from conventional to modern such as activated sludge, coagulation-cum-flocculation, sedimentation, filtration, membrane process, and advanced oxidation process (Table 8.1) were used to minimize the consequences. In every treatment, the pollutants may be separated from the mainstream whether in the solid or liquid phase and further degradation or decomposition of that segregated pollutants was considered to be a tedious step; the cost involved in this process was found to be high and all the above processes are designed to target single pollutant. Treatment processes such as anaerobic/aerobic digestion, incineration, composting, landfill for last-stage treating are not 100% efficient in set

Table 8.1 Data on the biosorption of dye, heavy metal, phenol, and radioactive element by various biosorbents

S. no.	Treatments	Merits	Demerits
1	Activated sludge treatment	Biological pollutants are removed using this method	Short circuits occur in due course of time
			Maintenance problem
2	Coagulation-cum-flocculation	Lightweight particles are bonded up for removal in the combination of fast and slow mixing	The chemicals added are added up to the sludge
3	Sedimentation	Not suitable for heavy metal and hazardous pollutant removal	Cleaning and biomass accumulation are time-consuming
			Not suitable for targeted pollutants
4	Filtration	Clarifies liquid are efficiently removed by this method	Less dense pollutants are removed using this method
			Live or dead biomass are accumulated in this method
5	Membrane process	Pure effluents	Membrane fouling and cleaning are tedious
			The retentate formed consists of enormous toxic substance
6	Adsorption	Conventional sorbents	Not efficient for the removal of heavy metals
7	Advanced oxidation process	Used with the combinations of conventional treatments	Targeted pollutants are only removed
			Very costly

backing the components which are taken from our earth, i.e., not converted to its primary form which is considered to be harmless. For example, trivalent chromium (Cr(III)) in trace amount is needed for optimal health and found to be biologically active in food. It enhances the insulin. It is a hormone used for storage for carbohydrate, fat, and proteins whereas hexavalent chromium (Cr(VI)) is considered as pollutants due to the discharge into the environment from industries, mainly from leather industries.

Bioremediation is a technique by which microorganisms are used for cleaning up of contaminants from our environment, and the addition of nutrients and electron acceptors increases the removal efficiency, generally, oxygen or nitrogen will be used as electron acceptor. The microorganisms use contaminants as a food source and convert the contaminants into biomass and harmless by-products such as CO_2 and other inorganic salts. The combination of bioremediation with different techniques increases the efficiency in removal. Biosorption is a process in which biomolecules are used to bind the ions, especially non-degradable contaminants. Bacteria, fungi, algae, industrial, and agricultural waste are generally used as biosorbents. Biosorption techniques have good potential to replace all other conventional technologies where no secondary pollutants were accumulated.

8.2 Potential of Biosorption

The mechanism for biosorption is difficult to predict because several factors such as the biosorbent used, the substance to be sorbed, and the environmental conditions are to be included. In laboratory level, we can determine the above factors when coming to a real end except for biosorbent dosage nothing cannot be predicted because day by day, the pollutants will be accumulated and the environmental condition changes time to time. Bio refers to the biological activity of dead or living organisms, and sorption refers to absorption and adsorption especially. The metabolic inactive materials are passively bonded to the biomass. Fast-growing biomass and also dead cell biomass do not leave toxicity effects after treatment. The same biomass used can be used for many adsorption cycles (Volesky 2003; Norton and Baskaran 2004). The toxicants are taken up by live or dead biomaterials passively. Figure 8.1 gives detailed explanation regarding the biosorption process. Two types of phases are seen in biosorption; solid phase, generally refers to biosorbent and a liquid phase, refers to solvent. It is considered as a potential mechanism for removal of metal ions and the various functional groups on the cell wall offer certain attraction forces and provide high removal efficiency. The adsorbate is attracted and bonded over the adsorbent due to higher affinity, and this process is continued until equilibrium is reached. The distribution between solid and liquid phases determines the adsorbent affinity. It is known as a physicochemical process which includes absorption, adsorption, ion exchange, precipitation, and surface complexation, and biosorption is carried out using microbial systems such as bacteria, fungi, algae, toxic metals, and radionuclides (Macek and Makova 2011). Detoxification and transformation of organic as well as inorganic pollutants are the major properties of microorganisms. Biosorption is widely used in the removal of metal ions. The removal of metal ions mostly depends on the (1) type and availability of biomass, (2) composition of the wastewater, and (3) type of biomass preparation. When compared to ion exchange process, biosorption is considered as 1/10 times cheaper. Biosorption has number of advantages, they are:

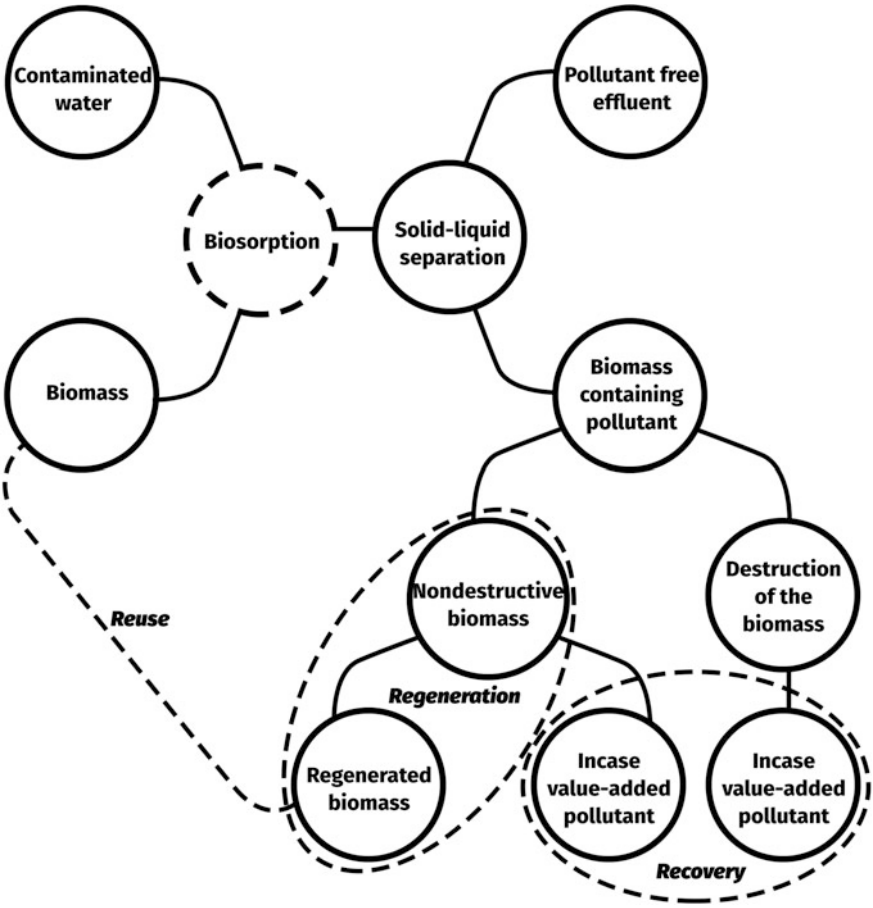


Fig. 8.1 Flowchart explaining about biosorption process

Cost efficiency: Biosorbents are used from waste materials which are abundant in our environment.

Process selective: Depends on the nature and quantity of pollutant, specific biomass is selected for removal of particular pollutants.

Regeneration: Used biosorbents can be used for a number of times, and the efficiencies are decreased after many numbers of times.

Formation of sludge: Secondary pollutants are not formed in sludge, and there are no chemical pollutants present in the sludge.

Recovery of metals: After the removal of pollutants using biomass, the pollutants are taken back to original form as there is no any chemical intervention.

Proficiency in performance: Biosorption is considered as an efficient technique than other techniques. Ion exchange was considered as equal and efficient technique to biosorption but it is costly.

No additional nutrient requirement: Other than biomass, other compounds are not added so additional nutrients are not added for their growth as it utilizes from the pollutants.

Efficiency: A large volume of wastewater can be treated at a time.

Operational conditions: Consideration of pH, temperature, and other physio-chemical parameters are found to be in wide range. Biosorption used to treat water which especially contains mixed wastes and heavy metals.

The drawback of biosorption process are as follows (Rao and Prabhakar 2011),

- So far all studies are carried out in laboratory-/small scale due to difficulties in scale-up
- The major problem associated with disposal of used adsorbent
- When dealing with wastewater, the mechanism involved in the biosorption process is difficult to predict
- Several parameters should be optimized in order to achieve higher removal efficiency
  - temperature has to be maintained in the range of 20–35 °C throughout the process and
  - pH influences the affinity of the functional groups in the biomass and the competition of metallic ions
  - biomass concentration, for low concentrations the specific uptake will be higher
- Biosorption is mainly used in treating wastewater where varieties of contaminants are already present. The removal of one contaminant may be influenced by the other contaminant.

8.3 Biosorption and the Pollutants

Dye, heavy metal, phenols, and radioactive waste are considered as major toxic pollutants in our environment, and their biosorbent capacities are listed in Table 8.2. Their occurrence due to human activities and the importance of biosorption techniques are highlighted below.

8.3.1 Biosorption and Heavy Metal

Heavy metal contamination may result from natural activities such as forest fire, volcanic eruption, and anthropogenic activities such as mining and industrial manufacturing companies. Cadmium and zinc contamination are found in paddy field (Simmons et al. 2003). The industrialization leads to a sudden increase of

Table 8.2 Data on the biosorption of dye, heavy metal, phenol, and radioactive element by various biosorbents

Compounds	Biosorbents	Compound removal	Biosorption capacity (mg/g)	Reference
Dye	*Stoechospermum marginatum*	Acid blue 25	42.0	Daneshvar et al. (2012a, b)
		Acid orange 7	36.7	
		Acid black 1	23.8	
	Chitosan film	Acid Red 18	194.6	Dotto et al. (2013a)
		FD & C blue no. 2	154.8	
	NaOH treated husk	Brilliant green dye	58.5	Mane and Babu (2011)
	Posidonia oceanica	Astrazon dye	68.97	Cengiz (2012)
	Lignocellulosic waste	Reactive red 2	23.6	Akar et al. (2013)
	Pyracantha coccinea	Orange G	128	Ari et al. (2013)
	Jackfruit leaf powder	Amido black 10B dye	3.7	Ojha and Bulasara (2014)
	White rice husk ash	Brilliant green dye	85.56	Tavliveva et al. (2013,
	Tannery solid waste	Yellow 194, red 357, black 210	300	Piccin et al. (2012)
	Sargassum glaucescens	Acid black 1	30.9	Daneshvar et al. (2012a, b)
	Pumpkin seed hull	Methylene blue	141.1	Hameed and El-Khaiary (2008)
	Coal fly ash	Reactive black	54.3	Pengthamkeerati et al. (2008)
Heavy metal	*Sargassum sinicola*	Copper	3.44	Prado et al. (2010)
	Staphylococcus xylosus	Cadmium(II)	250	Zigova et al. (2007)
		Chomium(VI)	278	
	Pseudomonas sp	Cadmium(II)	143	Zigova et al. (2007)
		Chomium(VI)	95	
	Fucus spiralis	Cadmium	114.5	Romera et al. (2007)
	Pseudomonas Putida	Lead	50.9	Pardo et al. (2003)
		Copper	32.5	
		Cadmium	46.2	
	Enterobacter sp.	Lead	50.9	Lu et al. (2006)
		Copper	32.5	
		Cadmium	46.2	
	Myriophyllum spicatum	Lead(II)	55.12	Yan et al. (2010)

(continued)

Table 8.2 (continued)

Compounds	Biosorbents	Compound removal	Biosorption capacity (mg/g)	Reference
	Nostoc linckia	Chromium(VI)	42.6	Mona et al. (2011)
	Candida sp.	Chromium(VI)	44.38	Jimenez et al. (2009)
	Streptomyces rimosus	Cadmium	63.3	Seletnia et al. (2004)
	Gelatinous colonies	Copper(II)	27.78	Tran et al. (2016)
		Cadmium(II)	28.57	
		Lead(II)	76.92	
	Sargassum oligocystum	Mercury(II)	60.25	Delshab et al. (2016)
		Cadmium(II)	153.85	
		Copper(II)	45.25	
	Saccharomyces cerevisiae	Copper(II)	29.9	Amirnia et al. (2015)
		Lead(II)	72.5	
	Lepiota hystrix	Copper(II)	74.8	Kariuki et al. (2017)
		Lead(II)	62.52	
	Nizamuddin zanardini	Lead(II)	50.41	Montazer et al. (2011)
		Cadmium(II)	19.42	
		Nickel(II)	10.06	
	Sargassum ilicifolium	Lead(II)	195	Tabaraki et al. (2014)
	Chlamydomonas reinhardtii	Mercury(II)	2.2 ± 0.67	Tuzun et al. (2005)
		Cadmium(II)	42.6 ± 0.54	
		Lead(II)	96.3 ± 0.86	
	Laminaria hyperborea Bifurcaria bifurcata Sargassum muticum	Cadmium(II)	23.9 − 39.5	Vilar et al. (2005)
		Zinc(II)	18.6 − 32.0	
	Fucus spiralis	Lead(II)	32.3 − 50.4	
		Lead(II)	64	
	Cystoseira baccata	Cadmium(II)	101	Lodeiro et al. (2006)
		Lead(II)	186	
	Cystoseira crinitophylla	Copper(II)	160	Christoforidis et al. (2015)
Phenol	Date-pit activated carbon	Phenol	262.3	Naas et al. (2010)
	Activated carbon	Phenol	278	Wu and Yu (2006)
	Chitosan calcium alginate blended beads	Phenol, O-chlorophenol	108.69	Siva Kumar Nadavala et al. (2009)
	Organobentonite	Phenol	193.0	Perez et al. (2011)

(continued)

Table 8.2 (continued)

Compounds	Biosorbents	Compound removal	Biosorption capacity (mg/g)	Reference
	Activated carbon (tea industry waste)	Phenol	142.9	Gundogdu et al. (2012)
	Funalia trogii pellets	2-chlorophenol	147.0	Bayramoglu et al. (2009)
	Fungal mycelia	Chlorophenol	5.0	
	Phanerochaete chrysosporium	Phenol	13.5	Farkas et al. (2013)
	Spirulina sp. LEB18	Phenol	159.33	Dotto et al. (2013a)
Radioactive element	Biocomposite	Uranium	50.3	Sule et al. (2011)
	Dictyopteris polypodioides	Uranium	62.5	Bampaiti et al. (2015)
	Saccharomyces cerevisiae	Uranium	113.5	Faghihian and Peyvandi (2012)
	Trichoderma harzianum	Uranium	612	Akhtar et al. (2007)
	RD256	Uranium	354	Akhtar et al. (2007)
	RD257		408	
	Cladophora hutchinsiae	Uranium	152	Bagdaa et al. (2017)
	Rhizopus arrhizus	Americium-241	88.6	Jalali et al. (2004)
	Chitosan-Polyvinyl alcohol	Cobalt(II)	14.39	Zhu et al. (2014)
	Crab shell	Selenium(II)	144.9	Vijayaraghavan and Balasubramanian (2010)
		Europium(II)	49.5	

metal in the soil as well as in the aquatic environment. Plating, battery, dye, tannery, pharmaceutical, nuclear power industries discharge heavy metal such as nickel, chromium, cadmium, mercury, lead, zirconium, uranium, thorium. Cadmium is a lustrous metal, comes from various sources like battery manufacturing, metal plating, mineral processing, fertilizers. Cadmium health risk was first detected in Japan in the time of 1950s when the municipal sewage sludge was used as fertilizer for crops. Cadmium metal causes cancer and produces serious health hazards, and it is mobile in soil and accumulates to the roots, leaves, and stems of the plants (Chakravarty et al. 2010; Vullo et al. 2009; Cruz et al. 2004). Mercury, arsenic, and lead are found naturally on earth; when they react with the earth

components, they create health hazards to the living things. When mercury mixes with water, the bacteria convert mercury to methylmercury and it is accumulated into the fish, and it reaches to the higher level of organisms with the help of food chain. Mercury is found in fluorescent lamps, thermostat switches, and medical equipment. Arsenic are found in some pesticides, and lead are found along with PVC pipes; lead affects the brain in children and also damages the kidney, central nervous system, and liver. Severe exposure leads to abortion and sterility. In industrial concentrations, the outcome of lead is in the range of 200–500 mg/l which is very high when compared to WHO standards (Kwon and jeon 2013; Masoudzadeha et al. 2011; Prado et al. 2010). Properties like the interaction with intramolecular proteins and nucleic acid, high solubility in water, permeability through biological membranes make chromium (VI) more toxic than chromium (III). Due to its toxicity, the discharge standard fixed by pollution control board is 0.05 mg/l (Congeevaram et al. 2007). Chromium is generally used in industries like chrome plating, tanning, paper and wood pulp, and textile. In the Gulf of California due to human activities, pollutants are increased in coastal areas. A research work was done on the shores of sea which is located at Santa Rosalia and in La Paz provides an evidence for the bioaccumulation of copper and phosphorite deposition along the sea shore due to mining activities (Figueroa et al. 2008; Méndez et al. 2006). Many technologies namely precipitation, filtration, ion exchange, electrochemical treatment are found to be ineffective when dealing with the factors like volume, concentration of metals, salinity, temperature of the waste water. Many of these technologies are ineffective when the concentrations are less than 100 mg/l (Ahluwalia et al. 2007; Mulligan et al. 2001).

In recent times, biosorption has been used as alternative technology because of its low cost, biosorbent recovery, minimization in chemical sludge, and biosorption has particular techniques such as acting like a chemical substance in the removal of metal and reusability of biosorbents may be reused and metals are recovered (Özdemir et al. 2009; Vijayaraghavan and Yun 2008; Volesky 2007; Pagnanelli et al. 2000). Extracellular polysaccharides present in the cell wall are responsible for metal adsorption, but it depends upon the species and the growth condition. Cell composition is the most influencing factor in deciding the biological properties of biomass. In gram-positive bacteria, anionic functional groups such as peptidoglycan and teichoic acids and in gram-negative bacteria phospholipids and lipopolysaccharides are the anionic characters which are responsible for the metal binding nature of bacteria (Pagnanelli et al. 2010). The choice of biomass is based on origin, type, and the targeted composition of the solution. This type of biomass consists of different functional groups such as carboxylic, amino, sulfates which are responsible for the binding of metals to the cell wall. The modification of biomass is done using simple pretreatment techniques and modification techniques. Some of the agricultural waste such as peat, coconut shell, coffee leaf, nutshell and industrial waste from various industries are also used as biosorbents (Chuber et al. 2004).

8.3.2 Biosorption and Dyes

Dyes are synthetic organic colorants with complex aromatic structures, having applications in the various industrial fields. More than 9,000 types of dyes have been incorporated in color index. 7 × 105 tonnes of dyes are produced worldwide among them two-thirds have been used by textile industries. Textile industries are ranked one in the usage of dyes for fibers and other industries like pharmaceutical, food, agro industries (Preetha et al. 2015). The textile industries contribute 10–15% used dyes to the effluents. The effluents from dye consuming and manufacturing industries have high biological and chemical demand, without preliminary treatment discharged to the streams, and the esthetic values as well as the characteristics of the water get disturbed. The color hindrances the passage of sunlight to the water bodies; as a result, photosynthesis is affected and the plants and aquatic organisms get affected. In due course of time, the biomass in the water bodies will be increased due to the dead and decay of living substances; as a result, the aquatic ecosystem gets affected. Recently, pollution control board of Delhi sets minimum control standards for dye effluents including decolorization of effluents before emission (Bekc et al. 2008; Garg et al. 2004; Mohan et al. 2002). Among all the dyes water soluble, reactive dyes and bright-colored acid are found to be dangerous and it was not disturbed when treated with conventional treatment systems.

Dyes generally have a complex aromatic structure which makes them difficult to degrade. Dyes are classified as anionic-direct, acid and reactive dyes, cationic-basic dyes, non-ionic disperse dyes (Fu et al. 2001). The anionic- and ionic-based chromophores consist of azo groups. Toxic amines are formed due to the reductive cleavage of amine groups. Reactive dyes are a combination of azo groups and different reactive groups like difluorochloropyremidine, vinyl sulfane, chlorotriazine; they are mostly used in textile industries for its simple applications and low energy consumption. Acid dyes tend to be problematic because they pass through the conventional treatments unaffected. Basic dyes are very bright in low concentrations (O'Mahony et al. 2002; Robinson T et al. 2001). Dye effluents are generally treated with physical or chemical treatment process. This include chemical coagulation, flocculation, sedimentation, filtration, membrane process, ion exchange, oxidation, precipitation, adsorption; these technologies show effective color reduction, but there are some constraints like cost for the plant treatment and maintenance, accumulation of sludge with disposal problems, addition of unwanted chemicals during the process, sensitive to unwanted compounds present in the wastewater. In recent years, a number of studies were focused on microorganisms and the dyes. A wide variety of microorganisms like bacteria, fungi, algae are used in aerobic, anaerobic, and sequential anaerobic–aerobic process (Manu and choudri 2001; Robinson et al. 2001). Many researches show that degradation of biological pollutants mainly depends on the parameter conditions such as aeration, temperature, redox potential, pH to obtain the maximum dye reduction. The efficiency in removal of particular microorganisms on concern dye has to be investigated before treatment as the composition of dye wastewater consists of salts, inorganics,

nutrients, and organic compounds (Donmez 2002; Aksu 2003). Another biological treatment is known as bioaccumulation in which living biomass is used for degrading the dyes, and it has limitations such as energy has to be provided externally, and there is inhibition in cell growth during higher concentrations of cell growth.

Biosorption was found to be a potential treatment for removal of dye. It can be defined as sequestering of organic pollutants using dead biomass which may be bacteria, fungi, yeast, seaweeds; it is considered as low-cost treatment when comparing to other ones. So it can be used as removal techniques for non-biodegradable pollutants such as dyes (Vijayaraghavan and Yun et al. 2008; Khataee and Dehgha 2011; Khataeea and Kasi 2010). Low-cost adsorbents such as barley husk, rice husk, and citrus biomass are employed for the removal of dyes like methylene blue and red BA (Bhatti et al. 2012; Sun et al. 2007; Haq et al. 2011). Due to large surface area, algae also used a biosorbent. The presence of functional group such as amino, phosphate, carboxylic, hydroxyl in the cell wall contributes to a major part in the degradation of dyes (Daneshwar et al. 2007; Tien 2002; Srinivasan and Viraraghavan 2010).

8.3.3 Biosorption and Phenol

Phenol is an organic pollutant and considered as a priority pollutant by US Environmental Protection Agency (USEPA) with a considerable limit of 0.1 mg/l, and World Health Organization permissible limit is 1 µg/l. It is a combustible substance with a pungent smell, and they are considered as protoplasmic toxins. It is soluble in oil, water, and many organic solvents (Yousef et al. 2011; Busca et al. 2008; Ahmaruzzaman 2008). The occurrence of phenols to the environment is due to paint, pesticide, petroleum, and petrochemical industries. Chlorophenols are used in manufacturing process of fungicide, herbicide, pharmaceuticals, etc. (Rubin and Rodiguez 2006; Wu and Yu. 2006). The intrusion of phenol to human body impulses tissue erosion, paralysis in the nervous system, and protein degeneration. Phenol can be removed using physical, chemical, and biological treatments. Treatments like solvent extraction, electrochemical methods, and chemical oxidation are widely used for the removal of phenol compounds. High cost, toxic, and secondary products are some of the drawbacks found in these types of treatments. Because of low cost and wide availability, biosorption is found to be efficient technique.

The use of microorganisms like algae, bacteria, fungi, and agricultural and industrial wastes is used for the removal of phenolic compounds (Bayramoglu and Arica 2009; Navarro et al. 2008). Sargassum multicum algae have been used for the removal of phenol and 2-chlorophenol, and 2, 4-chlorophenol is removed using non-living fungal pellets of Phanerochaete chrysosporium (Sampedro et al. 2007; Aranda et al. 2006). Compounds like Caulerpa scalpelliformis, Funalia trogii, Pleurotus sajor caju, Bacillus subtilis, Phanerochaete chrysosporium, Ca-alginate beads are tried for the removal of phenols, and their adsorption capacity was found to be efficient.

8.3.4 Biosorption and Radioactive Waste

Uranium, Thorium, Selenium, and cobalt are some of the radioactive components. The derivatives are found in various forms in our environment. Uranium is in different isotopes such as U-238, U-235, and U-235. The sources of uranium are from mining and nuclear power generation (Kahouli 2011). Rare earth elements such as lanthanides, scandium, and the elements coming under the group17 in chemical element table and the source of outcome are from wind turbines, batteries in the car, magnets used in a computer. The element cerium plays a role in polishing glass and in the manufacturing of glass and magnets. Americum-234 and Cobalt-60 are also used as radio element in the nuclear industry along with uranium and thorium. Cobalt (II) component is used in paint and pigment, electronic manufacturing, and nuclear power plants (Baun and Christensen 2004). Strontium-90 is seen in the fission products of Fukushima nuclear accident happened on 2011; it is considered as a hazardous substance to our environment and to the living organisms (Nagaoka et al. 2014; Nagaoka et al. 2015). Rare earth elements, as well as radioactive elements, enter the food chain, and bioaccumulation occurs in living organisms (Tai et al. 2010). The potential applications of biosorption using microorganisms attracted researchers in the removal of radioactive elements. The size of the biosorbents may be smaller, but it has high adsorption capacity. Different species such as Pinus brutia, Platanus orientalis, Turbinaria conoides, Agrobacterium sp. HN1 are used in the removal of rare earth metals.

8.4 Factors Consideration in Biosorption Process

8.4.1 Cost of Biosorbents

In biosorption process major amount of investments are done on the processing of biosorbents in order to improve its sorption efficiency. The preprocessing and drying of real biomass will increase its sorption efficiency. This stage includes a collection of industrial biomass as well as natural biomass from various environments, for example, marine algae are collected from high seas or offshore areas. It is a well-known fact that different biomaterial compounds are made ready to use as biosorbents using different technologies and treatment. The overall treatment depends upon the cost of the prepared biosorbent. The cost of biosorbents should be lower than that of ion exchange, membrane process, electrochemical treatment, advanced oxidation process, etc. Harvesting and drying of algal biomass seem to be of high value. The cost constraint makes the preparation of biomass in laboratory level and converting into real time if it gives beneficiary results. Type and source of a biomass play major role in determining the cost of biosorbents. Manufacturers will take into account of both production and maintenance of biomass if the biomass has to be cultivated. Choosing naturally available biosorbents decreases the

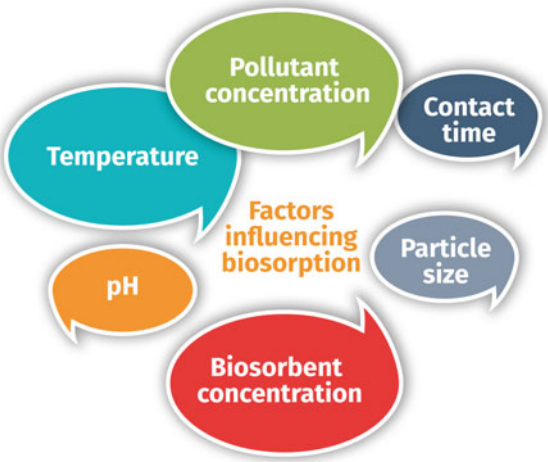


Fig. 8.2 Some of the factors influencing the adsorption process

production cost. The waste from industries such as food, leather, pharmaceutical, and enzyme are free of cost because of disposal problems faced by these industries. The only cost spent are in the places of transportation and further treatment (Volesky and Naja 2007; Bagdaa et al. 2017; Tran et al. 2016) (Fig. 8.2).

8.4.2 Biosorbent Regeneration

Regeneration of biosorbents is an important step due to the investments in preparation and generation of biosorbents. The recovery options are to be cheap so that the used biomass can be utilized for multiple cycles. Depending upon the type and the mechanism of biosorbents, eluents are chosen. The following requirements are necessary for choosing the eluants, they are (1) it should not affect the biomass, (2) eco-friendly, (3) cost-efficient (Vijayaraghavan and Yun 2008; Zhu et al. 2014). Both acids and base medium were used for desorption process. The eluents like $CaCl_2$ with HCl, EDTA, NaOH are reported.

8.4.3 Biosorbent Immobilization

The biosorbents which are formed from microorganisms are of small in size; densities, mechanical strength, and rigidity are found to be low. Even though they have many advantages like low process cost, rapid equilibrium attainment, high adsorption capacity, improving biomass–liquid separation, mechanical strength, stability, efficiency in the removal of metal ions, increase in lifetime of adsorbent (Shashirekha et al. 2008; Wang and Chen 2009), the main drawback in using

biomass as it includes solid–liquid separation problems, swelling of biomass (Vijayaraghavan and Yun 2007a, b). Among many techniques, immobilization technique was found to be practical.

Three processes such as biochemical reaction, adsorption, and mass transfer occur simultaneously as well as within the adsorbent. Packed or fluidized bed reactors and usage of polymeric matrix make immobilization more efficient. The benefits like biomass regeneration, particle size control, liquid–solid separation, and minimal clogging. The polymeric matrix used in biosorbents includes sodium alginate, polysulfone polyacrylamide, and polyurethane (Vijayaraghavan et al. 2007). The polymeric matrix plays a key role in immobilization, and it determines the mechanical and chemical strength of the biosorbent. However, there are some limitations like mass transfer and additional processing cost, i.e., the amount invested on immobilization process. Mass transfer is a key factor in determining the equilibrium attainment of biosorbent.

Two types of immobilization were found normally; they are entrapment and attachment. Natural polymers (alginate, rubber, cellulose derivatives) and synthetic polymers (nylon, teflon, polyester, polyethylene) which are derived from petroleum and oil coming under the category of entrapment are also used. Alginate is used in major extent for cell entrapment because of its non-toxicity in nature (Couto 2009; Dalel et al. 2013). Bark, leaf, flower, and stem of trees have large surface area and cavities that are due to the biostructural matrix of plant materials, for example, papaya wood, loofa sponge from the dried fruit of Luffa cylindrica, activated carbon (Podder and majumder 2016; Saeed et al. 2009; Iqbal and Saeed 2007) are used. In some cases, volcanic rock matrix is used for immobilization technique. Bacteria are immobilized using volcanic rock matrix, and it consists of the compounds such as SiO_2, Al_2O_3, Fe_2O_3, CaO, Na_2O, MgO, K_2O, FeO, and TiO_2. The volcanic rock matrix is grounded and sieved according to the need (Ni et al. 2012). The active sites in silica gel are found to be high, and it is also used as immobilization matrix (Akar et al. 2009). Immobilization of microorganisms in natural or synthetic polymeric matrix increases its mechanical strength, porosity, size, and resistance. Entrapment is the most commonly used technique for immobilization of organisms. While using the microorganisms in reactors, immobilized biosorbents are found to be more efficient in avoiding clogging and solid–liquid separation, regeneration of biomass are made easy etc., (Li et al. 2007; Wu and Yu 2007).

8.4.4 Charge of Biomass

The cell wall of the biomass plays a major role in sorption. The binding sites within the cell wall are to be induced in order to improve the biosorption capacity. The positive charge pollutant has to be attracted to negative charged binding sites. Phosphonate, sulfonate, hydroxyl, amine are some of the functional groups available for binding the dyes. When the binding site strength is low, the biomass exhibits low adsorption capacity. To overcome this, the functional groups which are

less important are to be converted to active binding sites. Many chemicals, as well as thermal treatments, are available (Won et al. 2008). Sludge which has high negative charge exhibits high biosorption. Proteins, carbohydrates, nucleic acid, and lipids are responsible for the negative charge. The net charge of the biomass has to be examined in order to provide the binding sites, and the binding sites also depend on specific applications (Farkas et al. 2013; Dotto et al. 2013a, b).

8.4.5 Biosorption Process Design

Three types of design are followed while using biosorption experiments. They are packed bed, fluidized bed, and continuous stirred bed reactors. From the earlier studies, it is well known that packed bed columns are suitable for biosorption process. Liquid–solid separation is found to be good in this type, and the effluent quality was good (Chu 2004; Aksu and Gonen 2004). Scaling was found to be minimal. The efficiency will be increased if immobilized biosorbents are used in packed bed and clogging will be eliminated when biosorbents are used in pelletized form. Regeneration of biomass was found to be easy. Occasionally, fluidized and continuous stirred bed reactors are used. Biomass should be in powdered form while using stirred bed reactors, and the cost, operation, and maintenance are found to be high. Fluidized bed requires high flow rate. There are some difficulties in achieving high flow rate when using fluidized bed (Aksu 2005; Vijayaraghavan and Yun 2008).

8.5 Biomass Types

Availability has to be taken into account when choosing the type of biomass. Biosorbents can be obtained either by nature such as fungi (molds, mushrooms, yeast), bacteria (gram-positive bacteria, gram-negative bacteria, *Cyanobacteria,* etc.,), algae (micro-algae, macro-algae, seaweed), industrial wastes (activated sludge, food industries, fermentation industries, etc.,), agricultural wastes (bark, leaves, stem of trees, fruit/vegetable waste, rice and wheat straws, and husk). Table 8.3 represents different species and their adsorption capacity for different pollutants. Many types of research are conducted under metal and dye treatments using biosorbents. Some biosorbents are seemed to be metal-specific and some bind over a wide range of metals without priority (Volesky and Holan 1995; Vieira 2000). The origin of biomass is from various sources such as (i) organisms which grow quickly, (ii) organisms which are available in a large amount, (iii) industrial and agricultural waste which are available at low cost. Generally, biosorbents are found naturally, mainly from algae, fungi, and bacteria. They are modified using acids, base, or thermal treatments. Figure 20.1 refers to the classification and processing steps in preparing biosorbent (Fig. 8.3).

Table 8.3 Data on the various types of biosorbents used for the treatment purpose

Biosorbent types	Biosorbents	Compound removal	Biosorption capacity (mg/g)	Reference
Algae	*Spirulina*	Yellow 12	714	Marzballi et al. (2017)
	Chlorella vulgaris	Cd (II)	85.6	Aksu (2001)
	Spirogyra	Copper	38.2	Lee and Chang (2011)
	Ceramium virgatum	Chromium	26.5	Sari and Tuzen (2008)
	Cladophora hutchinsiae	Selenium	74.9	Tuzen and Sari (2010)
	Spirogyra condensate, Rhizoclinium hieroglyphicum	Chromium	14.82 11.81	Onyancha et al. (2008)
	Spirogyra neglecta	Lead(II)	116.1	Singh et al. (2007)
		Copper(II)	115.3	
	Tricho viride	Lead(II)	1825.2	Singh et al. (2010)
		Cadmium(II)	1597.92	
		Copper(II)	1215.84	
	Ulva fasciata, Sargassum sp.	Copper(II)	73.5 72.5	Karthikeyan et al. (2007)
	Chlorella vulgaris	Uranium(VI)	26.6	Vogel et al. (2010)
	Scenedesmus obliquus	Zinc	209.6	Monteiro et al.2009)
	Cladophora fascicularis	Lead(II)	198.5	Deng et al. (2006)
	Anabaena spharica	Cadmium(II)	111.1	Abdel et al. (2013)
		Lead(II)	121.95	
	Chlorella vulgaris	Cadmium	68.5	
		Nickel	28.3	
	Chlamydomonas reinhardtii	Lead(II)	96.3	
Bacteria	*Rhodococcus opacus*	Nickel(II)	7.63	Cayllahua et al. (2009)
	Enterobacter sp.	Copper	32.5	Lu et al. (2006)
		Cadmium	46.2	
	Pseudomonus putida	Lead(II)	271.7	Ulsu and Tanyol. (2006)
		Copper(II)	46.8	
	Arthrobacter sp.	Copper(II)	175.87	Hasan and Srivastava. (2009)

(continued)

Table 8.3 (continued)

Biosorbent types	Biosorbents	Compound removal	Biosorption capacity (mg/g)	Reference
	Bacillus sp.	Copper(II)	89.62	Zheng et al. (2008)
	Geobacillus toebii	Cadmium(II)	9.2	Ozdemir et al. (2009)
		Copper(II)	48.5	
		Nickel(II)	21	
		Zinc(II)	21.1	
		Manganese(II)	13.9	
	Geobacillus thermoleovorans	Cadmium(II)	38.8	Ozdemir et al. (2009)
		Copper(II)	41.5	
		Nickel(II)	42	
		Zinc(II)	29	
	Bacillus sp. FM1	Chromium(VI)	64.102	Masood and Malik (2011)
		Copper(II)	78.125	
	Sphaerotilus natans	–	5.4	Beolchini et al. (2006)
Fungi	*Pleurotus eryngii*	Flouride	66.6	Amin et al. (2015)
	Saccharomyces cerevisiae	Fluoride	12.227	Qiu and Feng (2017)
	Fomes fomentarius	Methylene blue	204.38–232.73	Maurya et al. (2006)
	Phellinus igniarius		25.12–36.82	
	Paecilomyces lilacinus	Cadmium	41.99	Zeng et al. (2013)
	Schizophyllum commune	Phenolic	120	Kumar et al. (2011)
		2-chlorophenol	178	
		4-chlorophenol	244	
	Lactarius scrobiculatus	Lead(II)	56.2	Anayurt et al. (2009)
		Cadmium(II)	53.1	
	Coriolus versicolor	Zirconium	71.0	Sana et al. (2013)
	Aspergillus flavus	Lead(II)	20.75–93.65	Iram and Abrar (2015)
	Aspergillus niger		3.25–172.25	
	Saccharomyces cerevisiae	Manganese(II)	41.3	Fadel et al. (2017)
Industrial waste	Spent grain	Copper	10.47	Lu and Gibbs (2008)
	Activated sludge	Mercury(II)	31.6	Kilic et al. (2008)

(continued)

Table 8.3 (continued)

Biosorbent types	Biosorbents	Compound removal	Biosorption capacity (mg/g)	Reference
	Chlorella sorokiniana	Chromium(III)	9.26 ± 1.28	Nasreen et al. (2008)
			58.80 ± 1.76	
	Egg shell	Methylene blue	94.9	Abdel et al. (2017)
		Congo red	49.5	
Agricultural waste	Sawdust	Malachite green dye	52.610	Deniz et al. (2017)
	Pinus roxburghii	Arsenic	3.27	Shafique et al. (2012)
	Saccharum bengalense	Congo red	125	Din et al. (2013)
	Sorghum straw (SS)	Chromium(III)	6.96	Bernardo et al. (2009)
	Oats straw (OS)		12.97	
	Agave bagasse (AB)		11.44	
	Sugarcane bagasse	Zirconium(IV)	107.4	Kausar et al. (2016)
	Rice husk	Tetracycline	8.37	Chen et al. (2016)
	S clerocarya birrea	Lead(II)	14.02	Moyo et al. (2015a, b)
		Copper(II)	7.22	
	Phytolacca americana L.	Lead(II)	2.66	Moyo et al. (2015a, b)
	Musa paradisiaca	Cadmium	67.2	Vargas et al. (2012)
	Citrus limonum	Copper	12	
	Citrus sinensis	Lead	28.8	
	Peanut shells	Lead(II)	39	Tasar et al. (2014)
	Kappaphycus alvarezii	Chromium(III)	0.86	Kang et al. (2011)
	hanerochaete chrysosporium	Lead(II)	76.33	Xu et al. (2012)
	Eucalyptus citriodora	Uranium	7.75	Bhatti and Hamid (2014a, b)
	Sugarcane bagasse	Mercury	35.71	Bhatti and Hamid (2014a, b)
	Solanum lycopersicum	Copper(II)	46.04	Yargic et al. (2014)

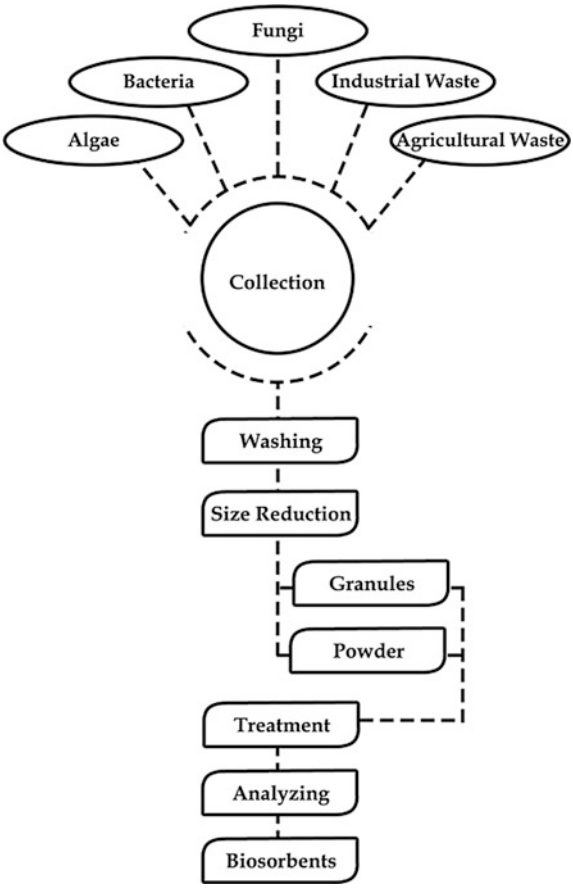


Fig. 8.3 Various types of biomass and its processing steps

8.5.1 Biosorption Using Algae

The cell wall of micro-algae includes several macromolecules such as polysaccharides which have charged groups, which are responsible for the binding of pollutants. The amphoteric properties of the cell wall are due to the anionic and cationic sites. However, the use of micro-algae for wastewater has been limited due to the separation of algae from treated water. Generally, centrifugation and industrial filtration are used for harvesting micro-algae but they are not cost-effective. Immobilization of algae using a polymeric matrix such as silica gel, carrageenan, and alginate increases the efficiency. The immobilized algae can be used for several cycles. Several mechanisms like surface adsorption, biosorption, adsorption on extracellular biopolymers, and adsorption on extracellular biominerals are studied. The functional groups like hydroxyl, carboxyl, carbonyl, and amino groups are available in the algal cell wall (Chojnacka et al. 2005).

The use of algae is economically viable because of its wide availability, economic and eco-friendly, less chemical or biological sludge, high volume-to-surface area ratio, regeneration capacity. The chemical structure such as porosity, swelling effects, flexibility, rigidity, and the chemical composition refers to the chemical groups that are generally known as active sites for the binding of metals. The electrostatic attraction and the complexation on the cell wall of algae play an important role. Alginic acid or alginate is embedded in Phaeophyta with sulfated polysaccharide in a smaller amount and the Rhodophyta consist of sulfated galactan and amorphous embedded polysaccharides matrix. These properties make algal groups excellent biosorbents (Davis et al. 2003; Plazacazon et al. 2014). They are eukaryotic organisms produce their food by means of photosynthesis, and they are known as primary producers and it determines the hydrophobic organic compounds in an aquatic system. They are generally known as thallophytes, and thallophytes are responsible for photosynthesis in autotrophic organisms (Torres et al. 2008).

From many research it was concluded that, algae was used as a biosorption substance inorder to remove mainly heavy metals and it has many advantages like high removal efficiency, efficiency in cost and minimum sludge obtainment which is found to be non-toxic. Hydroxyl, carboxyl, amino, and sulfhydryl are the binding sites generally seen in algae (Gonzalez et al. 2011). Algae are the ancestor for nutrient organic matter (NOM), and NOM consists of rubbery and glass domain. Algae are known by its good low-cost sorbivity and mainly used for the production of biofuel (Carro et al. 2011; Singh et al. 2011). Due to the presence of cellulose and protein on the cell wall with a lot of proteins bounded on green algae, these are helpful for binding of metals. The polysaccharides consist of amino, carboxyl, sulfate, and hydroxyl groups, which are helpful in biosorption process. Brown algae consist of three components in its cell wall; they are cellulose, sulfated polysaccharides, and alginic acid. Due to its aligate content, brown algae show higher uptake to different metal ions (Bulgariu and Bulgariu 2012; Karthikeyan et al. 2007; Lee and Chang 2011; Romera et al. 2007).

Different reactions such as coordination, chelation of metal, ion exchange, adsorption, and microprecipitation on the cell wall are responsible for metal removal. Two types of uptake are possible, active and passive. During active uptake, ion transports across the cell membrane and it is metabolism-dependent and in passive uptake metabolism where metal ions absorb onto the cell surface. In some cases, metal ion transportation occurs due to passive diffusion. The factors such as metal concentration, biomass, pH, temperature, and metabolism are considered when using algae. Studies are made using living as well as non-living biomass, and the attraction has shifted toward non-living algae because inactive biomass has the greater metal binding capacity, low cost, abundantly available; they do not require nutrient to grow. Before proceeding with biosorption, in order to improve the adsorption capacity as well as binding ability of biomass prior treatment should be provided. $CaCl_2$, HCL, NaOH, NaCl, ethanol.

8.5.2 Biosorption Using Bacteria

In recent times, cyanobacteria have been used for the removal of Cr(VI) heavy metal. Around 80% of Cr(VI) are removed (Kiran et al. 2007). The properties of bacteria such a nutrition in autotrophic mode, smaller size are able to grow in controlled conditions uses bacteria in the wider range of environmental conditions. Fast growth and non-toxic nature make bacteria favorable for the use of biosorbents. Instead of using biomass as a dry powder, immobilized form gives better results in adsorption. The recovery of biomass was made easy if the biomass is in rigid form and it also avoids biomass-liquid density. Biomass is converted into a polymeric matrix or in the form of gel or beads to increase its mechanical strength, which increases the removal efficiency as compared to the free cell biomass (Garido et al. 2005). A lot of *cyanobacteria* are dumped after using it as biosorbent. Many types of researches are done in order to extract hydrogen from this biomass but the biomass after the extraction of hydrogen is again a serious issue (Dutta et al. 2005; Yan and Vijayaraghavan 2003). *Rhodococcus opacus* is a gram-positive bacteria which consist of polysaccharides, mycolic acid, lipid groups, and carboxylic groups on the surface, and the behavior of these compounds impacts on cell wall (Botero et al. 2007).

Different shapes of bacteria are seen; they include cocci, rods, spiral, and filamentous. Eubacteria do not have cell nuclei but have a cell wall. The strength of bacterial cell wall depends upon peptidoglycan, and the cell shape depends on peptidoglycan. All cell walls of the bacteria are not the same. The cell wall is the important component that differentiates the species. Two types of bacteria exist in common; gram negative and gram positive. Gram positive consist of thick peptidoglycan layer which is connected by amino acid bridges and consist of 90% of peptidoglycan. The substance teichoic acid gives overall negative charge, that is due to the phosphodiester bonds between the teichoic acid monomers and another type is known as gram-negative bacteria which consists of 10–20% peptidoglycan, and the cell wall additionally consists of phospholipids and lipopolysaccharides. Chemical modification and genetically modified bacteria are generally used as biosorbents (Vijayaraghavan and Yun 2008). Depending on binding mechanisms, a number of surface binding sites in bacteria are selected for pollutant removal. Due to its small size and capability to grow under certain conditions and its adaptability over a wide range of environmental conditions, bacteria are used as biosorbents (Eman. 2012; Kinoshita et al. 2013).

8.5.3 Biosorption Using Fungi

Fungi are non-pathogenic and robust for humans and animals, and it can be easily produced using fermentation process or from the industrial waste. Cell walls are responsible for the sequestration of heavy metal, and cell wall alone does not

contribute to the removal of pollutants. Various components such as polysaccharides contribute to 90% constituents to the cell wall. The ultrasonic studies reveal that the outer layer consists of glucan, mannans, and the inner layer made up of parallel arrangements of chitin chain, cellulose chain, the yeast of non-cellulosic glucan, and there will be a continuous transition between two layers.

Many studies showed that fungi as a good biosorbent for the removal of metals because of its efficiency when compared to conventional adsorbents like activated carbons, brown algae, polymers. Many fungal biosorbents are found in abundant, and they have less nutrient requirement. The separation of biomass from the treated medium requires a simple operation. Modification of biomass is possible using physical and chemical treatments. Fungi is a eukaryotic, non-photosynthetic organism, macromolecular structures consist of glucans, mannans, proteins, and chitins, and other substances such as polysaccharides, pigments, and lipids are responsible for binding efficiency. It depends on organic substance as their whole source for their growth and metabolic activity. Molds are known as filamentous fungi and mycelial structures are seen on molds. The sexual pore available on mold provides resistance against heat, freezing, drying, etc. Rhizopus arrhizus is a type of mold. The size of mold varies from 5 to 20 μm. Molds are generally formed as pellets, and the size of pellet varies from 50 μm to 1 mm; it depends on the type of mold and growth conditions. Fungi are classified into four categories such as phycomycetes, ascomycetes, basidiomycetes, and deutromycetes. In many research, the phenolic compounds have been removed using the fungal biomass namely, *bacillus subtilis*, *fungal trogil*, *Trametes versicolor*, *Emericella nidulans* and *Penicillium miczynskii*. Phanerochaete chrysosporium (Wu and Yu 2006; Bayramoglu et al. 2009; Kumar et al. 2009; Matinari et al. 2007).

Fungi easily grow in a faster manner, and they can be modified genetically as well as morphologically. The fungi groups are mostly robust than bacteria. Bioremediation occurs in the tropical forest than in temperate because of climatic conditions (Gadd 2001). Aspergillus efficiently removes nickel and chromium. Recent research provides information regarding the fungi species involved in treating the sludges (Lacina et al. 2003). The inter- and intracellular enzymes are useful for the absorption of pollutants. These enzymes are helpful in degradation of dye. *Pleurotus eryngii* are edible species seen in the oyster of the mushroom genus which utilize dyes as a carbon source and use it for growth. If the carbon or nitrogen source is not available, fungi will secrete an enzyme which degrades the complex molecules to simple molecules (Hadibarata et al. 2011; Gao and Liu 2010). White rot fungi secretes lignin peroxidase, manganese peroxidase, and laccase which are known as ligninolytic enzymes, these enzymes have the ability to degrade the pollutants (Hadibarat and Kristanti 2012). The white rot fungi species such as *coriolopsis sp, Penicillium simplicissimum,* and *pleurotus* show degradation of dye. Many disadvantages like long growth phase, enzyme unreliable production, and large reactor size are seen. Usage of fungi alone has a disadvantage, i.e., the system is no stable and after 20–30 days, bacteria will grow and dominate the system (Anastasi et al. 2011; Gadd 2009; Quan et al. 2004).

8.5.4 Biosorbents from Agricultural Waste

Recent studies reveal that biosorbents from agricultural waste such as bark of trees, hazelnut shell, rice bran, wheat husk, rice husk, wheat bran, sawdust (sawdust was naturally available abundant biomass in forest as well as in agriculture), tea leaves, maize corn cob, sugarcane bagasse, apple, banana, orange peels, sugar beet pulp, soybean hulls, grapes stalks, sunflower stalks have been tried (Cimino et al. 2000). Agricultural adsorbents consist of cellulose and hemicellulose and lignin which are responsible for hydroxyl groups. Cellulose is pure organic polymer consist of anhydroglucose bound together in a large straight-chain molecule. By means of b-(1, 4) glycosidic linkages, anhydroglucose are held together, due to these linkages, cellobiose forms unit of cellulose chains. The intramolecular and intermolecular hydrogen bonds are formed in between OH groups; microfibril is formed from the bundles of linear cellulose which are the base for cell wall (Demirbas 2000; Hashem et al. 2007). Apart from hydroxyl groups, it has a variety of other functional groups such as carboxyl, phenolic, polysaccharides, amido, amino, alcohol, acetamido. These functional groups donate an electron pair to form complexes with a metal ion or substitute hydrogen ions instead of metal ions. Agricultural waste has abundant binding groups (Cedillo et al. 2013; Marin-Rangel et al. 2011; Zafar et al. 2007).

Many studies reported the biosorption of metal ion using agricultural waste. Strong affinity and high selectivity toward heavy metal are the reason for the usage of agricultural waste (Banerjee et al. 2012). As agricultural wastes usually are low of cost because of abundantly available agricultural originating materials and more over agricultural waste can be processed, used for treatment and recovered without any harmful effects on the environment. It is known as an effective adsorbent for the removal of metal and metal ions and the recycling of agricultural waste and their by-products for heavy metals and also believed to be economically friendly (Ngah et al. 2008; Okoro and Okoro. 2011). Choosing a good agricultural biosorbent is not an easy task for researchers. Many attempts were made in this area. Abundant availability and cheapness are the main key factors in selecting the biosorbents and some other factors like desorption rate, regeneration capacity, and the negligible release of unexpected compound (Park et al. 2010). The adsorption capacities of heavy metals depend on the type of agricultural waste and the pretreatment process provided. In a study, it is released that the removal of heavy metal decreases from cotton stalks, maize stalk, and rice straw. The removal efficiency of the cotton stalk is high; this is due to the presence of cellulose, hemicellulose, and lignin (Mosa et al. 2011). In another research, zinc, cadmium, and iron were investigated with the biosorbents such as rice hull, sawdust, sugarcane bagasse, and wheat straw, and the higher adsorption was found in rice hull; this is because of silanol group presence (Osmon et al. 2010).

Zerovalent iron particles are an environmentally friendly agent used for removing the pollutants present in the environment. Zerovalent iron particles generally have large surface area and high reactivity. This property makes them unstable in an aqueous environment. In order to stabilize, zerovalent iron particles

are blended with biochar as it increases the efficiency of removal. Rice husk consists of floristic fiber, protein, and dome functional groups such as hydroxyl, amidogen, and carboxyl (Han et al. 2004), and additionally, rice husk is found abundant as a by-product from agriculture. Agricultural waste can be easily obtained because of its low cost. (i) Commercial value is low and it is readily available in nature. (ii) They are coming under the category of renewable sources. (iii) They are high affinity toward removal of metals (Santos et al. 2013; Chatterje et al. 2010).

8.5.5 Biosorption from Industrial Waste

The contamination of heavy metal in water bodies is a major issue, and it is harmful to the living things. The discharge from industries like battery manufacturing, automotive, metal plating, tanneries, welding, paper, and pulp industries releases a different concentration of metals and other pollutants in a large amount (He and Chen. 2014). The trace amount of this substance also produces harmful effects to the environment. Among many processes, biosorption was found to be efficient technique due to its regenerative property, efficiency in removal (Vijayaraghavan and Yun 2008). It is found to be a suitable technique for our environment. Drawbacks such as pH of biomass and slow removal rate are to be rectified. The use of natural biosorbents is generally specific to particular ion removal, in order to remove multi-metal ion different biosorbent need to be applied. The industrial waste from whiskey, brewery, leather, wastewater treatment plants, poultry is used as biosorbents after treatment. As it is a sludge, it consists of active as well as dead biomass, and at the cell wall of biomass, different types of chemical compositions are seen which helps in fast removal of metal ion when comparing to microorganism biomass. In some cases, the microbial organisms are separated from activated sludge or from contaminated soil and used as biosorbents due to its high efficiency in removal (Abdel et al. 2017; Ramrakhiani et al. 2017; Nasreen et al. 2008).

8.6 Application of Biosorbents

Many natural biosorbents are used with little modification in the preparation. In recent times, industrial waste is also used as biosorbents after treatment. Biosorption is known as cost-effective and potential technique, and it treats a large amount of wastewater including heavy metals. Two trends are followed; one is using hybrid technologies and another one using commercial biosorbents just like ion-exchange resin (Volesky 2007). The difficulties in using biosorption induce people to use other technologies such as bioreduction and bioprecipitation. The research on living cells is also under process instead of dead cells. For large-scale treatments, the combination of the biological process as well as the process like chemical

precipitation, membrane technology, and electrochemical process is also done. High-value pharmaceutical is purified using biosorption process, compounds such as proteins, pharmaceuticals, and drugs are recovered using biosorption. The advantages of biosorptions are (1) cost-efficient, (2) more versatile and flexible, (3) metal concentration reduction level is efficient, (4) regeneration capacity, and (5) biosorption capacity. In developing countries, biosorption is used as most efficient technology due to following reasons such as (1) large availability of biomaterials, (2) shortage of advanced water treatment systems, (3) biosorption was considered as efficient and cheaper method when comparing to other advanced technologies. Biosorption was compared with the ion-exchange resins in the market. Ion exchange is selective and gives anticipated removal efficiencies. In the case of biosorption, many functional groups are available so there is no problem in selectivity and the efficiencies are achieved. Instead of using biosorbents in powdered form, granulated forms are used. It avoids clogging, and there is a good separation between liquid and solid (Bhatti and Hamid 2014a, b; Xu et al. 2012).

8.7 Conclusion

Biosorption technique for the removal of toxic pollutants such as hazardous waste, radioactive waste, fertilizers, pharmaceutical was found to be efficient when comparing to conventional techniques. The cost of the raw materials for the preparation of biosorbents is found to be low, and there is no huge capital investments required. As biosorbents have regeneration capacity, it can be utilized for several cycles without any compromise in efficiency. It was found that the combination of biosorption with other techniques like membrane technology, electrochemical treatment, photo catalysis, and ozonation increases the removal efficiency tremendously. To increase the mechanical strength, chemical strength and rigidity of biosorbents which are derived from living species such as fungi, bacteria, and algae and from agricultural and industrial waste are immobilized using synthetic and natural polymeric matrix. Immobilization is another higher end technique in biosorption which increases the resistance, surface area, and porosity of biosorbents. On the whole, biosorption was found to be suitable and a cheaper technique for all sorts of pollutants. The selectivity and specificity of suitable biosorbents for particular pollutant matters.

References

Abdel-Aty AM, Ammar NS, Ghafar HHA et al (2013) Biosorption of cadmium and lead from aqueous solution by fresh water alga *anabaena sphaerica* biomass. J Adv Res 4:367–374

Abdel-Khalek MK, Abdel Rahman MK, Francis AA (2017) Exploring the adsorption behavior of cationic and anionic dyes on industrial waste shells of egg. J Environ Chem Eng 5(1):319–327

Ahluwalia S, Goyal D (2007) Microbial and plant derived biomass for removal of heavy metals from wastewater. Bioresour Technol 98:2243–2257

Ahmaruzzaman M (2008) Adsorption of phenolic compounds on low-cost adsorbents: a review. Adv Colloid Interface Sci 143:48–67

Akar ST, Yilmazer D, Celik S et al (2013) On the utilization of a lignocellulosic waste as an excellent dye remover: modification, characterization and mechanism analysis. Chem Eng J 229:257–266

Akar T, Kaynak Z, Ulusoy S et al (2009) Enhanced biosorption of nickel(II) ions by silica-gel-immobilized waste biomass: biosorption characteristics in batch and dynamic flow mode. J Hazard Mater 163:1134–1141

Akhtar K, Akhtar MW, Khalid AM (2007) Removal and recovery of uranium from aqueous solutions by *richoderma harzianum*. Water Res 4:1366–1378

Aksu Z (2001) Equilibrium and kinetic modelling of cadmium(II) biosorption by C.6 *ulgaris* in a batch system: effect of temperature. Sep Purif Technol 21:285–294

Aksu Z (2003) Reactive dye bioaccumulation by *saccharomyces cerevisiae*. Process Biochem 38:1437–1444

Aksu Z (2005) Application of biosorption for the removal of organic pollutants: a review. Process Biochem 40:997–1026

Aksu Z, Gönen F (2004) Biosorption of phenol by immobilized activated sludge in a continuous packed bed: prediction of breakthrough curves. Process Biochem 39:599–613

Amin F, Talpur FN, Balouch A et al (2015) Biosorption of fluoride from aqueous solution by white —rot fungus *pleurotus eryngii* ATCC 90888. Environ Nanotechnol Monit Manage 3:30–37

Amirnia S, Ray MB, Margariti A (2015) Heavy metals removal from aqueous solutions using saccharomyces cerevisiae in a novel continuous bioreactor–biosorption system. Chem Eng J 264:863–872

Anastasi A, Parato B, Spina F et al (2011) Decolourisation and detoxifcation in the fungal treatment of textile wastewaters from dyeing processes. New Biotechnol 29:38–45

Anayurt RA, Sari A, Tuzen M (2009) Equilibrium, thermodynamic and kinetic studies on biosorption of Pb(II) and Cd(II) from aqueous solution by macrofungus (Lactarius scrobiculatus) biomass. Chem Eng J 151:255–261

Andleeb S, Jiang Z, Rehman K, Olajide EK, Ying Z (2016) Influence of soil pH and temperature on atrazine bioremediation. J Northeast Agric Univ 23(2):12–19

Aranda E, Sampedro I, Ocampo JA, García-Romera I (2006) Phenolic removal of onative-mill dry residues by laccase activity of white-rot fungi and its impact on tomato plant growth. Int Biodeter Biodegr 58:176–179

Ari AG, Celik BS (2013) Biosorption potential of orange G dye by modified *pyracantha coccinea*: batch and dynamic flow system applications. Chem Eng J 226:263–270

Bagdaa E, Tuzen M, Sar A (2017) Equilibrium, thermodynamic and kinetic investigations for biosorption of uranium with green algae (*cladophora hutchinsiae*). J Environ Radioact 175–176:7–14

Bampaiti A, Yusan S, Aytas S (2015) Investigation of uranium biosorption from aqueous solutions by *dictyopteris polypodioides* brown algae. J Radioanal Nucl Chem. https://doi.org/10.1007/s10967-015-4289-9

Banerjee K, Ramesh ST, Nidheesh PV, Bharathi KS (2012) A novel agricultural waste adsorbent, watermelon shell for the removal of copper from aqueous solutions. Iranica J Energy Environ 3:143–156

Baun DL, Christensen TH (2004) Speciation of heavy metals in landfill leachate: a review. Waste Manage Res 22:3–23

Bayramoglu G, Arica MY (2009) Construction a hybrid biosorbent using *scenedesmus quadricauda* and ca-alginate for biosorption of Cu(II), Zn(II) and Ni(II): kinetics and equilibrium studies. Bioresour Technol 100:186–193

Bayramoglu G, Gursel I, Tunali Y, Arica NY (2009) Biosorption of phenol and 2-chlorophenol by *funalia trogii* pellets. Bioresour Technol 100:2685–2691

Bekc Z, Ozveri C, Seki Y, Yurdakoc K (2008) Sorption of malachite green on chitosan bead. J Hazard Mater 154:254–261

Beolchini F, Pagnanelli F, Toro L (2006) Ionic strength effect on copper biosorption by *sphaerotilusnatans*: equilibrium study and dynamic modelling in membrane reactor. Water Res 40:144–152

Bernardo GRR, Rene RMJ, Catalina ATM (2009) Chromium (III) uptake by agro-waste biosorbents: chemical characterization, sorption–desorption studies, and mechanism. J Hazard Mater 170:845–854

Bhatti HN, Akhtar N, Saleem N (2012) Adsorptive removal of methylene blue by low-cost Citrus *sinensis bagasse*: equilibrium, kinetic and thermodynamic characterization. Arab J Sci Eng 37:9–18

Bhatti HN, Hamid S (2014a) Mercury biosorption from aqueous solutions by sugarcane bagasse. J Taiwan Inst Chem Eng 44:266–269

Bhatti HN, Hamid S (2014b) Removal of uranium(VI) from aqueous solutions using eucalyptus citriodora distillation sludge. Int J Environ Sci Technol 11(3):813–822

Botero AEC, Torem ML, de Mesquita LMS (2007) Fundamental studies of *rhodococcus opacus* as a biocollector of calcite and magnesite. Min Eng 21:1026–1032

Bulgariu D, Bulgariu L (2012) Equilibrium and kinetic studies of heavy metal ions biosorption on green algae waste biomass. Bioresour Technol 103:489–493

Busca G, Berardinelli S, Resini C (2008) Technologies for the removal of phenol from fluid streams: a short review of recent developments. J Hazard Mater 160:265–288

Carro L, Barriada JL, Herrero R, Sastre de Vicente ME (2011) Adsorptive behavior of mercury on algal biomass: competition with divalent cations and organic compounds. J Hazard Mater 192:284–291

Cayllahua RJEB, Carvalho RJ, Torem ML (2009) Evaluation of equilibrium, kinetic and thermodynamic parameters for biosorption of nickel(II) ions onto bacteria strain. Miner Eng 22:1318–1325

Cedillo MJI, Olguí MT, Fall C, Cruz AC (2013) As(III) and As(V) sorption on iron-modified non-pyrolyzed and pyrolyzed biomass from Petroselinum crispum (parsley). J Environ Manage 117:242–252

Cengiz S, Aksu FTS (2012) An alternative source of adsorbent for the removal of dyes from textile waters: *posidonia oceanica (L)*. Chem Eng J 189–190:32–40

Chakravarty P, Sarma NS, Sarma HP (2010) Biosorption of cadmium (II) from aqueous solution using heartwood powder of areca catechu. Chem Eng J 162:949–955

Chatterje S, Lim SR, Woo SH (2010) Removal of reactive black 5 by zero-valent iron modified with various surfactants. Chem Eng J 160:27–32

Chen Y, Wang F, Duan L et al (2016) Tetracycline adsorption onto rice husk ash, an agricultural waste: Its kinetic and thermodynamic studies. J Mol Liq 222:487–494

Chojnacka K, Chojnacki A, Gorecka H (2005) Biosorption of Cr^{3+}, Cd^{2+} and Cu^{2+} ions by blue-green algae *spirulina sp*: kinetics, equilibrium and the mechanism of the process. Chemosphere 59:75–84

Chrisman JR, Koifman S, Novaes Sarcinelli P et al (2009) Pesticide sales and adult male cancer mortality in brazil. Int J Hyg Environ Health 212:310–321

Christoforidis AK, Orfanidis S, Papageorgiou SK (2015) Study of Cu(II) removal by *cystoseira* crinitophylla biomass in batch and continuous flow biosorption. Cheml Eng J 277:334–340

Chu KH (2004) Improved fixed bed models for metal biosorption. Chem Eng J 97:233–239

Chubar N, Carvalho JR, Correi MJN (2004) Heavy metals biosorption on cork biomass: effect of the pre-treatment. Colloids Surface A: Physicochem Eng Aspects 238:51–58

Cimino G, Passerini A, Toscano G (2000) Removal of toxic cations and Cr (VI) from aqueous solution by hazelnut shell. Water Res 34:2955–2962

Congeevaram S, Dhanarani S, Park J, Dexilin M, Thamaraiselvi K (2007) Biosorption of chromium and nickel by heavy metal resistant fungal and bacterial isolates. J Hazard Mater 146:270–277

Couto SR (2009) Dye removal by immobilised fungi. Biotechnol Adv 27:227–235

Cruz CCV, da Costa AVA, Henriques CA et al (2004) Kinetic modeling and equilibrium studies during cadmium biosorption by dead *Sargassum sp* biomass. Bioresour Technol 91:249–257

Dalel D, Mechichi T, Nasri M (2013) Decolorization of the metal textile dye lanaset grey G by immobilized white-rot fungi. J Environ Manage 129:324–332

Daneshvar E, Kousha M, Mojtaba Jokar M et al (2012a) Acidic dye biosorption onto marine brown macroalgae: Isotherms, kinetic and thermodynamic studies. Chem Eng J 204–206:225–234

Daneshvar E, Kousha M, Sohrabi MS et al (2012b) Biosorption of three acid dyes by the brown macroalga *stoechospermum marginatum*: isotherm, kinetic and thermodynamic studies. Chem Eng J 195–196:297–306

Daneshvar N, Ayazloo M, Khataee AR, Pourhassan M (2007) Biological decolorization of dye solution containing Malachite Green by microalgae *cosmarium sp*. Bioresour Technol 98:1176–1182

Davis TA, Volesky B, Mucci A (2003) A review of the biochemistry of heavy metal biosorption by brown algae. Water Res 37:4311–4330

Delshab S, Kouhgardi E, Ramavandi B (2016) Data of heavy metals biosorption onto Sargassum oligocystum collected from the northern coast of Persian Gulf. Data Brief 8:235–241

Demirbas A (2000) Mechanisms of liquefaction and pyrolysis reactions of biomass. Energy Convers Manage 41:633–646

Deng L, Su Y, Su H (2006) Biosorption of copper (II) and lead (II) from aqueous solutions by nonliving green algae *cladophora fascicularis*: equilibrium, kinetics and environmental effects. Adsorption 12:267–277

Deniz F, Remziye A, Kepekc Y (2017) Bioremoval of Malachite green from water sample by forestry waste mixture as potential biosorbent. Microchem J 132:172–178

Din MI, Hussain Z, Mirza ML et al (2013) Biosorption of toxic congo red dye from aqueous solution by eco-friendly biosorbent *saccharum bengalense*: kinetics and thermodynamics. Desalin Water Treat 51:28–30

Dönmez G (2002) Bioaccumulation of the reactive textile dyes by *candida tropicalis* growing in molasses medium. Enzym Microb Technol 30:363–366

Dotto GL, Gonçalves JO, Cadaval TRS Jr et al (2013a) Biosorption of phenol onto bionanoparticles from *spirulina sp* LEB 18. J Colloid Interf Sci 407:450–456

Dotto GL, Moura JM, Cadava TRSl et al (2013) Application of chitosan films for the removal of food dyes from aqueous solutions by adsorption. Chem Eng J 214:8–16

Dutta D, De D, Chaudhuri S et al (2005) Hydrogen production by *cyanobacteria*. Microb Cell Fact 4:1–14

Eman ZG (2012) Production and characteristics of a heavy metals removing bioflocculant produced by *pseudomonas aeruginosa polish*. J Microb 61:281–289

Fadel M, Hassanein NM, Elshafei MM et al (2017) Biosorption of manganese from groundwater by biomass of *saccharomyces cerevisiae*. HBRC J 13(1):106–113

Faghihian H, Peyvandi S (2012) Adsorption isotherm for uranyl biosorption by *Saccharomyces cerevisiae* biomass. J Radioanal Nucl Chem 293:463–468

Farkas V, Felinge A, Hegedusova A (2013) Comparative study of the kinetics and equilibrium of phenol biosorption on immobilized white-rot fungus *phanerochaete chrysosporium* from aqueous solution. Colloids Surf B 103:381–390

Figueroa GMR, Shumilin E, Rodriguez IA (2008) Heavy metal pollution monitoring using the brown seaweed *padina durvillaei* in the coastal zone of the Santa Rosalía mining region, Baja California Peninsula Mexico. J Appl Phycol 21:19–26

Fu Y, Viraraghavan T (2001) Fungal decolourization of wastewaters: a review. Bioresour Technol 79:251–262

Gadd GM (2009) Biosorption: critical review of scientific rationale, environmental importance and significance for pollution treatment. J Chem Technol Biotechnol 84:13–28

Gao L, Liu X (2010) Effects of carbon concentrations and carbon to nitrogen ratios on sporulation of two biological control fungi as determined by different culture methods. Mycopathologia 169:475–481

Garg VK, Kumar R, Gupta R (2004) Removal of malachite green dye from aqueous solution by adsorption using agro-industry waste: a case study of *prosopis cineraria*. Dye Pigment 62:1–10

Garrido IM, Campana O, Lubian LM et al (2005) Calcium alginate immobilized marine microalgae: experiments on growth and short-term heavy metal accumulation. Mar Pollut Bull 51:823–829

Ggadd GM (ed) (2001) Fungi in bioremediation. Brit Mycol Soc

González F, Romera E, Antonio B et al (2011) Algal biosorption and biosorbents. In: Kotrba P, Mackova M, Macek T (eds). Springer, pp 159–178

Gundogdu A, Duran C, Basri Senturk HB et al (2012) Adsorption of phenol from aqueous solution on a low-cost activated carbon produced from tea industry waste: equilibrium, kinetic, and thermodynamic study. J Chem Eng Data 57(10):2733–2743

Hadibarata T, Kristanti RA (2012) Identification of metabolites from benzo[a]pyrene oxidation by ligninolytic enzymes of Polyporussp. S133. J Environ Manage 111:115–119

Hadibarata T, Yusoff ARM, Aris A et al (2011) Decoloration of azo, triphenylmethane and anthraquinone dyes by laccase of a newly isolated Armillaria sp.F022. Water Air Soil Pollut 223:1045–1054

Hameed BH, El-Khaiary MI (2008) Removal of basic dye from aqueous medium using a novel agricultural waste material: pumpkin seed hull. J Hazard Mater 155:601–609

Han RP, Li YH, Li HQ, Wu YJ, Shi J (2004) The elemental analysis and FT-IR comparison bet ween MDP and casting. Spectrosc Spect Anal 24:185–186

Haq Bhatti HN, Asgher M (2011) Removal of solar red BA textile dye from aqueous solution by low cost barley husk: equilibrium, kinetic and thermodynamic study. Can J Chem Eng 89:593–600

Hasan SH, Srivastava P (2009) Batch and continuous biosorption of Cu^2 by immobilized biomass of *arthrobacter* sp. J Environ Manage 90:3313–3321

Hashem A, Akasha RA, Ghith A, Hussein DA (2007) Adsorbent based on agricultural wastes for heavy metal and dye removal: a review. Energy Educ Sci Tech 19:69–86

He J, Chen JP (2014) A comprehensive review on biosorption of heavy metals by algal biomass: materials, performances, chemistry, and modeling simulation tools. Bioresour Technol 160:67–78

Hooper SW, Pettigrew CA, Sayler GS (2009) Ecological fate, effects and prospects for the elimination of environmental polychlorinated biphenyls (PCBs). Environ Toxicol Chem 9:655–667

Iqbal M, Saeed A (2007) Production of an immobilized hybrid biosorbent for the sorption of Ni(II) from aqueous solution. Process Biochem 42:148–157

Iram S, Abrar S (2015) Biosorption of copper and lead by heavy metal resistant fungal isolates. Int J Sci Res 5(1):2250–3153

Jalali-Rad R, Ghafourian H, Asef Y et al (2004) Biosorption of cesium by native and chemically modified biomass of marine algae: introduce the new biosorbents for biotechnology applications. J Hazard Mater B 116:125–134

Jimenez GFE, Netzahuatl-Munoz AR, Morales-Barrera L (2009) Hexavalent chromium removal by *candida sp* in a concentric draft-tube airlift bioreactor. Water Air Soil Pollut 204:43–51

Kahouli S (2011) Re-examining uranium supply and demand: new insights. Energy Policy 39:358–376

Kang OL, Ramli N, Said M et al (2011) *Kappaphycus alvarezii* waste biomass: a potential biosorbent for chromium ions removal. J Environ Sci 23(6):918–922

Kariuki Z, Kiptoo J, Onyancha D (2017) Biosorption studies of lead and copper using roger mushroom biomass *lepiota hystrix*. S Afr J Chem Eng 23:62–70

Karthikeyan S, Balasubramanian R, Iyer CSP (2007) Evaluation of the marine algae *ulva fasciata* and *sargassum sp* for the biosorption of Cu(II) from aqueous solutions. Bioresour Technol 98:452–455

Kausar A, Bhatti HN, MacKinnon G (2016) Re-use of agricultural wastes for the removal and recovery of Zr(IV) from aqueous solutions. J Taiwan Inst Chem Eng 59:330–340

Khataee AR, Dehghan G (2011) Optimization of biological treatment of a dye solution by macroalgae *cladophora sp* using response surface methodology. J Taiwan Inst Chem Eng 4:26–33

Khataeea AR, Kasiri MB (2010) Photocatalytic degradation of organic dyes in the presence of nanostructured titanium dioxide: influence of the chemical structure of dyes. J Mol Catal A: Chem 328:8–26

Kılıc M, Keskin ME, Mazlum et al (2008) Effect of conditioning for Pb(II) and Hg(II) biosorption on waste activated sludge. Chem Eng Process 47:31–40

Kinoshita H, Sohma Y, Ohtake F (2013) Biosorption of heavy metals by lactic acid bacteria and identification of mercury binding protein. Res Microbiol 164(7):701–709

Kiran B, Kaushik A, Kaushik CP (2007) Response surface methodological approach for optimizing removal of Cr(VI) from aqueous solution using immobilized *cyanobacterium*. Chem Eng J 126: 147–153

Kumar NS, Boddu VM, Krishnaiah A (2009) Biosorption of phenolic compounds by *trametes versicolor polyporus* fungus. Adsorp Sci Technol 27(1):31–46

Kumar NS, Kim Min K (2011) Phenolic compounds biosorption onto *schizophyllum commune* fungus: FTIR analysis, kinetics and adsorption isotherms modeling. Chem Eng J 168:562–571

Kwon T, Jeon C (2013) Adsorption characteristics of sericite for nickel ions from industrial waste water. J Ind Eng Chem 19(1):68–72

Lacina C, Germain G, Spiros AN (2003) Utilization of fungi for biotreatment of raw wastewater. Afri Biotech 2:620–630

Lee Y, Chang S (2011) The biosorption of heavy metals from aqueous solution by *spirogyra* and *cladophora* filamentous macroalgae. Bioresour Technol 102:5297–5304

Li G, Huang K, Jiang Y (2007) Production of (R)-mandelic acid by immobilized cells of *saccha romyces cerevisiae* on chitosan carrier. Proc Biochem 42:1465–1469

Lodeiro P, Barriada JL, Herrero R (2006) The marine macroalga cystoseira baccata as biosorbent for cadmium (II) and lead (II) removal: kinetic and equilibrium studies. Environ Pollut 142:264–273

Lu S, Gibb SW (2008) Copper removal from wastewater using spent-grain as biosorbent. Bioresour Technol 99:1509–1517

Lu W, Shi J, Wang C (2006) Biosorption of lead, copper and cadmium by an indigenous isolate *enterobacter sp*. J1 possessing high heavy-metal resistance. J Hazard Mater B 134:80–86

Macek T, Mackova M (2011) Potential of biosorption technology. In: Kotrba P et al (ed) Microbial biosorption of metals. Springer Science + Business Media BV

Mane VS, Babu PV (2011) Studies on the adsorption of Brilliant Green dye from aqueous solution onto low-cost NaOH treated saw dust. Desalin 273:321–329

Manu B, Chaudhari S (2001) Anaerobic decolourization of simulated textile wastewater containing azo dyes. Bioresour Technol 82:225–231

Marinari SS, D'Annibale A, Grego S, Ocampo JA, Garcia-Romera I (2007) Organic matter evolution and partial detoxification in two-phase onative mill waste colonized by white-rot fungi. Int Biodeter Biodegr 60(2):116–125

Marin-Rangel VM, Cortes-Martines R, Villanueva RAC, Garnica-Romo MG, Martinez-Flores HE (2011) As(V) biosorption in an aqueous solution using chemically treated lemon (*Citrus aurantifolia swingle*) residues. J Food Sci 71:10–14

Marzbali MH, Mir AA, Pazoki M et al (2017) Removal of direct yellow 12 from aqueous solution by adsorption onto spirulina algae as a high-efficiency adsorbent. J Environ Chem Eng 5 (2):1946–1956

Masood F, Malik A (2011) Biosorption of metal ions from aqueous solution and tannery effluent by *bacillus sp*. FM1. J Environ Sci Health A 46:1667–1674

Masoudzadeha N, Zakeria F, Lotfabada TB et al (2011) Biosorption of cadmium by brevundimonas sp ZF12 strain, a novel biosorbent isolated from hot-spring waters in high background radiation areas. J Hazard Mater 197:190–198

Maurya NS, Mittal AK, Cornel P et al (2006) Biosorption of dyes using dead macro fungi: effect of dye structure, ionic strength and pH. Bioresour Technol 97:512–521

Méndez L, Palacios E, Acosta B, Monsalvo-Spencer P et al (2006) Heavy metals in the clam megapitaria squalida collected from wild and phosphorite mine-impacted sites in Baja California Mexico. Bio Trace Elem Res 110:275–287

Mohan SV, Rao NC, Prasad KK, Karthikeyan J (2002) Treatment of simulated Reactive Yellow 22 (Azo) dye effluents using *spirogyra species*. Waste Manage 22:575–582

Mona S, Kaushik A, Kaushik CP (2011) Biosorption of chromium(VI) by spent *cyanobacterial* biomass from a hydrogen fermentor using box-behnken model. Int Biodeterior Biodegrad 65:656–663

Montazer-Rahmati MM, Rabbani P, Abdolali A et al (2011) Kinetics and equilibrium studies on biosorption of cadmium lead, and nickel ions from aqueous solutions by intact and chemically modified Brown algae. J Hazard Mater 185:401–407

Monteiro CM, Castro PML, Malcata FX (2009) Biosorption of zinc ions from aqueous solution by the Microalga *scenedesmus obliquus*. Environ Chem Lett 9(2):169–176

Mosa AA, El-Ghamry A, Trüby P (2011) Chemically modified crop residues as a low-cost technique for the removal of heavy metal ions from wastewater. Water Air Soil Pollut 217:637–647

Moyo M, Guyo U, Mawenyiyo G (2015a) Marula seed husk (*sclerocarya birrea*) biomass as a low cost biosorbent for removal of Pb(II) and Cu(II) from aqueous solution. J Ind Eng Chem 27:126–132

Moyo M, Guyo U, Mawenyiyo G et al (2015b) Removal of Pb(II) from aqueous solutions by *phytolacca americana L.* biomass as a low cost biosorbent. Ind Eng Chem 27:126–132

Mulligan CN, Yong RN, Gibbs BF (2001) An evaluation of technologies for the heavy metal remediation of dredged sediments. J Hazard Mater 85:145–163

Naas MHEC, Al-Zuhair S, Alhaija MA (2010) Removal of phenol from petroleum refinery wastewater through adsorption on date-pit activated. Chem Eng J 162:997–1005

Nagaoka M, Yokoyama H, Fujita H et al (2014) Spatial distribution of radionuclides in seabed sediments off ibaraki coast after the Fukushima daiichi nuclear power plant accident. J Radioanal Nucl Chem 303(2):1305–1308

Nagaoka M, Yokoyama H, Fujita H et al (2015) Spatial distribution of radionuclides in seabed sediments off ibaraki coast after the Fukushima daiichi nuclear power plant accident. J Radioanal Nucl Chem 303(2):1305–1308

Nasreen A, Muhammad I, Iqbal ZS (2008) Biosorption characteristics of unicellular green alga *chlorella sorokiniana* immobilized in loofa sponge for removal of Cr(III). J Environ Sci 20:231–239

Navarro AE, Portales RF, Sun-Kou MR et al (2008) Effect of pH on phenol biosorption by marine seaweeds. J Hazard Mater 156(1–3):405–411

Ngah WSW, Hanafiah MAKM (2008) Removal of heavy metal ions from wastewater by chemically modified plant wastes as adsorbents: a review. Bioresour Technol 99:3935–3948

Ni H, Xiong Z, Ye T (2012) Biosorption of copper(II) from aqueous solutions using volcanic rock matrix-immobilized pseudomonas putida cells with surface-displayedcyanobacterial metal-lothioneins. Chem Eng J 204–206:264–271

Norton L, Baskaran K, Mckenzie T (2004) Biosorption of zinc from aqueous solutions using biosolids. Adv Environ Res 8:29–35

O'Mahony T, Guibal E, Tobin JM (2002) Reactive dye biosorption by *rhizopus arrhizus* biomass. Enzym Microb Technol 31:456–463

Ojha AK, Bulasara VK (2014) Adsorption characteristics of jackfruit leaf powder for the removal of amido black 10B dye. Environ Prog Sustain Energy 34(2)

Okoro IA, Okoro SO (2011) Agricultural byproducts as green chemistry absorbents for the removal and recovery of metal ions from wastewater environment. Continental J Water Air Soil Pollut 2:15–22

Onyancha D, Mavura W, Ngila CJ (2008) Studies of chromium removal from tannery wastewaters by algae biosorbents, Spirogyra condensate and *rhizoclonium hieroglyphicum*. J Hazard Mater 158:605–661

Osman HE, Badwy RK, Ahmad HF (2010) Usage of some agricultural by-products in the removal of some heavy metals from industrial wastewater. J Phytol 2:51–62

Özdemir Kilinc E, Poli A, Nicolaus B, Güven K (2009) Biosorption of Cd, Cu, Ni, Mn and Zn from aqueous solutions by thermophilic bacteria, *geobacillus toebii subsp decanicus* and *geobacillus thermoleovorans subsp stromboliensis*: equilibrium, kinetic and thermodynamic studies. Chem Eng J 152:195–206

Pagnanelli F, Papini MP, Trifoni M, Toro L, Vegliò F (2000) Biosorption of metals ions on *arthrobacter sp*: biomass characterization and biosorption modeling. Environ Sci Technol 34:2773–2778

Pagnanelli F, Viggi CC, Toro L (2010) Isolation and quantification of cadmium removal mechanisms in batch reactors inoculated by sulphate reducing bacteria: biosorption versus bioprecipitation. Bioresour Technol 101:2981–2987

Pardo R, Herguedas M, Barrado E et al (2003) Biosorption of cadmium, copper, lead and zinc by inactive biomass of *pseudomonas putida*. Anal Bioanal Chem 376:26

Park D, Yun Y, Park JM (2010) The past, present, and future trends of biosorption. Biotechnol Bioprocess Eng 15:86–102

Pengthamkeerati P, Satapanajaru T, Singchan O (2008) Sorption of reactive dye from aqueous solution on biomass fly ash. J Hazard Mater 153:1149–1156

Perez R0, Ramos R, Barron JM et al (2011) Adsorption rate of phenol from aqueous solution onto organobentonite: surface diffusion and kinetic models. J Colloid Interface Sci 364:195–204

Piccin JS, Gomes CS, FeriS LA et al (2012) Kinetics and isotherms of leather dye adsorption by tannery solid waste. Chem Eng J 183:30–38

Plazacazon P, Viera M, Sala S et al (2014) Biochemical characterization of *macrocystis pyrifera* and *undaria pinnatifida* (phaeophyceae) in relation to their potentiality as biosorbents. Phycologia 53(1):100–108

Podder MS, Majumder CB (2016) *corynebacterium glutamicum* MTCC 2745 immobilized on Granular activated carbon/MnFe2O4 composite: a novel biosorbent for removal of As(III) and As(V) ions. Spectrochimi Acta Part A: Mol Biomol Spectrosc 168:159–179

Prado MP, Vargas BA, Aragoza ES et al (2010) Copper and cadmium biosorption by dried seaweed *sargassum sinicola* in saline wastewater Water. Air Soil Pollut 210:197–202

Preetha P, Latha MS, Koshy M (2015) Biosorption of malachite green dye from aqueous solution by calcium alginate nanoparticles: equilibrium study. J Mol Liq 723–730

Qiu L, Feng J (2017) Biosorption of the strontium ion by irradiated *saccharomyces cerevisiae* under culture conditions. J Environ Radioact 172:52–62

Quan X, Shi H, Zhang Y, Wang J, Qian Y (2004) Biodegradation of 2,4-dichlorophenol and phenol in an airlift inner-loop bioreactor immobilized with *achromobacter sp*. Sep Purif Technol 34:97–103

Ramrakhiani L, Halder A, Majumder A et al (2017) Industrial waste derived biosorbent for toxic metal remediation: mechanism studies and spent biosorbent management. Chem Eng J 308:1048–1064

Rao N, Prabhakar G (2011) Removal of heavy metals by biosorption-an overall review. J Eng Res Stud 2:17–22

Robinson T, Mcmullan G, Marchant R et al (2001) Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative. Bioresour Technol 77:247–255

Romera E, Gonzalez F, Ballester A et al (2007) Comparative study of biosorption of heavy metals using different types of algae. Bioresour Technol 98:3344–3353

Rubin E, Rodriguez P (2006) Biosorption of phenolic compounds by the brown alga *sargassum muticum*. J Chem Technol Biotechnol 81:1093–1099

Saeed A, Iqbal M, Zafar SI (2009) Immobilization of *trichoderma viride* for enhanced methylene blue biosorption: batch and column studies. J Hazard Mater 168:406–415

Sampedro I, Marinari S, D'Annibale A, Grego S, Ocampo JA, García-Romera I (2007) Organic matter evolution and partial detoxification in two-phase onative mill waste colonized by white-rot fungi. Int Biodeter Biodegr 60(2):116–125

Sana Sadaf S, Amin M, Haqbhatti (2013) Bioremediation of zirconium from aqueous solution by *coriolus versicolor*: process optimization. J Chem Soc Pak 35(3):692–698

Santos FA et al (2013) Cr(III) biosorption by forest wastes from *araucaria angustifolia* and pinus *elliottii*: biosorbent surface characterization and chromium quantification by spectrofluorimetry in micellar medium. Desalin Water Treat 51:5617–5626

Sari A, Tuzen M (2008) Biosorption of total chromium from aqueous solution by red algae (*ceramium virgatum*): equilibrium, kinetic and thermodynamic studies. J Hazard Mater 160:349–355

Selatnia A, Bakhti AMZ, Madani A et al (2004) Biosorption of Cd^{2+} from aqueous solution by a NaOH-treated bacterial dead *streptomyces rimosus*. Biomass Hydrometall 75:11–24

Shafique U, Ijaz A, Salman M et al (2012) Removal of arsenic from water using pine leaves. J Taiwan Inst Chem Eng 43:256–263

Shashirekha V, Sridharan MR, Swamy M (2008) Biosorption of trivalent chromium by free and immobilized blue green algae: kinetics and equilibrium studies. J Environ Sci Health Part A 43:390–401

Simmons RW, Pongsakul P, Chaney R, Saiyasitpanich D, Klinphoklap S, Nobuntou W (2003) The relative exclusion of zinc and iron from rice grain in relation to rice grain cadmium as compared to soybean implications for human health. Plant Soil 257:163–170

Singh A, Kumar D, Gaur JP (2007) Copper(II) and lead(II) sorption from aqueous solution by non-living *spirogyra neglecta*. Bioresour Technol 98:3622–3629

Singh A, Singh Nigam P, Murphy JD (2011) Renewable fuels from algae: an answer to debatable land based fuels. Bioresour Technol 102:10–16

Singh RR, Chadetrik RR, Kumar RR et al (2010) Biosorption optimization of lead(II), cadmium (II) and copper(II) using response surface methodology and applicability in isotherms and thermodynamics modeling. J Hazard Mater 174:623–634

Siva Kumar Nadavala SK, Swayampakula K, Boddu VM (2009) Biosorption of phenol and o-chlorophenol from aqueous solutions on to chitosan–calcium alginate blended beads. J Hazard Mater 162:482–489

Srinivasan A, Viraraghavan T (2010) Decolorization of dye wastewaters by biosorbents: a review. J Environ Manage 91:1915–1929

Sule Aytas S, Turkozu DA, Gok C (2011) Biosorption of uranium(VI) by bi-functionalized low cost biocomposite adsorbent. Desalin 280:354–362

Sun JH, Sun SP, Sun JY et al (2007) Degradation of azo dye acid black 1 using low concentration iron of Fenton process facilitated by ultrasonic irradiation. Ultrason Sonochem 14:761–766

Tabaraki R, Nateghi A, Ahmady-Asbchin S (2014) Biosorption of lead (II) ions on *sargassum ilicifolium*: application of response surface methodology. Int Biodeter Biodegr 93:145–152

Tai P, Zhao Q, Su Q et al (2010) Biological toxicity of lanthanide elements on algae. Chemosphere 80:1031–1035

Tasar S, Kaya F, Oze A (2014) Biosorption of lead(II) ions from aqueous solution by peanut shells: equilibrium, thermodynamic and kinetic studies. J Environ Chem Eng 2(2):1018–1026

Tavlieva MP, Genieva SD, Georgieva VG et al (2013) Kinetic study of brilliant green adsorption from aqueous solution onto white rice husk ash. J Colloid Interface Sci 409:112–122

Tien CJ (2002) Biosorption of metal ions by freshwater algae with different surface characteristics. Process Biochem 38:605–613

Torres MA, Barros MP, Campos SCG (2008) Biochemical biomarkers in algae and marine pollution: a review. Ecotoxicol Environ Saf 71:1–15

Tran HT, Vu ND, Matsukawa M (2016) Heavy metal biosorption from aqueous solutions by algae inhabiting rice paddies in Vietnam. J Environ Chem Eng 4:2529–2535

Tuzen M, Sari A (2010) Biosorption of selenium from aqueous solution by green algae (Cladophora hutchinsiae) biomass: equilibrium, thermodynamic and kinetic studies. Chem Eng J 158:200–206

Tuzun Bayramoglu G, Yalcin E et al (2005) Equilibrium and kinetic studies on biosorption of Hg (II), Cd (II) and Pb (II) ions onto microalgae *chlamydomonas reinhardtii*. J Environ Manage 77:85–92

Uslu G, Tanyol M (2006) Equilibrium and thermodynamic parameters of single and binary mixture biosorption of lead (II) and copper (II) ions onto Pseudomonas putida: effect of temperature. J Hazard Mater B 135:27–93

Vargas KK, Cerro-Lopez M, Reyna-Tellez S (2012) Biosorption of heavy metals in polluted water, using different waste fruit cortex. Phys Chem Earth 37–39:26–29

Vieira RHSF, Volesky B (2000) Biosorption: a solution to pollution. Int Microbiol 3:17–24

Vijayaraghavan K, Balasubramanian R (2010) Single and binary biosorption of cerium and europium onto crab shell particles. Chem Eng J 163:337–343

Vijayaraghavan K, Han MH, Choi SB (2007) Biosorption of Reactive black 5 by *corynebacterium glutamicum* biomass immobilized in alginate and polysulfone matrices. Chemosphere 68:1838–1845

Vijayaraghavan K, Yun YS (2007a) Chemical modification and immobilization of corynebacteriu glutamicum for biosorption of Reactive black 5 from aqueous solution. Ind Eng Chem Res 46:608–617

Vijayaraghavan K, Yun YS (2007b) Utilization of fermentation waste (*corynebacterium glutamicum*) for biosorption of Reactive Black 5 from aqueous solution. J Hazard Mater 141:45–52

Vijayaraghavan K, Yun YS (2008) Bacterial biosorbents and biosorption. Biotechnol Adv 26:266–291

Vilar VIP, Botelho CMS, Boaventura RAR (2005) Influence of pH, ionic strength and temperature on lead biosorption by *gelidium* and *agar* extraction algal waste. Process Biochem 40:3267–3275

Vogel M, Günther A, Rossberg A (2010) Biosorption of zinc ions from aqueous solution by the microalga *scenedesmus obliquus*. Sci Total Environ 409:384–395

Volesky B (2003) Sorption and biosorption. BV Sorbex Inc., Montreal-St Lambert, Quebec, Canada, p 320

Volesky B (2007) Biosorbents for the removal of synthetic organics and emerging pollutants: opportunities and challenges for developing countries. Water Res 41:4017–4029

Volesky B, Holan ZR (1995) Biosorption of heavy metals. Biotechnol Prog 11:235–250

Volesky B, Naja G (2007) Biosorption technology: starting up an enterprise. Int J Tech Transf Commer 6(2–4)

Vullo D, Ceretti H, Daniel M, ırez SR, Zalts A (2009) Cadmium, zinc and copper biosorption mediated by *pseudomonas veronii 2E*. Bioresour Technol 99:5574–5581

Wang J, Chen C (2009) Biosorbents for heavy metals removal and their future. Biotechnol Adv 27:195–226

Won SW, Vijayaraghavan K, Mao J (2008) An aminated bacterial biosorbent capable of effectively binding negatively charged pollutants in aqueous solution. Adsorpt Sci Technol 26 (8)

Wu J, Yu H (2006) Biosorption of phenol and chlorophenols from aqueous solutions by fungal *mycelia*. Process Biochem 41:44–49

Wu J, Yu H (2007) Biosorption of 2, 4-dichlorophenol by immobilized white-rot fungus *phanerochaete chrysosporium* from aqueous solutions. Bioresour Technol 98:253–259

Xie S, Rui W, Zhao W et al (2013) Atrazine biodegradation by arthrobacter strain DAT1: effect of glucose supplementation and change of the soil microbial community. Environ Sci Pollut Res 20(6):4078–4084

Xu P, Zeng GM, Huang DL et al (2012) Adsorption of Pb(II) by iron oxide nanoparticles immobilized *phanerochaete chrysosporium*: equilibrium, kinetic, thermodynamic and mechanisms analysis. Chem Eng J 203:423–431

Yan C, Li G, Xue P et al (2010) Competitive effect of Cu(II) and Zn(II) on the biosorption of lead (II) by *myriophyllum spicatum*. J Hazard Mater 179:721–728

Yan T, Viraraghavan T (2003) Heavy metal removal from aqueous solution by fungus *mucor rouxii*. Water Res 37:4486–4496

Yargi AS, Sahin RZY, Ozbay NY et al (2014) Assessment of toxic copper(II) biosorption from aqueous solution by chemically-treated tomato waste (*solanum lycopersicum*). J Clean Prod 1–8

Yousef RI, El-Eswed B, Al-Muhtaseb H (2011) Adsorption characteristics of natural zeolites as solid adsorbents for phenol removal from aqueous solutions: kinetics, mechanism, and thermodynamics studies. Chem Eng J 171:1143–1149

Zafar MN, Nadeem R, Hanif MA (2007) Biosorption of nickel from protonated rice bran. J Hazard Mater 143:478–485

Zeng X, Chai L, Tang J et al (2013) Different biosorption mechanisms of uranium(VI) by live and heat-killed *saccharomyces cerevisiae* under environmentally relevant conditions. Trans Nonferrous Met Soc China 23:2759–2765

Zhang Y, Ge S, Jiang M et al (2014) Combined bioremediation of atrazine-contaminated soil by pennisetum and arthrobactersp strain DNS10. Environ Sci Pollut Res 21(9): 6234–6238

Zhang DQ, Tan SK, Gersberg RM et al (2012) Removal of pharmaceutical compounds in tropical constructed wetlands. Ecol Eng 37:460–464

Zheng Y, Fang X, Ye Z et al (2008) Biosorption of Cu(II) on extracellular polymers from *bacillus sp.* F19. J Environ Sci 20:1288–1293

Zhu Y, Hu J, Wang J (2014) Removal of Co^2 from radioactive wastewater by polyvinyl alcohol (PVA)/chitosan magnetic composite. Prog Nucl Energy 71:172–178

Ziagova M, Dimitriadis G, Aslanidou D et al (2007) Comparative study of Cd(II) and Cr(VI) biosorption on *staphylococcus xylosus* and *pseudomonas sp* in single and binary mixtures. Bioresour Technol 98:2859–2865

Chapter 9
Bioremediation of Heavy Metals

P. Senthil Kumar and E. Gunasundari

Abstract Bioremediation technology is an effective and eco-friendly technology for removing toxic pollutants from the soil and aquatic environment. The lesser quantities of some heavy metals are important to humans and animals. However, the extensive usage of heavy metals for human purposes can change the geochemical cycles and biochemical balance. Due to this reason, the excess amount of toxic heavy metals like copper, lead, cadmium, nickel, and chromium is directly discharged into the soil and water bodies. The indiscriminate accumulation of heavy metals can be hazardous to the human life and aquatic biota. To overcome this problem, bioremediation technique has been developed for the treatment of heavy metals using biological agents like bacteria, fungi, algae, and plants. These biological agents can be used to change the metal bioavailability and toxicity in the soil and aqueous environment. The remediation of heavy metals in soil is further improved by the addition of organic amendments like biosolid, compost, and municipal solid waste, which is used as both nutrients and conditioner. Aim of this chapter is to investigate the role of microorganisms and plants to remediate the heavy metals and also to discuss the recent bioremediation technologies and methods for heavy metals in soil and aquatic environment.

Keywords Bioremediation · Biological agents · Heavy metals
Soil and aquatic environment

P. Senthil Kumar (✉) · E. Gunasundari
Department of Chemical Engineering, SSN College of Engineering, 603110
Chennai, India
e-mail: senthilchem8582@gmail.com

E. Gunasundari
e-mail: gunasundarielumalai2@gmail.com

S. J. Varjani et al. (eds.), *Bioremediation: Applications for Environmental Protection and Management*, Energy, Environment, and Sustainability,
https://doi.org/10.1007/978-981-10-7485-1_9

9.1 Introduction

In recent years, environment pollution is a major problem due to contamination of the earth's environment with undesirable recalcitrant and xenobiotic compounds. Every year, huge quantities of organic and inorganic compounds are discharged into the environment due to human activities (Kensa 2011). In these, heavy metals are the major toxic contaminants in environment, which are harmful to the human and ecosystem. The soil and water contaminations are occurred as a result of the accumulation of toxic compounds in excess of permissible level. The contaminated lands are potential risk to human health (Thiele 1995). To overcome these problems, the conventional technologies are used for the removal of heavy metals from contaminated environment including soil and water, which are carried out based on the physicochemical principles. These techniques are expensive and inefficient. In the conventional method, reagents are added to raise pH for the metal removal. The more addition of reagents results in conversion of metals from soluble to insoluble form that leads to the precipitation. Therefore, the more amounts of mud are formed in effluents by using the conventional technology, which is not easy to dispose. Single or multiple steps are available in complex processes and include precipitation, adsorption, redox chemistry, membranes usage, evaporation, electrolytic recovery, liquid–liquid extraction, and electro deposition (Volesky 1990). Nevertheless, the bioremediation is an alternative technology for removal of heavy metal for contaminated environment due to its low cost, simplicity, and efficiency (Garbisu and Alkorta 2003). It offers the possibility to destroy or render risk-free different pollutants using natural biological activities. Bioremediation is the best alternative to conventional cleanup technologies research in this area. The main objective of this chapter is to understand the environmental pollution and their effects and also to study the role of microorganisms using different bioremediation technologies and methods for heavy metals in soil and aquatic environment.

9.2 Heavy Metal-Polluted Environments

Heavy metals are generally described based on three different criteria such as density, chemical properties, and their atomic number. Heavy metal is examined that the density of specific metals is more than 5 g/cm^3. The density of heavy metal is five times greater than water. Heavy metals include chromium (Cr), lead (Pb), zinc (Zn), copper (Cu), cadmium (Cd), mercury (Hg), selenium (Se), nickel (Ni), gold (Au), uranium (U), silver (Ag), and arsenic (As). In general, the small quantities of heavy metal like Cu, Fe, Co, Mo, Zn, Ni, Mn, and V are essential for

living organisms, but above the permissible limit, they can be hazardous to living organisms. Some other heavy metals including Cd, Pb, As, and Hg are not even having any positive effect on living organisms. Therefore, they are extremely dangerous to environment. These metal ions are absorbed by the human body and can attach with the different biomolecules like proteins and nucleic acid and interfere with their functions (Hogan 2010). Heavy metals are pollutants from inorganic sources that mainly pollute the water due to the natural factors and human activities like agricultural runoff, discharge of wastes from different industries, and mining activities. Compare to other surplus variety of contaminants, these heavy metals are available all over the environment through water, soil, and air. The majority of the toxic pollutants which are in soluble forms are having an ability to pollute the environment. That is, the most of the metal ions are in the extremely stable oxidation state; they can react with biomolecules in bodies to form very stable biotoxic compounds. These compounds are not easy to dissociate. The greater distribution of these heavy metals in environment leads to soil pollution, water pollution, and air pollution. In water, heavy metals are distinguished as common contaminants because of their extensive use in different industries and their maximum concentration in the environment that leads to damage of life in the ecosystem (Gonzalez et al. 2007). Heavy metal pollution is the major problem due to its high toxic nature and the hazardous effects. Worldwide, soil and aquatic ecosystems are polluted directly or indirectly by the various heavy metals through different human activities and the anthropogenic activities. Human activities are such as the usage of metal-based pesticides and metal-enriched sewages sludge in agriculture, combustion of metallurgical industries and electronics, exploitation of mines and smelters, and military training and weapons. The anthropogenic activities include metal industries (e.g., Cu, Cr, As, Cd, Ni, Zn, and Hg), metalliferous mining industries (e.g., Pb, As, Cd, and Hg), agriculture (e.g., Cu, As, Cd, Pb, Se, Zn, and U), atmospheric deposition (e.g., Cu, Cr, As, Cd, Pb, and Hg), and waste disposal (e.g., Cu, Cr, As, Cd, Pb, Zn, and Hg). The contaminants (heavy metals) in soils of rural and urban environments can accumulate and remain for long period that is enough to cause hazard to plants, human health, animals, and ecosystem. Generally, contaminants (heavy metals) enter into the human body through inhalation, different food chain, and ingestion, and also through some human activities such as making pigments and metal alloy for paper, paints, rubber, and cement. If the heavy metals reach the human body, they can cause vomiting, nausea, anorexia, dermatitis, and gastrointestinal abnormalities. The toxic nature of heavy metal also has an ability to alter blood composition and disrupt or damage the metal and central nervous systems, livers, lungs, and kidneys.

9.2.1 Types of Heavy Metals and Their Toxicity

Some of the heavy metals and their toxicity are explained as follows.

9.2.1.1 Arsenic

Arsenic (As) is most commonly available contaminants in the water bodies around the world. The toxic level of the drinking water is increased predominantly due to the arsenic poisoning. The permissible limit of arsenic in primary drinking water and in soils is 0.05 mg/L and 1–50 mg/kg, respectively. These metals can cause carcinogenic damage to human bodies. The arsenic is lesser toxic to human skin due to the minimal dermal adsorption through the washing of hands and the bathing. The effluents from industries are having more amount of arsenic which is directly discharged into environment, which will create pollution. This toxic metal can affect the human being through water, inhalation of polluted air, and intake of polluted food. In general, arsenic is in either inorganic or organic forms that can affect the both peripheral and central nervous systems due to its neurotoxic effect. Neurotoxicity normally starts with sensory changes, tenderness after that gradual weakness from the proximal to distal muscle groups (Techounwou et al. 2014). Thus, arsenic is considered as a non-essential element to human and ecosystem that may be released into the environment via natural or anthropogenic activities.

9.2.1.2 Lead

Lead (Pb) is extensively distributed in the environment in biologically inactive form. It is mostly used in batteries, gasoline additives, bearing metals, cable covering, pesticide production, explosives, analytical agent, and antifouling paints that are the main sources of pollution (Flora et al. 2006). Exposure to lead can mainly occur through inhalation of lead-polluted dust, ingestion of lead-polluted food and water, and dermal absorption. Generally, the permissible limit of Pb in primary drinking water and in soils is 0.015 mg/L and 2–200 mg/kg, respectively. About 30–50% of lead is absorbed by the adult through drinking water. For children, the absorption rate may be more than 50%. These metals can affect numerous organs in human body such as liver, kidney, central nervous system, endocrine system, hematopoietic system, and reproduction system (Pirkle et al. 1998). The early symptoms of the effects of lead exposure on the central nervous system include headache, irritability, poor attention spam, memory loss, and dullness.

9.2.1.3 Zinc

Zinc (Zn) is the most abundantly available component in the earth's crust. The level of zinc in environment (soil, rock, and surface water) varies over an extensive range of concentrations. The level of zinc in soil, water, and air is increasing unusually through human activities (Herawati et al. 2000). Certain concentration of zinc is also present in many foodstuffs. In general, the permissible limit of zinc in secondary drinking water is 5.0 mg/L, and the average amount of zinc is about 80 mg/kg in lithosphere and ranging from 10 to 300 mg/kg in soil. Zinc is discharged as a waste material from the most of the industries such as manufacturing of batteries, electroplating, metallurgical industries, and galvanization. It is an essential element for human and plant growth. The deficiency of zinc compounds in the environment can reduce the growth of plant. Usually, the metallic form of zinc is not at all harm to environment such as soil and water, and its bioavailability is limited. But, zinc becomes toxic compound when it reacts with some other chemicals like oxygen and acids. This toxic compound can damage the biological systems (Herawati et al. 2000).

9.2.1.4 Cadmium

Cadmium (Cd) is widely available element that is detected at an average concentration of about 0.1 mg/kg in the earth's crust. The maximum concentration of cadmium compounds is present in sedimentary rocks and marine phosphates. It is often used in the different industries, especially in the production of pigment, alloys, and batteries (Wilson 1988). In general, the permissible limit of cadmium in primary drinking water and soil is 0.005 mg/L and 0.01–0.7 mg/kg, respectively. Exposure of cadmium is likely through ingestion of food, smoking cigarettes, employment in primary metal industries, and employment in cadmium-contaminated sites. Some additional sources of cadmium contain discharge from industrial activities, containing the making of pigment, alloys, batteries, and mining and smelting. It also exists in some quantities in certain foods like potatoes, leafy vegetables, seeds, and grains. Furthermore, cadmium-rich foodstuffs can increase the concentration of cadmium in human bodies and are mushroom, cocoa powder, mussels, and shellfish (Satarug et al. 2003). When it is inhaled or ingested, it can cause nausea, abdominal pain, burning sensation, muscle cramps, vomiting, salivation shock, loss of consciousness, hepatic, gastrointestinal tract erosion, and coma.

9.2.1.5 Copper

Copper (Cu) metal naturally exists in soil, water, rock, and also in all plants and animals. It is a significant constituent for all living organisms at low concentration.

But, at high concentration, it is toxic to the environment. It is released into the environment from copper mining and industries, domestic wastewater, dumps, wood production, and phosphate fertilizer production, combustion of fossil fuels and waste, and also from natural resources. After the discharge of copper into soil, it can be strongly bonded to the organic and some other elements such as sand and clay in upper layer of soil. When copper and their compounds are discharged into water, it can be dissolved with surface water in the type of either free copper or copper compounds or, probably, copper bound to particle suspended in water. Some water-soluble copper compounds may go into groundwater although copper strongly attaches to suspended particles and sediments. The permissible limit of Cu in primary and secondary drinking water and in soils is 1.3 mg/L, 1.0 mg/L, and 2–100 mg/kg, respectively. Above the permissible limit, Cu in water can cause nausea, headaches, diarrhea, dizziness kidney, and liver damage (Bsoul et al. 2014).

9.2.1.6 Chromium

Chromium (Cr) is naturally available element in the earth's crust with different oxidation states from chromium (II) to chromium (VI). The trivalent chromium (Cr^{3+}) is the most stable state of chromium compound that can occur in ores. Hexavalent chromium [Cr (VI)] is the second most stable state of chromium compounds, and the elemental chromium (Cr^{0}) is not naturally obtained from environment. In general, chromium enters into the environment (water, soil, and air) from natural and anthropogenic sources with maximum discharge from various industries including chromate production, stainless steel production, metal processing, and ferrochrome and chrome dye production. The high level of chromium is discharged as wastewater and air from chemical, refractory, and metallurgical industries. Chromium compounds are mostly discharged in the form of hexavalent chromium [Cr (VI)] through anthropogenic activity. The toxicity level of the chromium compounds depends on the oxidation states that increased from trivalent chromium [Cr (III)] to hexavalent chromium [Cr (VI)]. Trivalent chromium [Cr (III)] is lesser toxic in nature that is an essential nutrient for human and animal, which may be present in glucose, fat, and protein metabolism with potentiating the action of insulin. But, hexavalent chromium [Cr (VI)] is very toxic industrial contaminant that can be harmful to the human beings and animals, which creates the high risk of Cr-induced diseases to the workers in the industries. In general, the permissible limit of chromium in primary drinking water and soil is 0.1 mg/L and 1–1000 mg/kg, respectively (Cohen et al. 1993). Through ingestion or inhalation, it also forms a health effect to human body such as ulcers in stomach and in small intestine, irritation, anemia, male reproduction system damage and sperm damage, severe respiratory, gastrointestinal, cardiovascular, hepatic, hematological, and neurological effects as part of sequelae leading to death or the medical treatment needed to the patients for survival. Even though the trivalent chromium [Cr (III)] is not harmful to human, it causes allergic reactions such as severe redness and

swelling of the skin to some people who are very sensitive to chromium compounds.

9.2.1.7 Mercury

Mercury (Hg) is a transition metal in the series of periodic table, which is the only metallic element in the liquid form at standard conditions of pressure and temperature. It is naturally found in the three forms like elemental (metallic), inorganic, and organic forms, in which each individual form of mercury is containing its own degree of toxicity. Inorganic form of mercury may affect people through their occupation. Methyl mercury is the most commonly found organic form of mercury in the environment. It has been formed by the methylation of inorganic forms of mercury using microorganism obtained in the water and soil. It causes health effects to people through their diet. These mercury compounds affect several parts of human bodies such as lung, kidney, nervous, skin, eyes, digestive, and immune system. In general, mercury is naturally occurring in the earth's crust that can be discharged into environment from weathering rocks, volcanic activity, and because of human activity (Clarkson and Magos 2006). The high level of mercury metals is released mainly by the human activities such as residential burning of coal for cooking, mostly coal-fired power stations, mining, industrial process, and incinerators. In general, the permissible limit of mercury in primary drinking water and soil is 0.002 mg/L and 0.01–0.3 mg/kg, respectively. Mercury is frequently used in electrical industries including batteries, switches and thermostats, dentistry, several industrial processes such as in the caustic soda, as anti-fungal agents in wood processing, as a solvent for reactive and expensive metals in nuclear reactor, and also as preservative in vaccines and pharmaceutical products.

9.2.1.8 Silver

Silver (Ag) is a lustrous, soft white transition metal that is formed in the earth. It can be naturally available as an elemental metal in its metallic form and alloyed with some other elements including chloride, sulfide, and nitrate. The pure silver material is in a bright metallic white-gray color. The silver chloride and silver nitrate are in powdery color, but the silver oxide and silver sulfide are dark gray to black in color. In general, Ag is stable element in pure water and air, but it discolors rapidly, once it exposed to air which has elevated levels of ozone, hydrogen sulfide, or sulfur. It has been used in various applications like jewelery, silverware, currency, solar panels, medicine, chemical equipment, photography, nanoparticles, and catalysis. It can be released into the environment and can move long distance in water and air. The permissible limit of Ag in secondary drinking water and in soils is 0.1 mg/L and 0.01–5.0 mg/kg, respectively. Generally, silver is not poisonous compared to other heavy metals including mercury and lead. So, it is not identified to cause cancer, chromic adverse effect, or reproductive or neurological effects.

Solid silver spoons, coins, or bowls do not create any health effect to human in a day-to-day contact. Because, a silver metal is biologically inert, if it is consumed, it would directly pass through the human body without being absorbed into tissue. In a meantime, silver in the form of silver fumes or dust has some health effects to human when exposed to environment. Inhaling of silver fumes or dust can irritate the upper respiratory tract or mucous membranes. After contact to powdered silver, dental filling, or silver solutions, some sensitive persons get allergic reactions. In the same way, silver-containing skin creams initiate local skin discoloration in some sensitive persons. Consuming of silver compound-containing medicines may irritate the stomach. Continued exposure to silver compound-containing medicines can also cause a permanent blue-gray staining in eyes, mouth, nose, and skin. This blue-gray staining is medically named as "argyria," which is the major serious health effect of silver on human (Fowler et al. 1986).

9.2.1.9 Gold

Gold (Au) is the most bright, soft, dense, malleable, and ductile metallic compound that is found in the earth. It is the metal with a yellow color when in bulk, however it can be ruby, black, or purple in color when finely distributed. It is a transition metal and is the least reactive chemical compounds. Under standard condition, it is in solid form. Gold is generally available in free elemental form, in rock, and in alluvial deposits. It can be happened in the a solid solution series with the elemental silver and as well naturally combined with other metal such as palladium and copper. This gold is mostly used in jewelery, electronic, and glass manufacturing industry. Typically, pure elemental gold is harmless and non-irritating when it is ingested (Dierks 2005). Gold in leaf form is occasionally used as a food decoration. In the alcoholic drinks, metallic gold is one of the components, which is accepted as a food additive due to its relative chemical inertness. These gold ions are resistant to corrosion or conversion into soluble salts during any well-known process that can be encountered in the human body. Gold salts like gold chloride are dangerous to kidneys and liver. Potassium gold cyanide is normally used for gold electroplating, which is toxic due to the presence of both gold and cyanide content (Catherine and Olivier 2012).

9.2.1.10 Nickel

Nickel (Ni) is a hard, silvery-white, malleable, and ductile metal. Nickel naturally can exist in various oxidation states. But, in general, it is available in biovalence state. Majority of the nickel compounds are blue or green in color. It is used in various applications including manufacturing of stainless steel, alloy steel, catalysis, rechargeable batteries, electroplating, paint formation, and coinage. Few nickel compounds like nickel carbonyl are toxic and carcinogenic in nature that can easy to absorb through skin. At higher concentration, nickel can cause cancer of bone,

lungs and nose, lung embolism, respiratory failure, heart disorder, birth defect, asthma, and chronic bronchitis. Nickel fumes are generally respiratory irritants that can cause pneumonitis. Nickel metal is discharged into the air through power plant and trash incinerators. After reactions with raindrops, it will settle on the ground. This nickel compounds can easily absorb sediment and soil particles and turn out to be immobile. The permissible limits of the nickel in soil are 5–500 mg/kg (Goyer 1991). It can also end up in surface water when it is released through wastewater streams. In a small quantity, nickel metal is required foodstuff for animals. When the maximum tolerance limit of nickel is exceeded, it can also be a toxic compound.

9.2.1.11 Selenium

Selenium (Se) is a non-metallic compound and is hardly formed in its elemental state or as pure ore compounds in earth's crust. Generally, selenium is produced in the metal sulfide ores, where it partly exchanges the sulfur. It is commercially formed as a by-product in the refining of these ores. This selenium is used in various applications like pigments, glass manufacturing, and photocells. Small amount of the selenium is an essential element for cellular function in various organisms. At high level, selenium salts are dangerous. Inhalation of selenium is the reason for respiratory membrane irritation, bronchial inflammation, pneumonia, and pulmonary edema. Elemental selenium dust also causes mucous membrane irritation, coughing, and bleeding from nose. Some other health effects include nausea, cardiovascular effects, vomiting, headaches and malaise, and ophthalmic irritation. The intake of selenium for long term either organic or inorganic form of food or water may cause selenosis symptoms and gastrointestinal symptoms. Selenosis symptoms include a garlicky odor in breath or a metallic taste in the mouth. Gastrointestinal symptoms are tiredness, nausea or diarrhea, irritability, and joint pain (Sun et al. 2014).

9.2.1.12 Uranium

Uranium (U) is a lustrous, heavy, radioactive, and silvery-white metal in the actinide series of the periodic table. The density of the uranium is about 70% greater than lead and lesser than gold or tungsten. The availability of uranium in rock, soil, and water is in lower concentrations. It is mostly extracted from uranium-bearing minerals like uraninite. Uranium is generally available in water, air, and food, which inhaled daily by everyone in small amount. Uranium-235 is the single naturally forming fissile isotopes, which is extensively used in nuclear weapons and nuclear power plants. The uranous ion gives a harmful effect on the living cells through inhibiting the processes of metabolism of carbohydrates using the inhibition of enzyme systems (Seaborg 1968).

9.3 Bioremediation

9.3.1 Principle of Bioremediation

Bioremediation is the process to treat the environmental contaminants in water and soil using three different vital components such as microorganisms, food, and nutrients. In this process, biological compounds are used to impair or reduce the concentration of toxic heavy metals in the polluted environment. In other words, it can be defined as the technological process in which biological agents can be used to break down organic chemicals. In general, the microorganisms are abundantly available on earth. Therefore, the insufficient quantity of the food and nutrients for the microorganisms is commonly the missing element that hinders successful bioremediation (Kulshreshtha et al. 2014). Microorganisms can find the food from environment like soil or water. These microorganisms can also eat the pollutants in soil or water as an additional food source.

The major two reasons for the usage of these pollutants as food source are as follows:

- It gives a source of carbon for the growth microbes;
- The microbes got energy because of breaking of bonds and transferring electrons aside from the pollutants. It is called as an oxidation-reduction reaction.

Loses of electron by pollutants are described as an oxidation, and gains of electron by chemical are described as a reduction. The more cells are produced by the energy gained from the electron transfer along with the carbon and a few electrons. In the bioremediation, oxygen is most commonly used as an electron acceptor for microbes; however, sulfate, nitrate, CO_2 and iron are also used. The majority of the bioremediation systems are operated under aerobic condition. On the other hand, under the anaerobic conditions, the running of bioremediation system may allow the microorganisms to deteriorate or else recalcitrant molecules.

Bioremediation has effectively cleaned up most of the contaminated sites or environment. During the bioremediation process, microorganisms or plants are involved in different enzymatic action, which may decompose the contaminants that result in producing the harmless product. The microbes do not directly degrade heavy metals; however, the microbes can convert the valence states of metals into less toxic or immobile forms. The main advantages of the bioremediation are as follows:

- Natural and biological processes are used for cleaning of the polluted sites
- Bioremediation is an economical technique due to the minimum requirement of equipments and labors compared to the physical and chemical techniques.

9.3.2 Types of Bioremediation

In the bioremediation process, two basic methods used for the removal of pollutants from soil and water are (i) in situ bioremediation and (ii) ex situ bioremediation.

9.3.2.1 In Situ Bioremediation

In situ bioremediation consists of the stimulation of naturally occurring bacteria to degrade contaminants by the circulation of aqueous solution with oxygen and nutrients through contaminated soil or water. The remediation of polluted soil or water in the subsurface where it was found can be considered more suitable as compared to ex situ bioremediation. In situ bioremediation may be used in the unsaturated/vadose zone or in saturated soils and groundwater. In the in situ bioremediation, the removal rates and extent change depend on the pollutant of concern and site-specific properties. The removal rates are also based on variables like indigenous microbial populations and reaction kinetics; contaminants distribution and concentration; co-contaminants concentrations; and parameter such as moisture content, nutrient supply, pH, and temperature. This bioremediation technology is less expensive and more effective alternative to standard pump-and-treat methods that are used to clean up the polluted soils and aquifers using organic chemicals such as chlorinated solvents and fuel hydrocarbon, but has been extended in the breadth to discourse explosives, toxic metals like chromium and inorganic like nitrates. It is an excellent method to detoxifying the polluted environments as it is economical and uses toxic-free microorganisms to deteriorate the chemicals. It has the potential to offer benefits like complete elimination of contaminants, lesser equipment and operating costs, and lower risk to site workers (Hardisty and Ozdemiroglu 2005).

Types of In Situ Bioremediation

The in situ bioremediation is classified into two major types such as intrinsic and engineered in situ bioremediation.

Intrinsic in situ bioremediation is based on natural processes to degrade environmental contaminants without changing current conditions or enhancing amendments. This method can be dealt with stimulation of indigenously or naturally occurring microbial population via feeding those nutrients and oxygen to improve their metabolic activities (Rehm et al. 2000). It is the biological natural attenuation of pollutants in the environment.

Engineered in situ bioremediation can be used to expedite the degradation process through enhancing the physiochemical conditions to boost up the growth of microorganism. The natural conditions are not as much of important for engineered

bioremediation as for intrinsic bioremediation. This kind of in situ bioremediation can be used for treatment of soils, groundwater, and rarely aquatic sediments. Techniques involved in the in situ bioremediation for the treatment of contaminated soil and groundwater include pump-and-treat system, bioventing, percolation, biosparging, and bioslurping (Rehm et al. 2000).

Pump-and-treat systems are useful for the removal of polluted water from ground. The treatment may be carried out at either on site or off site, and it comes back to the polluted site. The limitations of this treatment are expensive in investment, maintenance, and more time-consuming process. But, it has an advantage to remove all pollutants from this system if it is water-soluble form.

Bioventing is the natural biological process, which can be used to biodegrade the aerobically degradable compounds by supplying oxygen to facilitate microorganisms. In this treatment, air is slowly pumped into the contaminated soil anywhere above the water table in the form of air through the injection wells. Air blower may be used for pulling or pushing the air into soil via the injection well. Heat, nutrients (e.g., nitrogen and phosphorous), water, and oxygen are pumped through the injection wells to improve the growth rate of microorganisms in the soil. The number of wells and location is based on the geological factor and engineering consideration. The major requirements of the bioventing process are adequate concentration of preexisting microorganisms; air flowing conditions: fast flow to maintain aerobic condition, but slow flow to reduce volatile compounds (VOCs) rising to the surface; warm temperature; and soil pH ranging from 6 to 8. Bioventing is used for the treatment of petroleum hydrocarbon, VOCs, non-chlorinated solvents, and some pesticides. However, this bioventing technique is not effective, when the water table is near to ground surface; moisture content is either extremely high or low; and temperature is low.

Percolation is the process of applying water with nutrients and microorganism to the surface of the contaminated area and let it to filter into soil and mingle with groundwater.

Biosparging is the treatment process for the injection of air and rarely gas-phase nutrients under pressure below the water table (saturated zone) to raise the oxygen concentration in groundwater and increase the rate of biodegradation of contaminants using naturally available bacteria. Biosparging is similar to the air-sparging in which volatile contaminants are also be separated from the saturated zone through desorption and volatilization process. Benzene, toluene, xylene, and ethylbenzene may be removed from the contaminated soil using this technology. Most widely, the biosparging technique can be used for the treatment of mid-weight petroleum hydrocarbon contaminants (e.g., diesel and jet fuels).

Bioslurping is an in situ bioremediation technology, also called as multi-phase extraction, which is used to remove the free product that is floating on the water table. It combines vacuum-enhanced free product recovery with bioventing. Vacuum-enhanced free product recovery extracts light, non-adequate-phase liquids (LNAPLs) from the capillary fringe and the water table. Bioventing is used for the bioremediation of contaminated soils. This system is mainly designed to control the

environmental release of soil gas and groundwater. Petroleum hydrocarbons and LNAPLs are treated by using bioslurping. Bioslurping is an economical technology, which can be applicable to the site with a deep water table.

Advantages and Disadvantages of In Situ Bioremediation

Advantages of in situ bioremediation are explained as follows:

- The in situ bioremediation may be possible to convert entirely the organic contaminants into harmless substances. Therefore, it ensures lesser exposure to public or site workers
- The simultaneous treatment of dissolved and sorbed contaminants is possible
- Time needed to treat subsurface pollution can be faster in the in situ bioremediation than pump-and-treat processes
- Because of these factors, it is cost-effective
- Bulk volume of soil can be treated by using this method.

Disadvantages of in situ bioremediation are explained as follows:

- It is a time-consuming process, seasonal difference in the microbial activity, and problematic usage of treatment additives in the natural environment
- In some situations, this bioremediation may turn into uncontrollable and minimum manageable.

9.3.2.2 Ex Situ Bioremediation

Ex situ bioremediation is the biological process, which can be applied to degrade organic contaminants from excavated soil or pumped out of water. In the bioremediation technique, contaminants in polluted soil can be excavated and located in a lined above-ground treatment area and aerated subsequent processing for improving the removal of contaminants concentration by indigenous microbial population. This technique generally depends on a number of site-specific factors such as type of contaminants, type of soil, the cost of treatment, depth of pollution, geographical location, and degree of pollution (Alvarez and Illman 2006). The effective bioremediation can be achieved based on the enhancement of the soil properties to allow uniform oxygenation, nutrients distribution, and optimal moisture control.

Type of Ex Situ Bioremediation

Ex situ bioremediation is also classified into two types as slurry-phase and solid-phase bioremediation.

Slurry-phase bioremediation is a controlled treatment process, which is also called as bioreactor. In this treatment, excavated contaminated soil is mixing with water and other additives, which is placed in the large bioreactor tank. This mixing is continued to keep the interaction of microorganisms with the contaminants in soil, which will create the optimum environment to the microorganisms to degrade contaminants (Alvarez and Illman 2006). The key of successful ex situ bioremediation may be the proper sampling technique and controlled conditions with collected core samples.

Solid-phase bioremediation can be used to excavate and place the contaminated soil into piles. The growth of microorganisms is stimulated by the network of pipes which are distributed through piles. The ventilation is essential for microbial respiration by pulling air via pipes. Water is sprayed on the soil to introduce moisture. This solid-phase bioremediation has some disadvantages, when compare to slurry-phase, which are large space requirement and more time needed for cleaning. There are three types of treatment processes; they are land farming, soil biopiles, and composting (Alvarez and Illman 2006).

Land farming is a biodegradation technique, which includes excavating the contaminated soil and distributing them on a thin surface. Tilling or plowing the soil aerobically can stimulate the degradation of pollutants. The indigenous microbial growth is to be enhanced by the nutrients and minerals (Alvarez and Illman 2006). Excavation of contaminants is not necessary if the polluted soil is lesser than three feet. Seasonal spraying soil with water can form moisture that becomes a barrier around the contaminated soil that controls erosion and also minimize the dust formation while tilling the soil to support aeration.

Soil biopiles is the immediate process, also called as biocells, in which the excavated soil and soil amendments are mixed and made into compost. It is a hybrid of land farming and compost which gives a favorable environment for the indigenous aerobic and anaerobic microorganisms. The height of biopiles is in the range of three to ten feet. The biopiles also uses oxygen for the stimulation of microbial growth. Engineering cells constructed as aerated composted piles can be used for the treatment of surface contaminants to control the physical losses of the contaminants by leaching and volatilization.

Composting is a biological process that can be used to produce optimum level of air and water to microorganism by mixing of the contaminated soil with a bulking agent like hay, straw, or corncobs. Composting is divided into three types: static pile composting, mechanically agitated composting, and window composting. The static composting which involves the contaminated soil is located into piles and then aerated them using either blower or vacuum pumps. It is also known as biopiles, which is an effective method to bioremediate the petroleum contaminants. In the mechanically agitated composting, the contaminated soil is placed in the treatment vessels in which it can be mixed to attain aeration. The window composting is carried out to place the contaminated soil in the long piles called as windows and seasonally blended by tractors (Alvarez and Illman 2006). This type of composting can be called as the most economical alternative, but the fugitive

emission of volatile organic compounds (VOCs) by the window composting may be in the greater level. The time required for cleaning up is faster for composting.

Advantages and Disadvantages of Ex Situ Bioremediation

- It is a simple and suitable treatment for a broad range of contaminants.
- It is not suitable for heavy metals pollution or chlorinated hydrocarbons like trichloroethylene.
- Further processing is essential for the non-permeable soil such as clay and silts.

9.3.3 Factor Affecting Bioremediation

The control and optimization of bioremediation processes for the removal of contaminate from polluted environment is a complex system of various factors. In general, there are three major factors affecting the bioremediation process and are microbial population, chemical factors (bioavailability and biodegradation of contaminants), and environmental factors (temperature, pH, nutrients, oxygen content, water availability, and soil type).

9.3.3.1 Microbial Populations for Bioremediation

Microorganisms are widely utilized for the bioremediation of pollutant for soil and water. These microbes are isolated from different environment conditions. Microbes can grow at frozen temperature, as well as heat, in the water with oxygen, and in anaerobic conditions (Fingerman and Nagabhushanam 2005). These microorganisms are generally classified into four types: aerobic, anaerobic, ligninolytic fungi, and methylotrophs. Aerobic bacteria can be grown in the presence of oxygen. Examples for the aerobic bacteria include *Sphingomonas, Pseudomonas, Alcaligenes, Mycobacterium* and *Rhodococcus.* They can eat the contaminants as the source of carbon and energy. Anaerobic bacteria are growing in the absence of oxygen. However, they are not mostly as used as aerobic bacteria. These kinds of bacteria can be utilized for the bioremediation of polychlorinated biphenyls (PCBs). Fungi are also having an ability to degrade the toxic pollutants in environment, e.g., *Phanaerochaete chrysosporium*. Methylotrophs are aerobic bacteria that can utilize methane as a source of carbon and energy.

9.3.3.2 Chemical Factors

Bioavailability of Pollutants

Bioavailability can be clearly defined as the quantity of pollutants, which can be easily consumed by microorganisms and degraded. Bioavailability is also explained as the possibility of effective contact between the pollutants and the microorganism. The maximum microorganism–pollutant interface leads to the better contact. In general, microorganisms absorb contaminants from aqueous medium and are not efficient to degrade a pollutant until it can be desorbed, diffused, or dissolved (Talley 2005). Therefore, these desorption, diffusion, or dissolution rates are controlling the biodegradation rates. Water-soluble and polar pollutants are more readily bioavailable. Surface active agents are used for the increase of microorganism–pollutant contact.

Biodegradability of Pollutants

The successful bioremediation generally depends on the chemical structure of the organic molecules existing in the polluted environment. Organic molecules (xenophores, e.g., substitutions of H with NO_2, Cl, CN, and SO_3 groups) are difficult to be metabolized with microorganisms. Thus, xenophores containing contaminants tend to be recalcitrant to microbial degradation. Several mechanisms have been explained for the degradation of organic molecules in contaminants (Talley 2005). Enzymes are used for most of the metabolic reactions. The *Oxygenase* enzymes have higher degradation capacity to attack aromatic hydrocarbons because of their non-specific substrate affinity.

9.3.3.3 Environmental Factors

Temperature

Temperature is an important factor that can affect the rate of degradation by controlling enzymatic reactions within microorganisms. Usually, the enzymatic reactions in the cell can be double for every 10 °C increase in temperature. Above a particular temperature, the cell will die. Most of the microorganisms are growing well at the temperature in the range of 10–38 °C (Rike et al. 2008). Temperature of the in situ process is very difficult to control, and temperature of ex situ processes can be slightly affected.

pH

The most of the bioremediation processes are carried out under pH ranging from 5.5 to 8. In this optimum pH range, the most of microorganisms, especially heterotrophic bacteria, are used in many bioremediation technologies. pH is effected by the complex relationship between microorganism, contaminant chemistry, and physical and chemical characteristics of local environment (Rike et al. 2008). There is a possibility of pH change during the bioremediation of contaminants, so the regular monitoring is required. The acidic or basic substances are added to adjust the pH in the desired range. pH is the important factor that affects the soil dispersion and its permeability.

Nutrients

Nutrients are essential for the stimulation of indigenous microorganism population in the contaminated soil or water. The major constituents in nutrients are carbon, oxygen, nitrogen, and phosphorous.

Moisture Content and Water Availability

Moisture content is the factor for the bioremediation. Moisture content in soil can affect the bioavailability of pollutants, the transfer of gases, the effective toxicity of pollutants, microorganism mobility, and their growth stages (Suthersan 1999). The presence of excess water content will make the entry of atmosphere oxygen to the soil difficult that can be a factor of limiting growth efficiency. The moisture content in soil is estimated to get some information about the water availability for microbial metabolism.

9.3.4 Bioremediation of Heavy Metals by Microorganism

Microorganisms play a vital role in the nutrient chains, which are the significant portion of the biological equilibrium in the life in our earth. These microorganisms are necessary for the closing of nutrient and geochemical cycles including carbon, sulfur, nitrogen, and phosphorous cycle. Bioremediation of heavy metals using microorganisms is an economical, efficient, and eco-friendly technique to minimize the utilization of chemical methods of bioremediation in industries. The heavy metal detoxification process is carried out using microorganisms via the valence transformation, extracellular chemical precipitation, and/or volatilization. Some heavy metals can also be degraded in the metabolic processes of microbes by the enzymatic reduction. The level and effectiveness of remediation depends on both heavy metal and microorganism. Some of the bioremediation techniques are

Table 9.1 Microorganisms that utilize heavy metals

Microorganisms	Species	Elements	References
Bacteria	*Bacillus substilis*	Cr, Zn, Se	Prasad and Freitas (1999)
	Desulfoviibrio desulfuricans	Cd, Zn	Banik et al. (2014)
	Pseudomonasaeruginosa	U, Cu, Ni	Prasad and Freitas (1999)
	Pseudomonas veronii	Cd, Zn, Cu	Vullo et al. (2008)
	Sporosarcina ginsengisoli	As	Achal et al. (2012)
Fungi	*Aspergillus versicolor*	Cr, Ni, Cu	Tastan et al. (2010)
	Cladonia rangiformis	Pb	Ekmekyapar et al. (2012)
	Penicillium canescens	Cr	Say et al. (2003)
Algae	*Spirogyra sp. and Cladophora sp.*	Pb, Cu	Lee and Chang (2011)
	Spirogyra sp. and Spirullina sp.	Cr, Cu, Zn	Mane and Bhosle (2012)
Yeast	*Saccharomycescerevisiae*	Cd	El-Sayed (2012)
	Candida utilis	Cu	Yuan-gang et al. (2006)

phytoremediation, bioleaching, bioventing, bioreactor, landfarming, composting, and biostimulation. Various types of microorganisms used for the bioremediation of heavy metal are listed in Table 9.1. And the microorganisms are bacteria (*Bacillus substilis*, *Enterobacter cloacae* and *Pseudomonas putida*, *Desulfoviibrio desulfuricans, rthrobacter* spp., *Pseudomonas veronii*, *Kocuria flava,* etc.), fungi (*Penicillium canescens*, *Aspergillus versicolor*, *Aspergillus fumigates, etc*.), algae (*Cladophora fascicularis*, *Spirogyra* spp., *Cladophora* spp., and *Spirullina* spp.), and yeast (*Saccharomyces cerevisiae* and *Candida utilis*). In these microorganisms, bacteria are widely used for the treatment of heavy metals in soil or water, because metal-reducing bacteria are involved to break down the pollutants and convert the very toxic soluble forms into less toxic forms in this remediation process (Banik et al. 2014). In general, metal ions may be adsorbed on the negatively charged carboxylic, hydroxyl, and phosphoryl groups in the bacterial cell wall. Some bacteria like *Bacillus substilis*, *Enterobacter cloacae,* and *Pseudomonas putida* are effectively used for the conversion of toxic Cr (VI) into less toxic Cr (III). These toxic metal ions will be dangerous to environmental and human health. The Gram-positive bacteria (*Bacillus substilis*) have an excellent adsorption capacity as a result of maximum peptidoglycan and teichoic acid content in their cell walls. Hence, it is also used for the reduction of selenite to less toxic elemental Se and for the reduction of non-metallic. The sulfate-reducing bacteria are used to treat the metal leachates formed by sulfuric acid-generating *Thiobacillus* sp. Another sulfate-reducing bacterium (*Desulfoviibrio desulfuricans*) indirectly changes sulfate into hydrogen sulfate through bioprecipitation that consequently interacts with heavy metals like Zn and Cd to produce insoluble form of these metal sulfides (Banik et al. 2014).

Widely, ex situ type of bioremediation may be carried out for microbe-assisted remediation. But, in situ type of bioremediation can easily convert the soluble mercuric ions Hg (II) into volatile metallic mercury. In this treatment, mercury resistant bacteria are used and the reduced Hg (0) may certainly volatilize from the environment and then that may be diluted in the surrounding.

A number of the microbes such as aerobes, anaerobes, and fungi are used as a biological agent in the enzymatic degradation process. The majority of bioremediation systems are carried out under aerobic conditions.

9.3.4.1 Mechanisms

The bioremediation of heavy metals from environment by microorganism can utilize various processes. The mechanisms involved in the bioremediation process are shown Fig. 9.1. In general, three types of mechanisms can be used for the remediation of heavy metals pollution in soil or water by microorganisms and are explained as follows:

- In first type, the biosorption (bioaccumulation) process is carried to concentrate microbes as well as integrate the metallic pollutants onto cell wall.
- In second type, the process of extracellular precipitation and uptake is carried out by purified biopolymers.
- And the third type, specific molecules derived from microbial cells may be involved in the process.

Bioremediation can be divided into two groups: biosorption and bioaccumulation. The biosorption is quick and reversible that is a passive adsorption process. The physicochemical interaction such as adsorption, ion exchange, complexation, crystallization, and precipitation may be taken between the metal ions and the functional groups available on the cell surface. The major factors affecting for the biosorption of heavy metals are biomass concentration, ionic strength, pH, presence

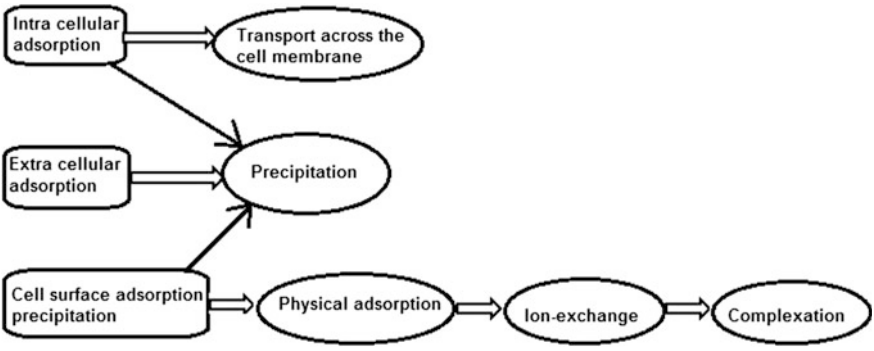


Fig. 9.1 Mechanisms involved in the bioremediation of heavy metals

of some other metal ions in solution, and particle size. The biosorption can be carried out with both living and non-living biomass due to the independent of cell of metabolism. But, the both intra and extracellular processes are involved in the bioaccumulation in which the minimum passive uptake may be taken place. Thus, living biomass is only involved in this bioaccumulation process. The biosorption process is an economical, recoverable, and reusable process. For this process, the biomass can be obtained from industrial waste. But, the bioaccumulation is very costly process. Living cells are involved in this process, and reuse of these cells is limited (Mani and Kumar 2014). Minimum selectivity is possible in the biosorption due to the physicochemical interaction, which can be improved by further modification of biomass. However, the bioaccumulation usually achieves greater compared to biosorption.

Fungi and yeast are also involved in bioremediation. The benefit of fungi used in bioremediation is extremely variable, varied in size from mushroom to microscopic molds. The growth of fungi is very fast and their biomass also produced extensively. Polysaccharide and glycoproteins are the major contents in the cell wall of fungi and comprise of phosphate, amine, sulfate, imidazole, and hydroxyl groups. A high concentration of these cell wall materials in fungi is an excellent binding element to heavy metals. On the other hand, yeast is containing more than 90% of polysaccharides in their cell wall, which is in the form of microfibrillar structure. The main functional groups present in the fungi are sulfate, amine, hydroxide, carboxyl, and phosphate.

9.3.5 Bioremediation of Heavy Metals by Plants

Phytoremediation is a part of bioremediation that uses the green plants to detoxify contaminated soil or water to absorb heavy metals. Either in situ or ex situ bioremediation is used for the phytoremediation process, in which in situ bioremediation is most commonly used as it can control the disturbance of soil and environment and as well as minimize the spread of pollution through airborne and waterborne wastes (Maiti et al. 2003). There are different mechanisms used for the phytoremediation of heavy metal-contaminated soils such as phytoextraction, phytostabilization, and phytovolatilization.

9.3.5.1 Mechanisms of Bioremediation by Plants

Phytoextraction

Phytoextraction is the widely used phytoremediation process that includes the sorption of heavy metals (contaminants) by the roots and shoot of plants along with

water and other nutrients. After the treatment, these plants are harvested and incinerated. The phytoextraction is also called as phytoaccumulation. The advantages of the phytoextraction using plants are high biomass, rapid growth rate, high tolerance to heavy metals, and large root system. The capacity to tolerate high amount of heavy metals using these plants may cause the metal accumulation in the harvestable part that may be difficult via contaminations of food chain. Based on the plant properties, the phytoextraction is carried out with two methods. In the first method, the natural hyperaccumulators (plant) are used that have more potential to accumulate heavy metals. These hyperaccumulators are very effective to accumulate the number of metal that is 10–500 times more metals compared to ordinary plants (Chibuike and Obiora 2014). Therefore, these hyperaccumulators are more appropriate for phytoremediation of heavy metals. In the second method, the high-biomass plant is used with chelates or soil amendment to improve the ability to accumulate metals from the environment. The soil amendment has more metal-mobilizing capacity.

In general, a plant is considered as a hyperaccumulator based on the following conditions:

- The metal (Se, Cu, Co, Cr, Al, and Ni) concentration in the shoot should be greater than 0.1%.
- The ratio of shoot to root concentration should be constantly greater than 1, which explains the ability to transfer metals from root to shoot and the formation hypertolerance capacity.
- The ratio of shoot to root concentration should be constantly greater than 1, which indicates the amount of plant metal uptake.

Commonly available plants (hyperaccumulators) for the accumulation of heavy metals contain *Aerollanthus subacaulis* (Cu hyperaccumulators), *Agrostis tenuis* (Pd hyperaccumulators), *Haumaniastrum robertii* (Co hyperaccumulators), *Maytenus bureaviana* (Mn hyperaccumulators), *Thlaspi tatrense* and *Thlaspi caerulescens* (Zn hyperaccumulators), *Lecythis ollaria* (Se hyperaccumulator), *Pteris vittata* (As hyperaccumulators), and *Streptanthus polygaloides* (Ni hyperaccumulators). Some plants have the capacity for the accumulation of more metal than one in the respective plant (hyperaccumulator), and the example is the hyperaccumulation of Zn and Cd by *Sedum alfredii*. The phytoextraction technique has some limitation, that is, the usage of hyperaccumulators involves the chances of contaminating the food chain. But, the hyperaccumulators of heavy metal from the family of *Brassicaceae* comprise more quantity of thiocyanates that make them unpalatable to animals, and hence, these plants can minimize the availability of metal ions in the food chain. Nowadays, hyperaccumulators are mostly used along with metal-chelating agents for increasing their growth with high biomass production. However, some hyperaccumulators from *Brassica genus* family such as *Brassica napus, Brassica rapa,* and *Brassica juncea* are rapid growers with high biomass. The chelating substances are also used to control the precipitation and metal sorption through the formation of metal chelate complexes that may lead to

improve the bioavailability of these metals. More addition of chelates to soil or water can influence the transportation of more metal ion within the soil solution via the suspension of precipitated elements and desorption of sorbed species. The chelates are also having an ability to translocate metal into the shoots of plants. The drawbacks of using the chelates in the phytoextraction are explained as follows:

- The groundwater contamination is possible through the leaching of these metals due to the use of chelate in the phytoextraction that can increase the presence of heavy metals in soil solution or water.
- More addition of chelates can be harmful to the both plants and microbes in soil.

Phytostabilization

Phytostablization or phytoimmobilization is the process of immobilizing metals by the plant, rather than degrading them. This technique can be used to decrease the bioavailability of metals in the environment through erosion and leaching. Thus, metals migrating to groundwater or metals passing into the food chain have to be prevented by this technique. Phytostabilization of metals in soils can be carried out using plants through sorption, precipitation, complexation, or metal valence reduction. The effectiveness of phytostabilization is based on the use of plants and soil amendments. Plant root systems benefit in stabilizing the soil; thus, the root systems prevent erosion as well as leaching. For phytostabilization, plants should have certain features such as capability to tolerate soil, fast growth to give sufficient ground coverage, dense rooting system, ease of establishment and maintenance under field conditions, and ability to self-propagate. The use of soil amendments in phytostabilization leads to control plant metal uptake and decrease biological activity (Chibuike and Obiora 2014). Most commonly, the organic type of soil amendments is used for this process. Some other amendments used for phytostabilization are lime, litter, phosphate, and biosolids. Compared to these amendments, organic amendments are mostly preferred due to their low cost, supply of nutrient for plant growth, and also enhancement of the physical properties of soil. This phytostabilization is more beneficial for the fast immobilization of heavy metal from soil and groundwater. However, the complete removal of heavy metals from the environment is not possible; thus, continuous monitoring is essential.

Rhizofiltration

Rhizofiltration is the form of phytoremediation, which is used to remediate extracted water with lesser concentration of contaminants by a mass of roots. This technique can be used for different metals such as Cd, Cu, Pb, Zn, Ni, and Cr that are mostly stored into the roots. Indian mustard, sunflower, tobacco, spinach, corn,

and rye are mostly used for the removal of lead (Pb) from water or soil. Generally, both terrestrial and aquatic plants involve in the rhizofiltration for in situ or ex situ purpose. The major drawback of this technique is the pH adjustment needed for regular period of time.

Phytovolatilization

In the phytovolatilization process, the uptake of the water by plants contains organic contaminants from soil; then, these contaminants are converted them into volatile form and are released into air through their leaves. The phytovolatilization can be used for organic pollutants as well as few heavy metals such as selenium (Se) and mercury (Hg). This technique could not remove the pollutant entirely; only it converts the toxic form of metal (pollutant) into less toxic form. The drawback of this process is the formation of new product that may be redeposited into lakes and rivers (Chibuike and Obiora 2014). For this technique, genetic engineered plants are widely used to uptake pollutants, especially Hg. *Brassica juncea* and *Brassica napus* have been used for phytovolatilization of Se from soil.

9.3.6 Bioremediation of Heavy Metals by Algae

The removal mechanisms for heavy metal comprise flocculation, sedimentation, precipitation, absorption, cation and anion exchange, oxidation/reduction, and microbial action and uptake. The remediation of heavy metals from polluted by microalgae is based on two major mechanisms and are explained as follows: (i) a metabolism dependent uptake into cells at low concentration and (ii) biosorption is a non-active adsorption process by biological materials. Nowadays, the aquatic plants particularly micro- and macro-algae are considered to be an effective biological agent for the degradation of heavy metals from polluted soil or water, because of their capacity to absorption of heavy metals and acceptance of harmful element from surroundings or converting them into less toxic compounds. The properties of the algae include large surface area/volume ratios, potential to grow both heterotrophically and autotrophically, high tolerance to heavy metals, ability for genetic manipulation, phototaxy, and phytochelatin expression.

In general, macro-algae are widely preferred for the treatment for the heavy metal pollution and marine environment all over the world. They have an ability to accumulate metal ions in their tissues. Green, brown, and red algae are utilized for the metal removal. The main advantage of algae is that the algae do not produce harmful substance compared to some other microorganisms including bacteria or fungi (Chekroun and Baghour 2013). Some of algal species are including *Chlorophyta*, *cyanophyta, Phaeophyta, Caulerpa racemosa* var. *cylindracea, Fucus vesiculosus, Phormidium, Spirogyra hyaline, Sargassum natans,* and

Dunaliella salina etc., *Chlorophyta* and *Cyanophyta* algae are hyper-phytoremediator, can be used to absorb and accumulate the metals such as arsenic and boron from the environment (Chekroun and Baghour 2013). *Chlorophyta* and *Cyanophyta* are called as hyper-absorbents and hyperaccumulators, respectively. *Phaeophyta* is one of the brown algae, predominantly effective accumulator for heavy metal owing to their availability of high level of sulfated polysaccharides and alginates inside their cell walls, in which metal can interact with them. *Caulerpa racemosa* var. *cylindracea* is also used for the removal of boron from aqueous solution due to its economical nature. Other brown algae (*Focus spp.*) accumulate the heavy metal-polluted habitats.

Phormidium, a blue green alga, is an efficient hyperaccumulator for removal of heavy metals such as Zn, Pb, Ni, Cd, and Cu. A green microalga (*Dunaliella salina*) is having more tendencies to accumulate zinc as well as cobalt and copper. For the reason that zinc act as hydrogen transferring element in photosynthesis. *Cladophora glomerata and Oedogonium rivulare* have been used for the copper, lead, and cadmium removal. Different freshwater microalgae such as *Anabaena sp., chlorella sp., westiellopsis sp., and synecococcus sp.* have high tolerant capacity to remove different contaminants. The adsorption capacity of various algae species and the algae of similar species may be different to adsorb heavy metals. The effective accumulation of these metal cations using algae can be due to their high negatively charged cell wall elements.

9.3.6.1 Mechanisms

The standard mechanism of metallic cation sequestration comprises the creation complexes among a metal ion and functional groups either on the surface or within the porous structure of the biomaterial. First, metals are rapidly adsorbed on the cell surface of algae that can be called as physical adsorption, and after these metal ions are slowly diffused within cytoplasm by the process, which is called chemisorption. For the accumulation of heavy metals, the different defense systems are used by algae and are exclusion, compartmentalization, making complexes and the production of binding proteins like phytochelatins (PCs) or metallothioneins (MTs) and translocate them within vacuoles. For heavy metals, carboxylic and amino acids are potential ligand that can play a role in tolerance and detoxification and are including malate, citrate, oxalate, phosphate derivatives (phytate), nicotianamine (NA), and histidine (Halder 2014).

9.3.7 Bioreactors

In bioremediation techniques, the best type of bioreactors is most commonly used, which can be used to attain most efficient process for the removal of heavy metals

from polluted effluents. In general, a bioreactor is a system in which a chemical process can be occured that contains organisms. In the heavy metal removal, microorganisms or other types of biomass are used in some bioreactor, which improve the performance of bioreactor and are explained below.

9.3.7.1 Stirred Tank Bioreactor (STRs)

The stirred tank bioreactors (STRs) are equipped with stirrers that can maintain the biomass in a suspension state. This system can be used in batch or continuous modes. Liquid phase is separated from solid phase either by sedimentation or by filtration. During sedimentation, mixing is stopped in bioreactor for phase separation. The bioremediation process carried out in STRs is not much complicated compared to other systems such as fixed-bed bioreactor or fluidized bed bioreactors. However, the operational costs are more for STRs system due to energy requirements.

9.3.7.2 Fluidized Bed Bioreactor (FBRs)

In the fluidized bed bioreactor, a microbial biofilm is formed on the solid particles which can support the bacterial growth. This operation is performed at short hydraulic retention time. The water is passed into the reactor to maintain the bed in the suspended state. The optimal hydrodynamic and mass transfer conditions are to be achieved by the continuous movement of particles in the fluidized state. The clogging is considerably minimized in the fluidized bed reactors (FBRs) than fixed-bed bioreactor (Howard 1989).

9.3.7.3 Airlift Reactors (ALRs)

Airlift reactors are usually the motionless bioreactor, in which the internal movement and mixing are performed by bubbling air. In these reactors, the medium of the vessels is separated into two interrelated zones by a draft tube or baffle tube. Two types of airlift bioreactor are generally available and are internal loop airlift bioreactor and external loop airlift bioreactor. The major advantages of ALRs are including low power consumption, no moving parts, fast mixing, better heat and mass transfer properties, improved oxygen solubility, excellent solid suspension, homogeneous shear, maximum efficiency of homogenization and minimal shear stress to cells, less pollution risk, and easy sterilization (Merchuk 2003).

9.3.7.4 Fixed-Bed Bioreactors (FXRs)

In this system, the biosorbent (organism or other biomass) is organized in a fixed-bed column. The solution polluted with heavy metals is allowed to pass

through the fixed-bed column. In these types of bioreactors, the regeneration of the fixed bed is required after attaining the maximum adsorption of pollutant on the biosorbent (fixed bed). For continuous operating condition, two columns are essential. If the biosorption process is carried out in the first fixed-bed column, simultaneously, the fixed bed is regenerated by using chemical substances to wash spent biomass in the second column (Viggi et al. 2010). Packed-bed column (PBCs) is one of the most commonly used fixed-bed bioreactors for the biosorption process. The major advantages of using PCBs are including countercurrent flow during process, simple in construction and operation. These columns are used in either continuous or batch settings.

9.3.7.5 Rotating Biological Bioreactor (RBC)

In rotating biological bioreactor (RBC), a fixed film of microorganisms is formed on the contactor that can be built from sets of discs placed to a central horizontal shaft. The shaft is rotated slowly, and the fixed biomass alternatively is submerged into the effluent, in which it can absorb heavy metals or absorb oxygen when it is raised out of liquid. The advantages of RBC include simple operation, low space requirements, low maintenance requirement, resistance to shock load, and excellent treatment efficient requirement (Grady et al. 1998).

9.4 Recent Trends

9.4.1 Application of Genetic Engineering

Genetic engineering is a recent technology, which is also called as recombinant DNA technology that allows modifying organisms (plant, animals, microorganisms, etc.) through natural genetic exchange between microorganisms. The modified organisms are generally referred as genetically modified organism (GMO) that has potential for bioremediation of polluted soil and water.

9.4.1.1 Genetically Modified Microorganisms

Genetic engineering technology has been focused on the development of genetically modified microorganisms that are used as microbial biosensors to determine the quantity of contaminants (heavy metals) in environment. Different biosensors are developed for the measurement of heavy metals such as, Hg, Cu, Cd, As, and Ni in the polluted sites quickly and accurately. Genetic modification of endophytes and rhizospheric bacteria involved in plant related decomposition of contaminants in soil which is used as an innovative technique for the removal of metals from

polluted sites (Kang 2014). *Escherichia coli* and *Moreaxella* sp. are expressing phytochelatin on the cell wall, which can accumulate more than 25 times of Cd or Hg compared to the wild-type strains (Zhang et al. 2015).

9.4.1.2 Genetically Modified Plants

In genetic engineering, the bacterial genes in genetically modified plants are mainly responsible for the degradation of metals. These bacterial genes can be introduced inside the plant tissues that lead to degrade metals inside the tissues. The advantages of plant-based bioremediation using the genetically engineered plants (GE) are sustainability and lesser toxic to human and environment. For the accumulation and detoxification of heavy metals, varieties of microbial genes are attached to the transgenic plant and some of the metal-detoxifying chelators include metallothioneins and phytochelatin. This chelator has been frequently utilized to enhance the metal-binding capacity, tolerance, or accumulation of bacteria and plants. The transgenic plants (*Brassica juncea*) overexpressing gene-encoding glutathione synthetase (*gshII*) suggestively accumulate large quantity of Cd. The transgenic plants transporting bacterial reductase can increase the volatilization of Se and Hg, although they are accumulating arsenic (As) in plant shoots. Poplar willow and *Jatropa* may be used for the phytoremediation and energy production due to their fast-growing and great-biomass production. The metal-contaminated plant materials are burning for energy production that may be released from soil or water to air. The toxic metal compounds are transferred from soil or water to air that can be harmful to environment. Therefore, the proper storage or disposal is required to overcome the harmful effect against the environment. The most of hybrid varieties of poplar are genetically modified by microbial catabolic genes and specific transporters to improve the remediation of metals (Sriprang and Murooka 2007). Genetically modified mercuric reductase and γ-glutamylcysteine synthetase genes can accumulate the higher concentration of heavy metals such Hg and Cd and Cu, respectively. The thiosulfate reductase gene from *Salmonella typhimurium* and *Escherichia coli*, which have been used to improve the effectiveness of heavy metal removal from aqueous solution. Nicotianamine (NA) is abundantly obtained from most of the plants. Nicotianamine synthase cDNA (TcDNA) sequestered from polymetallic hyperaccumulator *Thlaspi caerulescens* is expressed with *Arabidopsis thaliana*. Nickel tolerance is increased in these transgenic plants.

9.4.2 Rhizosphere Engineering

Engineered bioremediation approaches consist of either by adding of growth stimulators to the rhizosphere for heavy metal removal or by adding of nutrients to the polluted soil for improvement of bioremediation properties of microorganisms or genetically modified microorganisms and microbial growth. Several engineered

bacteria and genetically modified plants are known to form specific compounds that can support the rhizospheric transformation of heavy metals. Nowadays, these strategies have been used by various researchers to remove heavy metals from soil using the rhizosphere ecosystem (Dixit et al. 2015).

9.4.3 Application of Nanotechnology

Nanotechnology plays an important role in the bioremediation of heavy metals from environment. Nanoparticles are obtained naturally as well as artificially. The advantages of nanoparticles are sustainable and benign to environment. It can also be used to remove polluted constituents and chemical used as sensor of variations in the environment. Examples for the nanoparticles are nZVI (nano zerovalent ion), Fe_2O_3, SiC, SnO_2, and Al_2O_3. The nanoparticles have an ability to break down organic compounds like polychlorinated diphenyl (PCB) and poly chlorinated hydrocarbons (PCH). Fe/Pd biometallic nanoparticles in conjunction with surfactants such as saponin and Tween 80 are used for the removal of PCB from soil. The nanoparticles in the form of carbon nanotubes are used for the adsorption of dioxins in association with a conventionally activated carbon. Nanoparticles of hydroxyapatite are used for the reduction of bioavailability of Cu and Zn in the soil. Iron particles are predominantly used for the recovery and disposal of non-biodegradable materials (Juwarkar et al. 2010). The main mechanisms involved in the production of nanoparticles using microorganisms are comprising change of toxicity and solubility by reduction or oxidation, shortage of particular metal transport system, biosorption, extracellular complexation or precipitation of metals, bioaccumulation, and efflux system. The maximum activity of nanoparticles is generally based on their unique properties and maximum unfilled active surface areas. The nanoparticle catalysts are usually located in the cells to stimulate the activity of microorganisms that may improve the microbiological reaction rates.

Fungi are also used for the production of metal and metal sulfide nanoparticles because of the easy handling and availability of a range of enzymes in their cell wall. Compared to bacteria, fungi are producing more amounts of nanoparticles due to its huge quantity of proteins. The gold nanoparticles are also used for the removal of different heavy metals in environment. Nowadays, these nanoparticles are synthesized from the plants using chloroplast that acts as reducing agent as well as stabilizer. Iron phosphate nanoparticles, also called as vivianite, are used for the in situ immobilization of Pb^{+2} in soils. The bioavailability and mobility of Pb^{+2} may be decreased by using these nanoparticles. Even though nanoparticles are useful to remove heavy metals from contaminated environment such as soil and water, they can release toxic compounds that can develop health problems to human and environment.

9.4.4 *Effect of Plant–Microbe Symbiosis*

Phytoremediation is the effective method to remove heavy metal from polluted environment, but this method has some disadvantages. The accumulation and storage of contaminants in plant material and then process are insufficient when the multiple contaminants are present in polluted sites. To overcome this problem, this approach is used to unite plant–microbe symbiosis within the plant rhizosphere or to add microbes as endophytes within the plant rhizosphere for the degradation of contaminants (Dixit et al. 2015). The microbial population is greater in the rhizosphere than in the vegetation-less soil due to the simplification delivered by the plants. Still, this approach has been under the laboratory conditions. Some of bioremediation techniques like biostimulation and bioaugmentation approaches are also using the combination of microorganisms and plants for the heavy metal removal. Biostimulation consists of the addition of supplements into the polluted sites to stimulate the growth of microorganism for degrading the heavy metals. Bioaugmentation is the method, which is used to remove heavy metals from polluted soil or water by the addition of a group of naturally available microbial strains or genetically engineered variants.

9.5 Conclusion

Bioremediation is a successful remediation technique to clean up contaminated land and water using microorganisms. This remediation technology provides an efficient and cost-effective way to treat heavy metals in contaminated site. This chapter summarizes new or established research advances that expand the future capabilities of bioremediation. It evaluates the understanding of microbial processes for cleanup standards and their byproducts of stimulating materials to promote contact between contaminants and microbes. In such a way, biodegradation occurs at fast enough rates to ensure that cleanup goals are met.

References

Achal V, Pan X, Fu Q, Zhang D (2012) Biomineralization based remediation of As (III) contaminated soil by sporosarcinaginsengisoli. J Hazar Mater 201–202:178–184

Alvarez PJJ, Illman WA (2006) Bioremediation and natural attenuation: process fundamentals and mathematical models. Wiley

Banik S, Das KC, Islam MS, Salimullah M (2014) Recent advancements and challenges in microbial bioremediation of heavy metals contamination. JSM Biotechnol Biomed Eng 2:1035

Bsoul AA, Zeatoun L, Abdelhay A, Chiha M (2014) Adsorption of copper ions from water by different types of natural seed materials. Desalin Water Treat 52:5876–5882

Catherine L, Olivier P (2012) Gold nanoparticles for physics, chemistry and biology. World Scientific. ISBN 9781848168077

Chekroun KB, Baghour (2013) The role of algae in phytoremediation of heavy metals: a review. J Mater Environ Sci 4:873–880
Chibuike GU, Obiora SC (2014) Heavy metal polluted soils: effect on plants and bioremediation methods. Appl Environ Soil Sci. https://doi.org/10.1155/2014/752708
Clarkson TW, Magos L (2006) The toxicology of mercury and its chemical compounds. Crit Rev Toxicol 36:609–662
Cohen MD, Kargacin B, Klein CB, Costa M (1993) Mechanisms of chromium carcinogenicity and toxicity. Crit Rev Toxicol 23:255–281
Dierks S (2005) Gold MSDS, electronic space products international
Dixit R, Wassiullah Malaviya D, Pandiyan K, Singh UB, Sahu A, Shukla R, Singh BP, Rai JP, Sharma PK, Lade H, Paul D (2015) Bioremediation of heavy metals from soil and aquatic environment: an overview of principles and criteria of fundamental processes. Sustainability 7:2189–2212
Ekmekyapar F, Aslan A, Bayhan YK, Cakici A (2012) Biosorption of Pb(II) by nonliving lichen biomass of Cladonia rangiformishoffm. Int J Environ Res 6(2):417–424
El-Sayed MT (2012) The use of Saccharomyces cerevisiae for removing cadmium(II) from aqueous waste solutions. Afr J Microbiol Res 6(41):6900–6910
Fingerman M, Nagabhushanam R (2005) Bioremediation of aquatic and terrestrial ecosystem, Science Publishers Inc.
Flora SJS, Flora GJS, Saxena G (2006) Environmental occurrence, health effects and management of lead poisoning. In: Cascas SB, Sordo J (eds) Lead: chemistry, analytical aspects, environmental impacts and health effects. Elsevier Publication, Netherlands, pp 158–228
Fowler BA, Nordberg GF, Silver (1986). In: Friberg L, Nordberg GF, Vouk VB (eds) Handbook on the toxicology of metals. Amsterdam, Elsevier, pp 521–531
Garbisu C, Alkorta I (2003) Basic concepts of on heavy metal soil bioremediation (Review). Eur J Miner Process Environ Prot 1:58–66
Gonzalez I, Aguila E, Galan E (2007) Partitioning, bioavailability and origin of heavy metals from the Nado Laggoon sediments (Morocco) as a basis for their management. Environ Geol 52:1581–1593
Goyer R (1991) Toxic effects of metals. In: Amdur MO, Doull JD, Klaassen CD (eds) Casarett and Doull's Toxicology, 4th edn. Pergamon Press, Newyork, pp 623–630
Grady CPL, Daigger GT, Lim HC (1998) Biological wastewater treatment, 2nd edn. CRC Press. ISBN 0-8247-8919-9
Halder S (2014) Bioremediation of heavy metals through fresh microalgae: a review. Scholars Acad J Biosci 2(11):825–830
Hardisty PE, Ozdemiroglu E (2005) The economics of groundwater remediation and protecttion. CRC Press
Herawati N, Suzuki S, Hayashi K, et al (2000) Cadmium, copper and zinc levels in rice and soil of Japan, Indonesia and China by soil type. Bull Env Contam Toxicol 33–39
Hogan CM (2010) Heavy metal. In: Monosson E, Cleveland C (eds) Encyclopedia of earth. National Council for Science and the Environment, Washington, DC
Howard JR (1989) Fluidized bed technology: principles and applications. Adam Higler, New York, NY
Juwarkar AA, Singh SK, Mudhoo A (2010) A comprehensive overview of elements in bioremediation. Rev Environ Sci Biotechnol 9:215–288. https://doi.org/10.1007/s11157-010-9215-6
Kang JW (2014) Removing environmental organic pollutants with bioremediation and phytoremediation. Biotechnol Lett 36:1129–1139. https://doi.org/10.1021/es203753b
Kensa VM (2011) Bioremediation—an overview. J Ind Pollut Contr 27(161):168
Kulshreshtha A, Agrawal R, Barar M, Saxena S (2014) A review on bioremediation of heavy metals in contaminated water, IOSR. J Environ Sci Toxicol Food Technol 1(44):50
Lee YC, Chang SP (2011) The biosorption of heavy metals from aqueous solution by Spirogyra and Cladophora filamentous macroalgae. Biores Tech 102:5297–5304

Maiti RK, Pinero JLH, Oreja JAG, Santiago DL (2003) Plant based bioremediation and mechanisms of heavy metal tolerance of plants: a review. Pro Indian natn Sci Acad B70(1):1–12

Mane PC, Bhosle AB (2012) Bioremoval of some metals by living Algae Spirogyra sp. and Spirullina sp. from aqueous solution. Int J Environ Res 6(2):571–576

Mani D, Kumar C (2014) Biotechnological advances in bioremediation of heavy metals contaminated ecosystems: an overview with special reference to phytoremediation. Int J Environ Sci Technol 11:843–872

Merchuk JC (2003) Airlift bioreactors: review of the recent advances. Can J Chem Eng 81:324–337

Pirkle JL, Kaufmann RB, Brody DJ, Hickman T, Gunter EW, Paschal DC (1998) Exposure of the U.S. population to lead: 1991–1994. Environ Health Perspect 106:745–750

Prasad and Freitas (1999) Removal of toxic metalsfrom solution by leaf, stem and root phytomass of Quercus Ilex L. (Holly Oak). J Environ Pollut 110:277–283

Rehm HJ, Reed G, Puhler A. et al (2000) Environmental processes II—soil decontamination. Wiley—WCH

Rike AG, Schiewer S, Filler DM (2008) Bioremediation of petroleum hydrocarbons in cold regions. In: Filler DM, Snape I, Barnes DL (eds.), Cambridge University Press

Satarug S, Baker JR, Urbenjapol S, Haswell-Elkns M, Reilly PEB, Williams DJ, Moore MR (2003) A global perspective on cadmium pollution and toxicity in non-occupationally exposed population. Toxicol Lett 137:65–83

Say R, Yimaz N, Denizli A (2003) Removal of heavy metal ions using the fungus Penicillium canescens. Adsorpt Sci Technol 21:643–650

Seaborg GT (1968) Uranium. In: The Encyclopedia of Chemical Elements. Skokie, Illinois: Reinhold book corporation, pp 773–786. LCCCN 68-29938

Sriprang R Murooka Y (2007) Accumulation and detoxification of metals by plants and microbes. In: Singh SN, Tripathi RD (eds) Environmental bioremediation technologies. Springer, Berlin, Heidelberg, pp 7–100. https://doi.org/10.1007%2F978-3-540-34793-4

Sun HJ, Rathinasabapathi B, Wu B, Luo J, Pu LP, Ma LQ (2014) Arsenic and selenium toxicity and their interactiveeffects in humans. Environ Int 69:148–158

Suthersan SS (1999) Remediation engineering design concepts, CRC Press Lewis Publishers

Talley JW (2005) Bioremediation of recalcitrant compounds. In: Talley J (ed), Taylor & Francis

Tastan BE, Ertug˘rul S, Do˙nmez G (2010) Effective bioremoval of reactive dye and heavy metals by Aspergillus versicolor. Bioresour Technol 101(3):870–876

Techounwou PB, Yedjou CG, Patlolla AK, Sutton DJ (2014) Heavy metal metals toxicity and the environment. PMC. https://doi.org/10.1007/978-3-7643-8340-4_6

Thiele DJ (1995) Metal detoxification in eukaryotic cell, Crisp. Data Base of National Institute of Health, Washington D.C

Viggi CC, Pagnanelli F, Cibati A, Uccelletti D, Palleschi C, Toro L (2010) Biotreatment and bioassessment of heavy metal removal by sulphate reducing bacteria in fixed bed reactors. Water Res 44(1):151–158

Volesky B (1990) Biosorption of metal recovery. Trends Biotechnol 5:96–101

Vullo DL, Ceretti HM, Hughes EA, Ramyrez S, Zalts A (2008) Cadmium, zinc and copper biosorption mediated by Pseudomonasveronii 2E. Bioresour Technol 99:5574–5581

Wilson DN (1988) Association Cadmium. In: Cadmium—market trends and influences. Cadmium 87 Proceedings of the 6th International Cadmium Conference. London, pp 9–16

Yuan-gang Zu, Xia-hua ZHAO, Mao-sheng HU, Yuan REN, Peng XIAO, Lei ZHU, Zhang Yao (2006) Biosorption effects of copper ions on Candida utilis under negative pressure cavitation. J Environ Sci 18(6):1254–1259

Zhang R, Xu X, Chen W et al (2015) Genetically engineered Pseudomonas putida X3 strain and its potential ability to bioremediate soil microcosms contaminated with methyl parathion and cadmium. Appl Microbiol Biotechnol. https://doi.org/10.1007/s00253-015-7099-7

Chapter 10
Pesticides Bioremediation

P. Senthil Kumar, C. Femina Carolin and Sunita J. Varjani

Abstract In the developing countries, the usage of pesticides in the production of crops, fruits, and vegetables increases the economic status which establishes the major success in this field. Although the pesticide is an important aspect of the agricultural practices, the vast handling of harmful pesticides is an ultimate concern to the air, water, soil, and public health. Due to high impacts on human health, their application has been limited and different scenarios are developed to clean up the stubborn pesticides at different contaminated sites. Biological techniques like bioaugmentation, biostimulation, biosurfactants, bioremediation contaminated sites are available for degrading the pesticides, but the last one was found to be a most preferred method to mitigate the hazardous pesticides. Bioremediation method uses biological agent like microorganisms to degrade the contaminants in the existence of sufficient nutrients and environmental conditions. Properties of polluted sites, temperature, pH, nature of the pollutants are important factors which play a major role in the bioremediation process. The intent of this chapter is to bring out the bioremediation technologies accessible for clearing of pesticides at contaminated sites; additionally, their fundamentals, advantages, limitations and the pesticides treated are also summarized.

Keywords Pesticides · Bioremediation · Bioaugmentation · Biostimulation Microorganisms

P. Senthil Kumar (✉) · C. Femina Carolin
Department of Chemical Engineering, SSN College of Engineering,
Chennai 603110, India
e-mail: senthilchem8582@gmail.com

S. J. Varjani
Gujarat Pollution Control Board, Sector-10A, Gandhinagar
382010, Gujarat, India

S. J. Varjani et al. (eds.), *Bioremediation: Applications for Environmental Protection and Management*, Energy, Environment, and Sustainability,
https://doi.org/10.1007/978-981-10-7485-1_10

10.1 Introduction and General Overview

Because of certain anthropogenic activities, the pollution in the environment is increasing nowadays which is found to be an inseparable cause. In recent years, the growth of industrial and agricultural enterprises leads to high contamination in soil and water. Many new technologies were developed and elevated which resulted in the greater capacity to manufacture the new products in order to satisfy the human demands. The use of chemical products like pesticides, fertilizers, pharmaceuticals is rising to preserve certain aspects of life. Among these organic compounds, the pesticides are widely used to control or prevent the crop pests in the agricultural production and they are highly dispersed in the surroundings, particularly in the land, water, food products, aquatic system, etc. (Alvarez et al. 2017). During the last decades, the food demand is growing rapidly which greatly stimulates the use of pesticides in the modern agricultural systems at an ever-increasing rate (Morillo and Villaverde 2017). More use of pesticides was followed as a historical pattern in the agricultural system in India about 5–6 decades back.

In order to preserve the agricultural products, pesticides are utilized by the individuals to wipe out the pests. Depending upon their activity toward the pests, pesticides are classified into several types. They are insecticides, herbicides, fungicides, acaricides, nematicides, molluscicides, and rodenticides (Rani et al. 2017). A brief list of an above-mentioned chemical group of pesticides is explained in Table 10.1. There are some particular guides available because of the usage of agrochemicals in nature. Alarmingly, it is well known that exposure to pesticides, during manufacture as well as application, poses severe health threats to human. Stringent regulations to limit the use of agrochemicals have necessitated to manufacture eco-friendly products. Besides, numerous pesticides remain in the ecosystem for longer duration, and they were distinguished and recognized in the seaside environment (Arienzo et al. 2013), birds (Hong et al. 2014), human beings (Moon et al. 2012), etc. Moreover, they diffuse easily into the surroundings through air and water by means of overflow, permeation, sewage, and also spills during equipment washing. The declination of natural sources like soil and water of the

Table 10.1 General chemical group of pesticides and their function

Chemical group	Functions	Examples
Fungicides	It constrains the growth of fungus	Captafol, ziram, vinclozolin
Herbicides	Used to kill noxious weeds	hexachlorobenzene (HCB), picloram, atrazine
Insecticides	Kills the insects which are toxic to humans and environment	Aldicarb, carbofuran, DDT, dicofol
Rodenticides	Used to kill rats and mice	Diphacinone, chlorophacinone, pindone
Nematicides	Kills plant-parasitic nematodes or nematode worms	Aldicarb, dibromochloropropane

environment is mainly due to the above-mentioned basic points. Various studies showed that there is an existence of low concentrated pesticides around the world (Moschet et al. 2014). Furthermore, treatment might be recommended to secure the public health and environment even for the low-concentration pesticides because pesticides can exceed the standardized pesticide concentration levels.

Physical, chemical, and biological processes along with adsorption, oxidation, catalytic degradation are widely applied as treatment technology in the remediation of pesticides (Rani et al. 2017). The physical and chemical methods generally have specific problems like deficiency, high cost, and control issues. The selection of relevant technology is based on the specific considerations like the site at which the pesticides are present, their aspects, concentration, and forms. The propelled treatment process like activated carbon is not reasonable to operate at all necessities because it provides the high cost to operate. Thus, another scenario is needed to successfully evacuate the pesticides from the natural sources. Usually, biological process is most preferable because the physical and chemical processes are highly expensive. In order to decrease the pesticides streams over the water and other places, bioremediation technology is widely explored which includes the utilization of microbes to diminish the pesticides. It has been suggested as a different eco-friendly remediation method of pesticides polluted sites. Bioremediation is an overwhelming mechanism happens in non-sterile situations which utilize organic specialists like microorganisms, plants, catalysts for the decrease of lethal toxins. In the case of natural bioremediation, nothing is added to the surface of the soil and water, but the degradation of pollutants occurring by the microbial actions is observed regularly. While developing the bioremediation techniques for pesticides, a remarkable trouble needs to look at with a specific end goal to draw out the ecological change. This chapter talked about the aspects of pesticides in the natural environment. It also indicated the remediation strategies and significant efforts involved in the elimination of pesticides from contaminated sites.

10.2 Pesticides

Globally, 40% of the agriculture will be lost if the pesticides are not employed in the agricultural field. They not only wipe out the pests additionally, but they also increase the crop yield. The term pesticide is a form of an active ingredient which is applied for the application of killing the pests, and sometimes, it holds the behavior of pests or crops during crop production or storage. The use of pesticides in all over the world has become a common system for the mitigation of pests. It is limited to agricultural practices as well as incorporated into homes in the form of sprays, powders to kill the insects, mosquitoes, rats, etc. Chemical pesticides are used in developing countries to decrease the pest growth in order to increase the crop productivity. The first organic chemical used as a pesticide is 1,1,1-trichloro-2,2-bis [4-chlorophenyl] ethane (DDT). This pesticide is generated by Muller and co-workers in 1939 which has high insecticidal properties. In 1946, DDT is applied

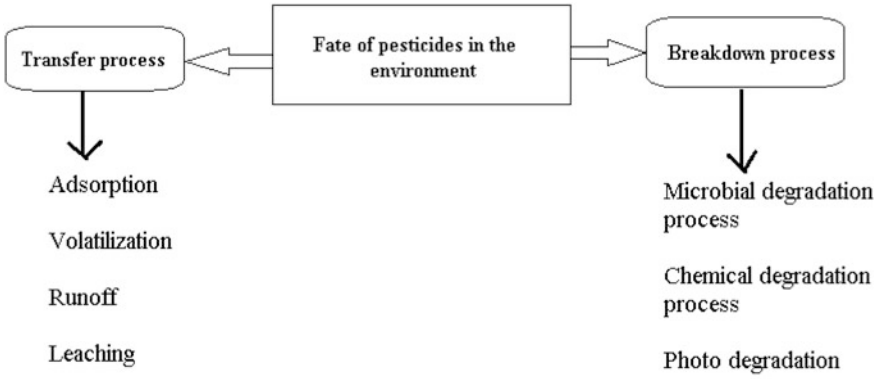


Fig. 10.1 Fate of pesticides into the environment

for the preservation of cotton, deciduous fruits, cereals, and potatoes. DDT completely abolished malaria in several parts of the world and provides the great success toward the crop protection and increased the use of pesticides among the world (Fig. 10.1).

10.3 Different Categories of Pesticides

Depending upon the structure, the pesticides are classified into two types. They are organic and inorganic pesticides. Organic pesticides consist of oxygen, phosphorus, sulfur, chloride, and fluorine heteroatoms with carbon, and these types of pesticides reside to modern pesticides. But in the case of inorganic pesticides, it consists of compounds like sulfur, copper, and other elements. Based on the functional groups, they are more classified into subcategories like organochlorine pesticides (OCPs), organophosphate pesticides (OPPs), and carbamates. Each pesticide has specific target site depending upon their mode of action which was shown in Table 10.2 (Fig. 10.2).

10.3.1 Organochlorine Pesticides

These pesticides are insecticides made up of carbon, hydrogen, and chlorine and represented by compounds like DDT analogs, benzene hexachloride (BHC), and cyclodiene. These are endocrine disruptor chemicals (EDCs) which result in poisoning of numbered about 1 million per year (Rani et al. 2017). Organophosphate pesticides are used not only in the agricultural field but also in medicine and some other industries. OPPs are highly toxic to vertebrates than the other families of

Table 10.2 Mode of action of different types of pesticides

Pesticides	Mode of action
Carbaryl, methyl parathion, carbofuran, tetrachlorvinphos, chlorpyrifos, propoxur, carbosulfan,	Inhibits acetylcholine esterase
chlordane, endosulfan, fipronil	Inhibits ligand-gated chloride channels
DDT, methoxychlor, cycloprothrin,	Modifies sodium channels
Acetochlor, metolachlor	Suppress the cell division

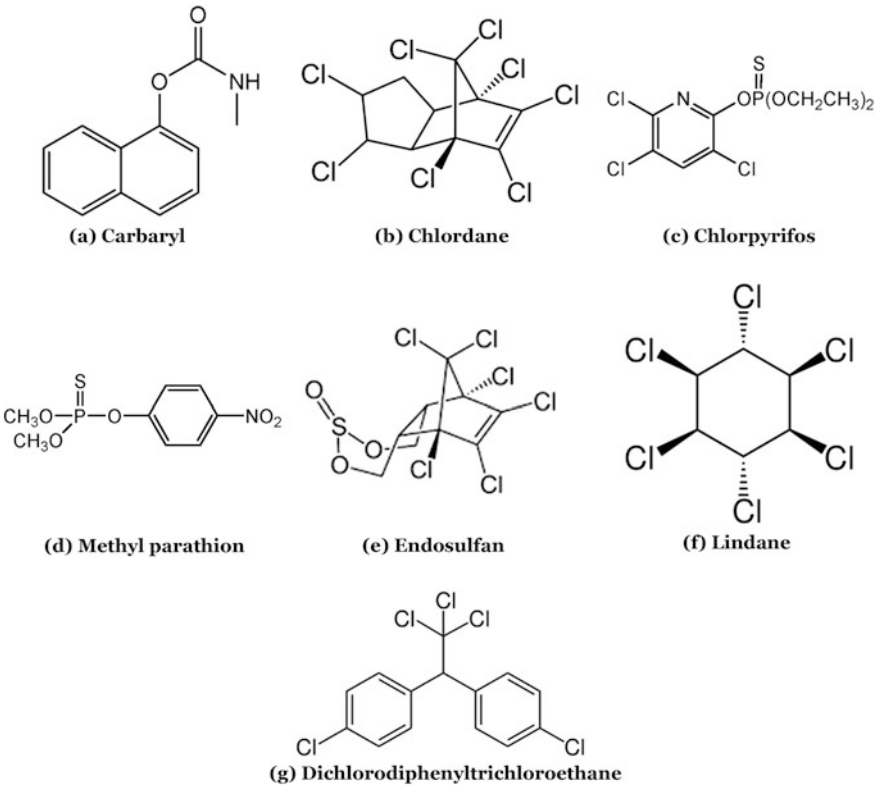


Fig. 10.2 Structure of various pesticides involved in agricultural practices

insecticides. It comes under the category of persistent organic pollutants (POPs). OCPs are high chemical stability and undergo abiotic and biotic transformations. In 1940, they have extensive application especially insecticides in several fields, but their usage is limited in developed countries from 1970 because of their harmful effects and persistence. Th sodium ion channel present in the nerve cell of insects is affected which leads to fire instantly. The half-life period of OCP is about 4–35 years but still rich in several developing countries of soil. They are

semi-volatile compounds and have the tendency to aggregate in the environmental circumstances and food crops. Mostly, OCPs are white crystalline organic compounds which are highly soluble in organic solvents and fats. Because of its solubility and chemical resistance, they get aggregated in adipose tissue biomagnification taking place consequently. Additionally, they also found in water, human breast milk, and foodstuffs. OCPs cause illness to the reproductive and immune system of the fetus. These organic compounds deposited in the phospholipid membrane and fatty tissues of living organisms. Though their extensive applications have been banned and restricted in several countries during the 1970s and 1980s, the organochlorine pesticides are still being used for agricultural purposes due to its low cost". The remediation technology available for the degradation of OCPs is bioremediation, photochemical, and adsorption.

10.3.1.1 DDT

DDT (dichlorodiphenyltrichloroethane) is an organic compound made up of aliphatic and aromatic structures. It is the first insecticide used all over the world. It acts against mosquito-borne malaria and being employed in several countries (Purnumo et al. 2011). It is low soluble and highly chemically stable. It rises hazard to humans in the form of cancer and endocrine disruption. It has been blocked in countries because of its long-term existence, water insolubility, and ecological effects. The metabolites like DDD (1,1-dichloro-2,2-bis(4-chlorophenyl)ethane) and TDE (1,1-dichloro-2,2-bis(4-chlorophenyl)ethylene) are widely distributed in various regions of world. Because of its high solubility in nonpolar solvents, it has the tendency to accumulate in food and adipose tissues. Henceforth, it is necessary to evacuate DDT from the environment. DDT has been applied as an antifouling paint in fishing ships. In the case of fungi remediation, DDT acts as a substrate to the organisms like *Phlebia lindtneri* and *Phlebia brevispora*. The remediation DDT is highly existing in many of the countries and research studies.

10.3.1.2 Lindane

Previously, the most widely used organochlorine pesticides are lindane (γ-hexachlorocyclohexane). Globally, in the case of a vector-borne disease like malaria and crop protection, the chlorinated insecticide like lindane was used extensively. From 1989, the lindane production in India reaches about 43%. It constitutes isomers such as α, β, γ, and δ that are found to be deeply persistent with high toxicity. The habitual of lindane are solubility, resistance, volatility, absorption which describe the transportation of lindane into the environment. The half-life period f lindane is more than 10 years because it is highly resistant to degradation. These lipophilic compounds can be easily transported and aggravated to tropical levels because of its high stability and persistence. An experiment was carried out to restrict the specific effects of lindane to human health and ecosystem through identifying the lipid

disruption, neurotoxic effects, the compounds reasoning for carcinogens and teratogens. It provokes negative effects to both human health and environment. Generally, the route of lindane pesticides are oral, dermal, intraperitoneal, or intra-muscular routes or inhalation through which it causes severe effects on the nervous system. The microorganisms like bacterial strains exist at the contaminated sites, also helpful in degrading lindane pesticide. The minute concentration of lindane remediation needs autotrophic microbes with the low requirement of nutrients. So, the most appropriate organism for this desire is *Anabaena/Nostoc*. Because of its wide range of applications, agricultural system is greatly affected and passed into the water bodies through surface runoff.

10.3.1.3 Chlordane

Chlordane (1,2,4,5,6,7,8,8-octachlor-2,3,3a,4,7,7a-hexahydro-4,7-methanol indane) is a toxic gaseous pesticide generally found in soil, water, and air. It has been widely used for both agricultural and other residential applications. The metabolite of chlordane compounds is found to be more carcinogenic and toxic and may contain estrogenic activities. The half-life period of chlordane is found to be 5 and 15 years. It has negative effects on humans and environment. According to UNER Stockholm Convention on POPs signed in 2001, chlordane pesticides are considered to be one of the 12 persistent organic pollutants (POPs) due to its long-time occurrence in the environment (Xiao et al. 2011a). Hundred and forty seven definite compounds are present in the technical chlordane with trans-chlordane (13.2%) and cis-chlordane (11.3%). Technical chlordane consists of compounds like cis-chlordane, trans-chlordane, trans-nonachlor, and heptachlor.

10.3.1.4 Endosulfan

Generally used chlorinated cyclodiene insecticide in agricultural field is endosulfan (6,7,8,9,10,10-Hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepine-3-oxide) which has been restrained in many countries. It is tested in crops like fruits, cotton, vegetables, tobacco, sugarcane, and tea. This pesticide cannot be degraded naturally and remains in the soil for several years. It constitutes α and β isomers and is widely used for the killing of insects and acarine mite pests on cotton, fruits, and vegetable crops (Kong et al. 2013). Endosulfan is usually employed in the control of tsetse flies, mites, home garden pests, and cabbage worms. In water, they get changed into diol, and in soil and sediment, they get changed into sulfate. Based on the persistence of endosulfan in the environment, the half-life period was determined and found to be 3–6 months or longer. It is highly harmful to microbial enzymes and also affects enzyme activities. α-endosulfan and β-endosulfan are two types of diastereoisomers present in technical-grade endosulfan of about 95%. But these isomers get converted into endosulfan diol, lactone, and hydroxy ether metabolites. The degradation substance obtained after the biodegradation process is

endosulfan sulfate. Agency for toxic substances and disease registry suggested that endosulfan is a toxic organic compound in 2001, and additionally, Stockholm Convention prescribed the same in 2011 (Mitton et al. 2016). Thus, the strategies are developed to remediate the endosulfan from the environment.

10.3.2 Organophosphate Pesticides

It is a common name for the esters of phosphoric acid. Because of its high solubility, within a week they get depleted from the soil after their usage in the agricultural field. Its effectiveness increases their application toward worldwide. About 36% of world population is using different classes of organophosphate pesticides. Organophosphate pesticides improve the quality and quantity of crops in agricultural practices, and their distribution leads to the contamination of environmental waters. It blocks the enzyme cholinesterase (ChE) which halts the signals coming from the nervous system. Though it has high degradation rates than OCPs, they show some acute toxicity to humans who exposed to larger amounts. Parathion, malathion, chlorpyrifos, diazinon, dichlorvos, phosmet, and azinphos-methyl are the types of OPPs widely used. These types of pesticides belong to phosphorothioate esters which were proposed to control the pests and have lesser usage in indoor use. In 1973, it has been proved that *Flavobacterium* sp. is able to grow in the presence of OP diazinon. The hydrolysis of OPs to less toxic compounds is an important consideration for environmental decontamination. Incineration and chemical hydrolysis/oxidation are the techniques used for the treatment of organochlorine pesticides, but these methods are not adequate in case of large contaminated water bodies (Gao et al. 2014). OPPs divide the chemical structure, and henceforth, the mechanism of toxicity is same. However, their usage is rising in all over the world.

10.3.2.1 Chlorpyrifos

Chlorpyrifos (0,0-diethyl 0-(3,5,6-trichloro-2-pyridyl)phosphorothioate) acts as an insecticide for cereal, cotton, fruit, nut, and vegetable crops. In many countries, it plays a major role in the control of pests over 40 years. Its application has been extended to fruit farming industry, particularly in blueberry crops. The soil contamination by chlorpyrifos is due to pesticide management in the farmyard and cleaning of containers. The usage of chlorpyrifos is proportional to the risk involved in environment and human health. The half-life of this pesticide is based on the soil type, moisture, pH, and initial concentrations. It is slightly tenacious in soil with time period of about less than 1 day to 240 days. The debris of this pesticide reduces the hormone thyroxine and rises estradiol levels in sheep. Bioremediation is an appropriate method to detoxify the chlorpyrifos. The other technologies to eradicate the pesticides are photolysis, chemical hydrolysis, and microbial degradation. Its degradation rate is based on initial concentration and soil

characteristics such as pH, temperature, moisture, and organic carbon content. The metabolic products obtained after degradation process is diethylthiophosphoric acid and 3,5,6-trichloro-2-pyridinol (TCP). TCP has an antimicrobial activity which prevents generation of chlorpyrifos-degrading microbes. Activities of microbes and carbon mineralization get reduced badly in the soil due to the chlorpyrifos. TCP has high mobility than chlorpyrifos because of its solubility and is distributed in polluted soil and aquatic system. The half-life period of TCP is 65–360 days. This pesticide is distinguished in lakes, rivers, rain, groundwater fog, sloughs, marine sediments. Methods available for the eradication of chlorpyrifos include chemical treatment, photodecomposition, volatilization, and incineration. But these methods are not adequate to treat low concentration of chlorpyrifos. Henceforth, biodegradation process was found to be most potential treatment of chlorpyrifos.

10.3.2.2 Methyl Parathion

Methyl parathion (O,O-Dimethyl-O-(4-nitrophenyl)phosphorothioate) is used as an insecticide for the control of aphids, mealybugs, and mites on the different types of crops like cereals, fruits, vegetables, ornamentals, and cotton. The activity of acetylcholinesterase is decreased by methyl parathion which leads to irreversible phosphorylation of esterase in insects and mammal's nervous system. It penetrates into the body through ingestion, inhalation, and dermal absorption. The widespread of methyl parathion in the environment is due to rain, fog, and the wind. Methyl parathion is categorized under 'Toxicity Category I' by the US Environmental Protection Agency (USEPA) and World Health Organization (WHO) under 'Category Ia' (Mishra et al. 2017). Its usage in many developing countries is increasing though it has been banned in several countries. The availability of this pesticide is found in soil, water bodies, and some other food materials. It is exceptionally dissolvable in natural solvents and insoluble in water. Through leaching and interaction between surface water and groundwater, methyl parathion gets transmitted easily from one to another. The half-life of methyl parathion is more than a month. Hence, bioaccumulation of this pesticide takes place in the environment and food chain (Liao et al. 2017). Therefore, it is necessary to evacuate this type of pesticides from the environment using the environmentally feasible method. The conventional analytical methods for the methyl parathion are high-performance liquid chromatography (HPLC), gas chromatography (GC), liquid chromatography (LC).

10.3.3 Carbamates

Carbamates were obtained from carbamic acid (NH_2COOH) and advanced during the nineteenth century. It is a preemergent herbicide applied to the soil which regulates grasses and leaves in the horticulture crops. In this class, pesticides such

as carbofuran, aldicarb, oxamyl, and methomyl are included. They are usually utilized in the form of sprays or baits to mitigate the pests. It was discovered in 1956 as an insecticide, and two different types were identified. In the last 50 years, carbamates were used an insecticide, fungicides, herbicides, or nematicides. Carbaryl is a first insecticide used which has low oral and dermal toxicity and ability to control a wide range of insects in the environment. The mode of action is similar like organophosphate pesticides. It abolishes neurotransmitter acetylcholine so that the transmission of signals from one cell to another cell gets affected and generally toxic to human beings (Schenk et al. 2016). The decrease in choline esterase activity can be measured in blood samples, and this reduction is present only about a short period of up to 48 h. The presence of carbamates in the urine gets metabolized and excreted; hence, it is easy to measure levels of carbamates in urine samples. Carbamates have a characteristic like water solubility which makes them absorb the pesticides by roots and leaves of plants. The diagnostic methods of carbamates are such as gas chromatography (GC), enzyme-linked immunosorbent assays (ELISAs), micellar electro-kinetic chromatography (MEKC), biosensor and high-performance liquid chromatography (HPLC). GC application is limited in carbamates because of its high thermal stability. The most commonly used method for the degradation of carbamates in soil is microbial degradation.

10.3.3.1 Carbaryl

In the 1950s, carbaryl (1-naphthyl *N*-methyl carbamate) was used as an insecticide. It is as yet being utilized as a part of the agricultural field in view of its low collection activity, high organic movement, and low poisonous quality (Bazrafshan et al. 2017). It plays a major role in the killing of insects and found to be carcinogenic to human beings. Aggregation of acetylcholine takes place in the nervous system due to inhibition of cholinesterase by carbaryl compound. Henceforth, their debris provides high risk to human health and their detection is needed. Carbamate pesticides get accumulated in tissues of animals and fish and in foods like nuts, vegetables. The diagnostic methods for the carbaryl detection are gas chromatography or high-performance liquid chromatography but highly expensive and time-consuming.

10.4 Risk Correlated with Pesticides

To raise the food production in the world, the pesticide usage was expanded recently in the agricultural circumstances which lead to human health and environmental problems. Moreover, pesticides are favored for the regulation of insects, weeds, and fungi in agricultural circumstances and implementing enormous benefits to the world. The main pathway for the widespread of pesticides to the environment and the human is the hydrological system. Water plays a major role in the transfer

of pesticides from one area to another. Owing to high persistence, long life span, and slower putrefaction rates, the pesticides are found to be harmful. The unlimited application of pesticides leads to depletion of terrestrial plants and aquatic organisms. Furthermore, the toxicity of pesticides also reaches the air, water, soil bodies. Pesticides and insecticides are recommended as highly toxic than the fungicides and herbicides. The most ambiguous pesticides found to be in the environment is organochlorine pesticides such as atrazine, dichlorodiphenyltrichloroethane (DDT), benzene hexachloride (BHC), lindane, and endosulfan. The most toxic pesticides found in the agricultural practices are organophosphorus pesticides (OPPs), carbamate (CB), and organochlorine pesticides (OCPs) (Alvarez et al. 2017). Based on the lethal concentration of pesticides, their toxicity is classified such as highly toxic, moderately toxic, and slightly relatively non-toxic. Though the pesticides are divided into moderately and slightly non-toxic, they can be precarious to the human health and environment.

10.4.1 Threats to Human Health

Though the human standard is increased in the India through the use of pesticides, the health effects are growing rapidly because of its persistent and extensive use resulted in the serious hazard. In particular, children and infants are highly accessible to the effects of the pesticide. Most of the health problems occurring in India are due to the pesticides. Mild level of organochlorine pesticides leads to anxiety, headaches, gastrointestinal problems, insensibility, and high irritation to individuals. Pesticides invade into the human body through dermal exposure, inhalation exposure, and oral exposure. Many chances are there to occur due to dermal exposure because liquid pesticides easily get absorbed into the body through the skin. Organochlorine pesticides like chlordane are familiar to acquire in the human adipose tissue through the dermal absorption. Inhalation exposure takes place due to the respiring of pesticide vapors or fumes during implementation and dust particles. Specifically, the breathing of fumigant pesticides is highly toxic to human beings than other pesticides, and inhaling the tobacco products is incorporated with the pesticide residues. Oral exposure results from eating pesticide-sprayed fruits and eating without washing the hands after the usage of pesticides. The toxicity depends on the types of pesticides, and their quantity and the toxicity are classified into acute and chronic toxicity. Long-time low-dose hazard causes the destruction of hormones, cancer, a decline of intelligence, etc. Even if the human body has the mechanisms of rejection of toxic substances, in some circumstances it gets to hold on the circulatory system because of its absorption process. Pesticide effects to children are high because of their immature metabolic process. Additionally, when the pesticides get exposed to pregnancy women, it affects the growth of the fetus and neural development. For some endorsed pesticides, studies were carried out on the development of neurotoxicity. It is noticed that there is a link between the pesticides and Parkinson's disease and Alzheimer's disease (Casida and Durkin

2013). Organochlorine pesticides (OCPs) are the chemicals come under the classification of hydrocarbon compounds. Human exposure to these types of chemicals is occurring mainly due to the fish meat, dairy products, and diet (Muller et al. 2017). Organophosphate pesticides (OPPs) are more toxic to human health, and the risk involved due to the exposure of OPPs is irregular sperms, the death of the fetus, variations in hormones, and DNA impairment. Also, when humans exposed to pesticides, they suffer from leukemia and brain cancer.

10.4.2 Threats to Plants

By drifting and volatilization process of pesticides such as phenoxy herbicides, the adjacent trees and shrubs are affected. The characteristics of seeds are afflicted through the herbicide glyphosate and raise the plant's susceptibility to diseases. Trace concentrations of herbicides, sulfonylureas, sulfonamides, and imidazolinones have an overwhelming effect on undesired crops, natural plant goods, and wildlife. The distributed forms of glyphosate-based herbicides scare the endangered species like *Dimorphandra wilsonii*. This herbicide prohibits the 5-enolpyruvylshikimate-3-phosphate synthase which acts as an enzyme in the shikimate pathway of aromatic amino acids and additionally destructs the secondary compounds of plants (Gomes et al. 2017). Photosynthesis process of plants is also affected by the herbicide glyphosate. Based on the type of herbicide, the phytotoxic effects are generated in the physiology and morphology of plants and the effects like leaves drying, chloroses, deformity, stunting, and delay in flowing and maturity.

10.4.3 Threats to Aquatic System

The extensive use of pesticides in worldwide causes high burden and high risk to the wildlife. The penetration of pesticides into the environment is mainly due to the surface runoff after the rainfall or by leaching through the soil or spray drift. Due to the drifting process, aquatic systems are facing the considerable damage. A small portion of pesticide existence can affect the aquatic. Almost, they are appearing in the form of separate chemicals or blended forms of diverse chemicals at low concentrations, but the effects due to them vary to the aquatic system (Schreiner et al. 2016). Depending upon the dissolved oxygen compounds and other physiological and behavioral factors associated with the aquatic habitat and the environment, the fish can withstand in that specific circumstances or increase its populations. Several attempts were focusing on the indirect and indirect pathways of excessive pesticides into the aquatic system. The pesticides like acetochlor, atrazine, carbaryl, chlorpyrifos, diazinon, malathion, and metolachlor develop as complicated blended forms. Other than fish, the immune system of amphibians also indirectly gets affected by the usage of pesticides like atrazine. The minute

concentration of carbaryl and herbicide glyphosate is toxic to tadpoles and juvenile which causes high mortality. Malathion brings out the modifications in plenty of phytoplankton and therefore affects the tadpole and frogs.

10.4.4 Threats to Soil

The organisms and earthworms present in the soil make the soil to function based on the physical and chemical properties and are considered to be most significant creatures (Ye et al. 2016). The distribution of pesticides in the soil due to the agricultural practices raises the burden over the soil and their organisms. Pollutants exist in the soil present in the form of single or group of mixtures. The interaction between these multiple pollutants leads to transformation of toxic substances in the soil and causes adverse effects to the earthworms present in the soil (Yang et al. 2017). Fenobucarb (FEN), chlorpyrifos (CPF), clothianidin (CLO), acetochlor (ACE) are the pesticides widely used in the agricultural crops which act as an inhibitor of the photosynthetic electron transport. The enterprises of soil enzymes are deactivated by the pesticides which are the key factors for stimulating many biochemical transformations in the soil. The availability of pesticides causes the reduction of soil respiration that follows the decrease in the microbial biomass. Repeated application of pesticides in the soil leads to the elimination of useful organisms. Several microbes present in the soil involved in the nitrogen cycle in the atmosphere which converts the nitrates into nitrogen. The organisms constituted with nitrogen cycle get disturbed due to the existence of chlorothalonil and dinitrophenyl fungicides in the soil because nitrification and denitrification bacteria play a major role in this nitrogen. The earthworms act as a bioindicator in the soil and are used for soil toxicity measurement. The destroying effects like DNA damage take place at the earthworms by glyphosate and chlorpyrifos. *Eisenia foetida* species is a common name of earthworm which gets assassinated through neonicotinoids that were found to be harmful to both animals and the environment (Goulson 2013). Chlororganic compounds, aldrin, heptachlor, endosulfan, and DDT, cause adverse effects on soil flora and fauna.

10.5 Bioremediation History

In many of the events, the scientific world was behind in the past humanity. In order to enhance the quality of human life, the biological process used to produce a good quality of products is called biotechnology. Previously, this technique was applied only in the food, but recently, the usage of biotechnology has been increased in several fields (Alvarez et al. 2017). An environmental biotechnological tool called bioremediation utilizes living organisms in air, soil, and water for the removal of pollutants. Since 1972, Romans were the first people who used bioremediation

method for cleaning up contaminants. For the treatment of wastewater, natural remediation has been utilized by civilizations to reduce the hazardous substances from the water (Uqab et al. 2016). In 1960, George Robinson employed microbes into the coast of Santa Barbara, California, to degrade the oil spills (Uqab et al. 2016).

10.6 Classes of Bioremediation

Two main classes of bioremediation process are in situ and ex situ processes.

10.6.1 In Situ Process

In situ bioremediation is a process of adding nutrients to the soil surface, water reservoirs, and other water bodies for the organisms to degrade the pollutants in it. Initially, in situ bioremediation was generated as a less expensive method for the declination of organic pollutants, inorganics, toxic metals, etc., at different contaminated sites. It brings out the favorable circumstances like low working expenses, a low hazard to working laborers at contaminated sites, and total destruction of pollutants. It is a preferable technique because less distribution of contaminants avoids the transportation of pollutants from one area to another. This process provides the ambiguous relationships between biomass and pollutants and gives the relevant actions between them. Additionally, it also strengthens the microbial degradation of organic components at the contaminated zone. The capability of in situ bioremediation is based on various factors like biogeochemical and hydrogeological conditions which regulate the biodegradation process (Verardo et al. 2017). The major advantage of the in situ process is that there is no need to remove or transfer the polluted site. But the efficiency is lower than the ex situ remediation process and depth of the soil cannot be treated properly by an in situ method.

10.6.2 Ex Situ Process

Ex situ bio remediation is a natural process which transfers the media from one site to another and handles the pollutants at the particular location. Because of its probable utilization in the environment, easiness, and consent with the governmental regulations, this strategy is used for the soil remediation. The defects like poor regulation of performing conditions and lengthy remediation time can be conquered by this methodology (Tomei et al. 2013). The downside of the ex situ process is the excavation of contaminated sites that are required for the treatment

process which leads to high cost and health risks to individuals. This technique can be observed very easily, and mostly time requirement is low in this process for cleaning the contaminants.

10.7 Bioremediation of Pesticides

The presence of pesticides causes several impacts to human and environment, and hence, remediation technologies are needed. The bioremediation is one of the biotechnological tools to decrease the toxicity and concentration of pesticides. It is suggested as less expensive and environmentally safe technique to remediate the pesticide-contaminated environmental sites, the most favored method over the other physical and chemical methods for the remediation of pesticides. Bioremediation techniques use microorganisms and plants for the elimination or conversion of pesticides into less toxic substances by the mechanisms like degradation, biotransformation, and confinement from the surroundings. The pesticide remediation rate relies upon pesticide accessibility, the limit of microorganisms to take up the pesticides, rate at which the enzyme degrades the pesticides and rate at which the organisms grow by utilizing pesticides as an energy. The efficiency of bioremediation is calculated from the outcomes of sorption, degradation, and volatilization. The satisfactory rate of bioremediation process can be obtained when the pesticide and its metabolite remediation reach the acceptable level in a limited time. The metabolic pathway involved in the degradation of pesticides is based on oxidative transformation, hydrolytic transformation, reductive transformation, conjugation reaction, and reductive dehalogenation (Odukkathil and Vasudevan 2013). Some contaminants exist in the polluted site are resistant to microbial attack; instead, they get degraded slowly. Finally, the substances resist the microbial process, and the bioremediation process is not efficient method to treat such type of compounds. A various number of researches have been carried out to degrade a number of contaminants by the bioremediation process. The organic pollutants generated from the chemical process need to be treated successfully, and the biological methods to meet the standards are described by the Environmental Protection Act, 1986 (Rani and Dhania 2014).

10.8 Upside of Bioremediation

It is considered as an adequate treatment for the entire eradication of pesticides. Usually, bioremediation process carried out in on-site which reduces the transportation of waste from one place to another, for example, from land to water or air. The legally restricted toxic compounds can be converted into less harmful compounds. It utilizes the naturally available compounds exist at the site for the degradation of pollutants. The debris from the bioremediation process is harmless

which includes carbon dioxide, cell mass, and water. The major benefits of bioremediation process are less expensive and less interruption than the other treatment technologies. Although bioremediation has many advantages, it is still in the developmental phase.

10.9 Downside of Bioremediation

There are chances to generate less toxic and volatile compounds due to the partial degradation of pesticides. Remediation process time is high when compared with the thermal treatment and takes the time to reach an acceptable level of pollutants. It is very tough to hypothesize from laboratory scale to field study. Field monitoring is required to record the degradation process of pesticides. In the case of in situ and ex situ processes, there are chances to form additional pollutants. The efficiency of bioremediation process depends upon some site conditions like microbial populations, appropriate environmental conditions, and concentration of nutrients and contaminants. In some cases, the products obtained after the degradation process are more toxic than the initial concentration of pollutants. Certain limited conditions are required: Some of them are bacterial and nutrient concentration, non-toxic conditions, and limited carbon source. It is also problematic in hypothesizing the results from pilot-scale to full-scale operations. The growth of microorganisms is limited during the transportation of contaminated sites.

10.10 Strategies of Pesticides Bioremediation

10.10.1 Involvement of Microbes in Bioremediation of Pesticides

Microbial remediation happens when pesticides are utilized as a nourishment sources by the microorganisms like fungi and bacteria. Under suitable circumstances, the microbial remediation can be fast and complete degradation occurs in the environment. Not only the presence of innate microbes in the contaminated site is required to manage the pesticides, but also the external inclusion of other microbes is suggested to degrade the pesticides (Uqab et al. 2016). The particular type of different microorganisms can be used for the detoxification of pesticides which raises the advancement of techniques in the bioremediation. Depending upon the bioavailability of pesticides and its actions, the microbial bioremediation of pesticides gets controlled (Mandal et al. 2014). In addition, repeated usage of pesticides in the agricultural field increased the degradation process. Many studies related to the evacuation of persistent organic pollutants from the environment were carried out using microbial degradation which is noticed as an important process.

Table 10.3 Studies on pesticides detoxifying microbes

Pesticides	Microorganisms	References
Organochlorine pesticides		
Aldrin	*Phlebia*	Xiao et al. (2011a, b)
Lindane	*Streptomyces consortium*	Saez et al. (2014)
DDT	*Ochrobactrum* sp.	Pan et al. (2017)
Endosulfan	*Alcaligenes faecalis*	Kong et al. (2013)
Organophosphate pesticides		
Diazinon	*Chlorella vulgaris*	Kurade et al. (2016)
Chlorpyrifos	*Pseudomonas aeruginosa*	Kharabsheh et al. (2017)

Microbial degradation of pesticides from the environmental streams includes some advantages like easy to culture, high microbial population, and rapid mutation. The main way suggested to degrade the organophosphate pesticides (OPPs) is the utilization of microorganisms in the bioremediation process. Based on the limits recommended by the regulatory authorities, the toxic compounds get converted into low-level toxic compounds by the microbial degradation process and a large consideration of microbial remediation was increased as an effective tool to clean up the pesticide-polluted sites. The most commonly prescribed microbes for the bioremediation of pesticides are *Pseudomonas* sp., *Bacillus* sp., *Klebsiella* sp., *Pandoraea* sp., *Phanerochaete Chrysosporium, Mycobacterium* sp. (Rani and Dhania 2014). Good pH, warm temperature, air circulation, and suitable humidity are the circumstances influencing the remediation process. Various research works have been carried in the remediation of pesticides using microorganisms which were explained in Table 10.3.

10.10.1.1 Bacterial Bioremediation

In the case of bioremediation process, the microorganisms like bacteria have been applied broadly for the remediation of pollutants. Several reviews are concentrated on the bacteria for the look of biotransformation enzymes. Bacterial remediators are a most relevant method because of its less expensive, rapid growth, and easy manipulation. At aerobic and anaerobic states, the bacterial bioremediation occurs. Diuron is an herbicide or algicide which is an active pollutant present in the water, soil, and other sediments. This herbicide gets mineralized by the three-member bacterial consortium like *Anthrobacter sulfonivorans, Vario-vorax soli,* and *Advenella* sp. and achieved the mineralization from 22.9 to 69.0% (Villaverde et al. 2017). *Bacillus firmus* demonstrated that it can able to bioremediate the insecticide fipronil which is causing a serious hazard to the environment. It is powerful across an extensive variety of insects (Mandal et al. 2014). Nowadays, a new biotechnological tool like mixed microbial cultures is perceived that can effectively remove the pesticides, avoids their aggregation in the surroundings, and promotes the

Table 10.4 Various types of phytoremediation mechanisms and their purposes (Rani and Dhania 2014; Archaya et al. 2014)

Mechanisms	Name of the plant	Purposes
Phytoextraction/ phytoaccumulation	*Sedum alfredii, Rumex crispus*	Through roots, the contaminants raised from soil to the above segment of plants
Rhizofiltration	*Brassica juncea*	Precipitate the contaminants present at the root region
Phytodegradation/ phytotransformation	*Cannas*	By the activity of plant enzymes, the pollutants are transferred into plant tissues
Phytostabilization/ immobilization	*Anthyllis vulneraria, Festuca arvernensis*	Stability of pollutants gets reduced by plants
Phytovolatilization	*Arabidopsis thaliana*	It takes the pollutants from the soil and volatilizes into the atmosphere

degradation of blended forms of pesticides. The upside of the mixed consortia is that the competition and the contact between the microbial population and target compound will be high. Mostly, at the highly polluted site, the microbial consortia were chosen for the degradation process because microbes exist at that site can tolerate the high concentrated organic compounds and the microbial population will be high. A study was carried out on the removal of diazinon which is the organophosphate pesticide by using the single and mixed culture of *Streptomyces* sp. and reported that mixed culture showed the high removal of diazinon than the single culture (Briceno et al. 2016). The mixed cultures of *Streptomyces* sp. has the ability to evacuate diazinon as well as other organophosphates from fluids or from different frameworks in nature.

Additionally, in order to degrade the blended forms of pesticides, the pure culture is not adequate, and by employing mixed consortia of bacterial strains, the efficient evacuation of mixtures of pesticides can be obtained due to the interaction among the mixed culture (Geed et al. 2017). A low expensive technique against the chemical and physical process for the bioremediation of organophosphate pesticides is the utilization of bacteria in the bioremediation process. Among bacterial organisms, *Streptomyces* sp. plays a major role in the degradation of OPPs because of its mycelial development and fast growth rates (Fuentes et al. 2017). Therefore, bacterial remediation is an assuring technique for the bioremediation of various frameworks polluted with contaminants. When compared with the chemical process, the bacterial bioremediation is low cost-effective and can be carried out in on-site. Additionally, the specific bacteria are utilized in bioremediation process; henceforth, the formation of unsafe by-products is low. In a few cases, there are fewer chances to form lethal by-products. Due to the utilization of recombinant strains, the cloned genes lose its stability in the polluted environment. This issue is held on not only with the marine organisms but also with the bacterial strains. Techniques were created for the bioremediation of the highly persistent toxic

pesticide lindane from the environment using recombinant live/dead *E. coli* (Chaurasia et al. 2013).

10.10.1.2 Mycoremediation

Although the several studies were carried out using bacteria, the numerous genera associated with the fungal strains like *Aspergillus* (Mohamed et al. 2011) *Penicillium* (Peng et al. 2012), *Phanerochaete* (Chrinside et al.2011) get involved in the degradation of pesticides (Maqbool et al. 2016). Significance has been provoked in the fungal bioremediation because it has the capacity to transmit the different types of hazardous chemicals. Since the 1980s, the use of fungal technology has shown a pledge toward the removal of contaminants. Lengthy mycelial networks and the ability to use organic compounds as growth source make them a best appropriate method for the remediation of compounds like pesticides (Maqbool et al. 2016). Besides, enzymes secretion, an enormous amount of fungi and the durability of fungal spores in the natural surroundings are the benefits influencing the use of fungi in the elimination of xenobiotic compounds like pesticides. Chlorpyrifos is an organophosphate insecticide, and its metabolite 3,5,6-trichloro-2-pyridinol (TCP) was degraded by using the fungal strain *Ganoderma* separated from the agricultural soil which was found to be highly effective (Silambarasan and Abraham 2014). Nowadays, bioremediation process was carried out using microbial consortia like fungal–bacterial consortia. These organisms contribute to the microbial environment with each other for their development. Several studies were investigated to mineralize the pesticides like phenyl urea herbicide diuron using fungal–bacterial consortium (Ellegaard-Jensen et al. 2014). An important aspect needs to be considered in the biotransformation of pesticides, i.e., metabolic pathways of the pollutants. Recently, various studies were carried out to determine the intermediate metabolites generating during the fungal remediation (Leon-Santiesteban et al. 2016). Methylation and dichlorination were recognized in the fungal degradation of pesticide pentachlorophenol. By the production of intermediate metabolites, the metabolic pathways can be determined easily.

Fungal biotransformation of pesticides occurs by acquainting a small basic change to the pesticides (Rani and Dhania 2014). It is a kind of exclusive microorganisms which discharge an assortment of extracellular enzymes such as laccases, polyphenol oxidases, lignin peroxidases and play a major role in fungal bioremediation process. Also, intracellular enzymes, namely reductases, methyltransferases, and cytochrome oxygenase, get involved in the degradation of organic pollutants. The neonicotinoid pesticides like clothianidin (CLO) get degraded by the white rot fungus-like *Phanerochaete sordida* and converted CLO into non-toxic metabolite *N*-(2-chlorothiazol-5-methyl)-*N′*-methyl urea (TZMU) (Mori et al. 2017). This fungus excretes manganese peroxidase, a major ligninolytic enzyme, but the degradation is independent on this enzyme. Cytochrome P450 enzymes (CYPs) that act as a major factor get involved in the degradation of CLO by *P. sordida* (Mori et al. 2017). A study was carried out to diminish DDT

(1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane) from contaminated sterilized and unsterilized soil using brown rot fungi like *Gloeophyllum trabeum, Fomitopsis pinicola,* and *Daedalea dickinsii. G. trabeum* showed greater capacity in the bioremediation of DDT (Purnomo et al. 2011). Than the bacteria, the white rot fungi can regulate the high concentrated organic compounds, and hence, it is suggested as a biotechnological tool in microbial remediation. Due to the presence of extracellular enzymes such as lignin peroxidase, manganese peroxidase, and laccase in the white rot fungus, it is recommended as a good degrader of pesticides. Under aerobic conditions, the white rot fungus belonging to the genus *Phlebia* is capable of degrading the organochlorine pesticides (OCPs) like dieldrin and aldrin into several hydrophilic products, including hydroxylated and carboxylated products (Xiao et al. 2011a). Using the above-mentioned species, a study was evaluated to detoxify organochlorine pesticide trans-chlordane and its metabolite oxychlordane, and this is the first report to explain the biotransformation of oxychlordane using microorganisms (Xiao et al. 2011b). The ability of fungal strain, *Fusarium oxysporum,* was investigated to detoxify malathion in soil with the inclusion of carbon, nitrogen, and phosphate nutrients, and the results implied that this strain is a good biotechnological tool for the remediation of soil-contaminated pesticides (Peter et al. 2015). Though fungal remediation has some benefits, it has certain drawbacks like slow biodegradation process in soil, incomplete elimination of pollutants, and inflexible with the given environment. The slower process is due to the differences in the climatic and physical conditions of the soil. Another downside in fungal remediation is that partial degradation of pollutants can occur in the case of the high fungal population which causes the aggregation of secondary metabolites (Maqbool et al. 2016).

10.10.1.3 Phycoremediation

Organic compounds generated from the anthropogenic activities can be evacuated by the utilization of microalgae or macroalgae called phycoremediation. Algae are a photosynthetic microorganism that converts organic compounds into a new molecule with high economic value. Most of the phytoplankton is blue-green algae that have the ability to adapt to various forms of pollutants. Phytoplankton can develop with the sight of toxic compounds due to the acclimatization. Microalgal species is highly suggested for the site contaminated with the pesticide lindane because lindane is highly toxic to both human and the environment. Some researchers have been focused on the degradation of OPPs in the presence of microbial enzymes. For example, alkaline phosphatase is an enzyme secreted from *Spirulina platensis* which has the potential to hydrolyze the OPPs like chlorpyrifos to its primary metabolite 3,5,6-trichloro-2-pyridinol (TCP) (Thengodkar and Sivakami 2010). So, from these findings, the enzymes secreted by the organisms can be immobilized on the solid matrix to decontaminate the polluted sites. Microalgal remediation can handle different varieties and quantities of pollutants in the contaminated water. Atrazine is an herbicide which can be removed by certain techniques like

nanofiltration, photocatalytic degradation, adsorption, etc., but these methods are regarded as a less feasible due to its high energy costs. A study was carried out in the degradation of atrazine by the microalgal species *Chlamydomonas Mexicana*. Because of atrazine degradation, the carbohydrate content gets increased in the algae and also proved that *C. Mexicana* can evacuate the atrazine at polluted streams (Kabra et al. 2014).

10.10.2 Phytoremediation of Pesticides

For the large-scale process, phytoremediation is utilized to remediate the pollutants. Phytoremediation is the usage of growing plants or genetically modified plants to remediate the pollutants in the surroundings, and the contaminants exist in the polluted site get permeated through the plants of the radical system and deposited in the components of plants like stems, leaves, and roots. This innovation is based on the idea thatthe toxicants in the environment get converted into non-toxicants in the presence of plant oxidative enzymes. Microorganisms correlated with the roots and physiological and biological characteristics of the plants help the bioremediation to occur based on the plants. The term phytoremediation was initially employed in 1980; however, its growth in the remedy of organic pollutants is established at the end of the last century (Morillo and Villaverde 2017). Greater than 2400 plant species can control the pest, but some plants slaughter the toxic substances, while the other plants control the pest by certain actions like repellency, hindrance in growth without creating negative impacts on human health and environment (Rani and Dhania 2014). Few low molecular weight pesticides are carried away through plant membranes and expelled from the soil. By means of evapotranspiration process, the leaves get discharged. Non-toxic compounds can be produced by some mechanisms like alteration in enzymes, phytoextraction, rhizofiltration, phytostabilization, rhizodegradation. The different purposes involved in the phytoremediation mechanism are depicted in Table 10.4. Compared to traditional techniques, phytoremediation has been examined as an adequate method over the last 20 years (Pandey et al. 2016).

Due to the aerobic and anaerobic conversions, only a few bacterial strains can play out the detoxification of organochlorine pesticides. In order to degrade the organochlorine pesticides, the phytoremediation process is needed. Depending upon the phytoremediation mechanisms and the contact within the plant–microbe, the organic pollutants like organochlorine pesticides get evacuated from the environment. The factor which affects the phytoremediation process is the development of plants in the pesticide-contaminated site. Because toxicity at the polluted sites can affect the phytoremediation mechanisms and leads to membrane lipid peroxidation (LPO) (Mitton et al. 2016), the main cause for causing LPO in plants is due to the existence of endosulfan in the soil. Huge biomass production and uptake capacity of pesticides by the phytoextraction mechanism of sunflower plant revealed that it is a good phytoremediator due to the decrease of pesticide

endosulfan in the soil (Mitton et al. 2016). The plants placed near the water sediments or soil-like macrophytes are able to transform or stabilize the organic contaminates, which is regarded as an eco-friendly technology. Atrazine is an herbicide which has a phytotoxic effect toward the submerged macrophytes, such as *Elodea canadensis, Myriophyllum spicatum,* and *Potamo-geton lucens* (Knauert et al. 2010; Brain et al. 2012). Silica is a significant element which stimulates the plant resistance against the pathogens, pests, salinity, and toxicity of metal ions (Romeh and Hendawi 2017). In the removal of cyanophos from the soil, liquid silicon dioxide (SiO_2) a stimulating agent was employed to increase the efficiency of Plantago major L (Romeh 2015). The merits of phytoremediation process are low in corrosion rate, enhances the properties of soil, land creative advancements, avoids the transfer of polluted sites, controls the bacterial growth etc. There are some plant products which can be utilized as pesticide source for killing the pest. The substances like Bt gene, a protease inhibitor, lectins, chitinase are incorporated into the transgenic plants to produce plant products which destroy the intended pest (Kandpal 2014). The downside of this process is a lengthy period of bioremediation than the microbial remediation and it is based upon climatic state, concentration of toxic substances, contaminated site and dumping of plant wastes (Morillo and Villaverde 2017).

10.11 Future Recommendations

It is noticeable from the chapter that many bioremediation techniques are available for the degradation of pesticides. Although some existing techniques like bacterial remediation, fungal remediation, algal remediation, and phytoremediation are strongly entrenched, they are not feasible in case of treating a blended form of pesticides present at the polluted site. Additionally, more than one treatment technique is needed to remediate the polluted sites. Only fewer bioremediators are found in the degradation of pesticides. Not all types of pesticides can be treated by bioremediators, only same types of pesticides are proved by bioremediators. For example, the organism which degrades organophosphate pesticides (OPPs) like chlorpyrifos can degrade other OPPs like methyl parathion, but they are not able to degrade organochlorine pesticides (OCPs). Hence, it needs to be identified to degrade all kinds of pesticides by the same bioremediators.

Several studies revealed that the persistent of pesticides level is high in the soil, water, and air. This is because of utilization of a wrong dosage of pesticides in rural applications. So, people should aware by education or training utilizing pesticides onto the farming area. They should also know how to handle equipment which was used for spraying the pesticides so that excess use of pesticides can be avoided. Many studies are being carried out to reform the bioremediation customs in order to apply these technologies in industrial-scale studies. A number of data regarding bioremediation of pesticides are needed to make them more suitable for pilot scale. In the case of microbial remediation, metabolism reaction takes some time, and

hence, it is difficult to employ them into pilot-scale level. Recognition related to microbial diversity is required, so that best resistance toward physical and chemical stress and high degrading capacity of microorganisms can be diagnosed easily for remediation purposes. This will lead to the replacement of other techniques available for the treatment of pesticides.

10.12 Conclusion

Bioremediation techniques deal with some of the concerns like contaminants with organic and inorganic pollutants. To bypass the decontamination, industries are developing various remediation technologies. Varieties of techniques include in situ, ex situ bioremediation, especially physical, chemical, biological, and advanced engineering technologies. The selection of bioremediation technology depends on the types of pesticides and organisms and environmental surroundings. Developing countries still using pesticides need an additional alternative technique to remediate the contaminant sites. The application of pesticides is increasing rapidly, and therefore, more consciousness is needed due to adverse effects of pesticides in environment and individuals. Several remediation technologies are available to decontaminate the polluted sites, but bioremediation techniques are obtaining massive benefits than the other technologies. The remediation techniques explained in this chapter have some advantages and disadvantages which need to be established at the suitable area. This approach does not need dredging and capping like other conventional techniques. It acts as an important strategy for pesticide removal by metabolic pathways. Hence, it is considered as an eco-friendly method for safe future.

References

Alvarez A, Saez JM, Costa JSB, Colin VL, Fuentes MO, Cuozzo SA, Benimeli CS, Maria SF, Polti MA, Amoroso MJ (2017) Actinobacteria: current research and perspectives for bioremediation of pesticides and heavy metals. Chemosphere 166:41–62

Archaya S, Gopinath LR, Bhuvaneswari R (2014) A review on advances in phytoremediation of pesticide polluted soils. Int J Adv Interdiscip Res 1:1–6

Arienzo M, Masuccio AA, Ferrara L (2013) Evaluation of sediment contamination by heavy metals, organochlorinated pesticides, and polycyclic aromatic hydrocarbons in the Berre coastal lagoon (southeast France). Arch Environ Contam Toxicol 65:396–406

Bazrafshan A, Ghaedi M, Rafiee Z, Hajati S, Ostovan A (2017) Nano-sized molecularly imprinted polymer for selective ultrasound assisted microextraction of pesticide Carbaryl from water samples: spectrophotometric determination. J Colloid Interface Sci 498:313–322

Brain RA, Hoberg J, Hosmer AJ, Wall SB (2012) Influence of light intensity on the toxicity of atrazine to the submerged freshwater aquatic macrophyte *Elodea canadensis*. Ecotoxicol Environ Saf 79:55–61

Briceno G, Schalchli H, Rubilar O, Tortella GR, Mutis A, Benimeli CS, Palma G, Diez MC (2016) Increased diazinon hydrolysis to 2-isopropyl-6-methyl-4-pyrimidinol in liquid medium by a specific Streptomyces mixed culture. Chemosphere 156:195–203

Casida JE, Durkin KA (2013) Neuroactive insecticides: targets, selectivity, resistance, and secondary effects. Annu Rev Entomol 58:99–117

Chaurasia AK, Adhya TK, Apte SK (2013) Engineering bacteria for bioremediation of persistent organochlorine pesticide lindane (gamma-hexachlorocyclohexane). Bioresour Technol 149:439–445

Chrinside AE, Ritter WF, Radosevich M (2011) Biodegradation of aged residues of atrazine and alachlor in a mix-load site soil by fungal enzymes. Appl Environ Soil Sci. https://doi.org/10.1155/2011/658569

Ellegaard-Jensen L, Knudsen BE, Johansen A, Albers CN, Aamand J, Rosendahl S (2014) Fungal–bacterial consortia increase diuron degradation in water-unsaturated systems. Sci Total Environ 466:699–705

Fuentes MS, Raimondo EE, Amoroso MJ, Benimeli CS (2017) Removal of a mixture of pesticides by a *Streptomyces* consortium: Influence of different soil systems. Chemosphere 173:359–367

Gao Y, Truong YB, Cacioli P, Butler P, Kyratzis IL (2014) Bioremediation of pesticide contaminated water using an organophosphate degrading enzyme immobilized on nonwoven polyester textiles. Enzyme Microb Technol 54:38–44

Geed SR, Shrirame BS, Singh RS, Rai BN (2017) Assessment of pesticides removal using two-stage Integrated Aerobic Treatment Plant (IATP) by *Bacillus* sp. isolated from Agricultural field. Bioresour. https://doi.org/10.1016/j.biortech.2017.03.080

Gomes MP, da Silva Cruz FV, Bicalho EM, Borges FV, Fonseca MB, Juneau P, Garcia QS (2017) Effects of glyphosate acid and the glyphosate-commercial formulation (Roundup) on *Dimorphandra wilsonii* seed germination: Interference of seed respiratory metabolism. Environ Pollut 220:452–459

Goulson DJ (2013) An overview of the environmental risks posed by neonicotinoid insecticides. J Appl Ecol 50:977–987

Hong SH, Shim WJ, Han GM, Ha SY, Jang M, Rani M, Hong S, Yeo GY (2014) Levels and profiles of persistent organic pollutants in resident and migratory birds from an urbanized coastal region of South Korea. Sci Total Environ 470–471:1463–1470

Kabra AN, Ji MK, Choi J, Kim JR, Govindwar SP, Jeon BH (2014) Toxicity of atrazine and its bioaccumulation and biodegradation in a green microalga, *Chlamydomonas mexicana*. Environ Sci Pollut Res Int 21:12270–12278

Kandpal V (2014) Biopesticides. Int J Environ Res Dev 4:191–196

Kharabsheh HA, Han S, Allen S, Chao SL (2017) Metabolism of chlorpyrifos by Pseudomonas aeruginosa increases toxicity in adult zebrafish (Danio rerio). Int Biodeterior Biodegradation 121:114–121

Knauert S, Singer H, Hollender J, Knauer K (2010) Phytotoxicity of atrazine, isoproturon, and diuron to submersed macrophytes in outdoor mesocosms. Environ Pollut 158:167–174

Kong L, Zhu S, Zhu L, Xie H, Su K, Yan T, Wang J, Wang J, Wang F, Sun F (2013) Biodegradation of organochlorine pesticide endosulfan by bacterial strain *Alcaligenes faecalis* JBW4. J Environ Sci 25:2257–2264

Kurade MB, Kim JR, Govindwar SP, Jeon B (2016) Insights into microalgae mediated biodegradation of diazinon by *Chlorella vulgaris*: Microalgal tolerance to xenobiotic pollutants and metabolism. Algal Res 20:126–134

Leon-Santiesteban HH, Wrobel K, Revah S, Tomasini A (2016) Pentachlorophenol removal by *Rhizopus oryzae* CDBB-H-1877 using sorption and degradation mechanisms. J Chem Technol Biotechnol 91:65–71

Liao X, Zhang C, Wang Y, Tang M (2017) The abiotic degradation of methyl parathion in anoxic sulfur-containing system mediated by natural organic matter. Chemosphere 176:288–295

Mandal K, Singh B, Jariyal M, Gupta VK (2014) Bioremediation of fipronil by a *Bacillus firmus* isolate from soil. Chemosphere 101:55–60

Maqbool Z, Hussain S, Imran M, Mahmood F, Shahzad T, Ahmed Z, Azeem F, Muzammil S (2016) Perspectives of using fungi as bioresource for bioremediation of pesticides in the environment: a critical review. Environ Sci Pollut Res 23:16904–16925

Mishra A, Kumar J, Melo J (2017) An optical microplate biosensor for the detection of methyl parathion pesticide using a biohybrid of *Sphingomonas* sp. cells-silica nanoparticles. Biosens Bioelectron 87:332–338

Mitton FM, Gonzalez M, Monserrat JM, Miglioranza KSB (2016) Potential use of edible crops in the phytoremediation of endosulfan residues in soil. Chemosphere 148:300–306

Mohamed AT, El-Hussain AA, El-Siddig MA, Osman AG (2011) Degradation of oxyfluorfen herbicide by soil microorganism biodegradation of herbicides. Biotechnol 10:274–279

Moon HB, Lee DH, Lee YS, Choi M, Choi HG, Kannan K (2012) Polybrominated diphenyl ethers, polychlorinated biphenyls, and organochlorine pesticides in adipose tissues of Korean women. Arch Environ Contam Toxicol 62:176–184

Mori T, Wang J, Tanaka Y, Nagai K, Kawagishi H, Hirai H (2017) Bioremediation of the neonicotinoid insecticide clothianidin by the white-rot fungus *Phanerochaete sordida*. J Hazard Mater 321:586–590

Morillo E, Villaverde J (2017a) Advanced technologies for the remediation of pesticide contaminated soils. Sci Total Environ 586:576–597

Morillo E, Villaverde J (2017b) Advanced technologies for the remediation of pesticide contaminated soils. Sci Total Environ. https://doi.org/10.1016/j.scitotenv.2017.02.020

Moschet C, Wittmer I, Simovic J, Junghans M, Piazzoli A, Singer H, Stamm C, Leu C, Hollender J (2014) How a complete pesticide screening changes the assessment of surface water quality. Environ Sci Technol 48:5423–5432

Müller MH, Polder A, Brynildsrud OB, Karimi M, Lie E, Manyilizu WB, Mdegela RH, Mokiti F, Murtadha M, Nonga HE, Skaare JU, Lyche JL (2017) Organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) in human breast milk and associated health risks to nursing infants in Northern Tanzania. Environ Res 154:425–434

Odukkathil G, Vasudevan N (2013) Toxicity and bioremediation of pesticides in agricultural soil. Rev Environ Sci Biotechnol 12:421–444

Pan X, Xu T, Xu H, Fang H, Yu Y (2017) Characterization and genome functional analysis of the DDT-degrading bacterium *Ochrobactrum* sp. DDT-2. Sci Total Environ 592:593–599

Pandey VC, Bajpai O, Singh N (2016) Energy crops in sustainable phytoremediation. Renew Sust Energ Rev 54:58–73

Peng X, Huang J, Liu C, Xiang Z, Zhou J, Zhong G (2012) Biodegradation of bensulphuron-methyl by a novel *Penicillium pinophilum* strain BP-H-02. J Hazard Mater 213:216–221

Peter L, Gajendiran A, Mani D, Nagaraj S, Abraham J (2015) Mineralization of malathion by *Fusarium oxysporum* strain JASA1 isolated from sugarcane fields. Environ Prog Sustainable Energy 34:112–116

Purnomo AS, Mori T, Takagi K, Kondo R (2011) Bioremediation of DDT contaminated soil using brown-rot fungi. Int Biodeter Biodegr 65:691–695

Rani K, Dhania G (2014) Bioremediation and biodegradation of pesticide from contaminated soil and water—a noval approach. Int J Curr Microbiol App Sci 3:23–33

Rani M, Shanker U, Jassal V (2017) Recent strategies for removal and degradation of persistent and toxic organochlorine pesticides using nanoparticles: a review. J Environ Manage 190:208–222

Romeh AA (2015) Enhancing agents for phytoremediation of soil contaminated by cyanophos. Ecotox Environ Safe 117:124–131

Romeh AA, Hendawi MY (2017) Biochemical interactions between *Glycine max L.* silicon di-oxide (SiO_2) and plant growth-promoting bacteria (PGPR) for improving phytoremediation of soil contaminated with fenamiphos and its degradation products. Pestic. Biochem. https://doi.org/10.1016/j.pestbp.2017.01.001

Saez JM, Alvareza A, Benimeli CS, Amoroso MJ (2014) Enhanced lindane removal from soil slurry by immobilized *Streptomyces* consortium. Int Biodeterior Biodegradation 93:63–69

Schenk G, Mateen I, Ng TK, Pedroso MM, Mitic N, Jafelicci M, Marques RFC, Gahan LR, Ollis DL (2016) Organophosphate-degrading metallohydrolases: structure and function of potent catalysts for applications in bioremediation. Coord Chem Rev 317:122–131

Schreiner VC, Szocs E, Bhowmik AK, Vijver MG, Schafer RB (2016) Pesticide mixtures in streams of several European countries and the USA. Sci Total Environ 573:680–689

Silambarasan S, Abraham J (2014) Efficacy of *Ganoderma* sp. JAS4 in bioremediation of chlorpyrifos and its hydrolyzing metabolite TCP from agricultural soil. J Basic Microbiol 54:44–55

Thengodkar RR, Sivakami S (2010) Degradation of Chlorpyrifos by an alkaline phosphatase from the cyanobacterium Spirulina platensis. Biodegradation 21:637–644

Tomei MC, Angelucci DM, Annesini MC, Daugulis AJ (2013) Ex situ remediation of polluted soils by absorptive polymers, and a comparison of slurry and two-phase partitioning bioreactors for ultimate contaminant degradation. J Hazard Mater 262:31–37

Uqab B, Mudasir S, Nazir R (2016) Review on bioremediation of pesticides. J Bioremediat Biodegradation 7:343

Verardo E, Atteia O, Prommer H (2017) Elucidating the fate of a mixed toluene, DHM, metha nol, and i-propanol plume during in situ bioremediation. J Contam Hydrol. https://doi.org/10.1016/j.jconhyd.2017.04.002

Villaverde J, Rubio-Bellido M, Merchan F, Morillo E (2017) Bioremediation of diuron contaminated soils by a novel degrading microbial consortium. J Environ Manage 188:379–386

Xiao P, Mori T, Kamei I, Kiyota H, Takagi K, Kondo R (2011a) Novel metabolic pathways of organochlorine pesticides dieldrin and aldrin by the white rot fungi of the genus *Phlebia*. Chemosphere 85:218–224

Xiao P, Mori T, Kondo R (2011b) Biotransformation of the organochlorine pesticide trans-chlordane by wood-rot fungi. N Biotechnol 29:107–115

Yang G, Chen C, Wang Y, Peng Q, Zhao H, Guo D, Wang Q, Qian Y (2017) Mixture toxicity of four commonly used pesticides at different effect levels to the epigeic earthworm, *Eisenia fetida*. Ecotoxicol Environ Saf 142:29–39

Ye XQ, Xiong K, Liu J (2016) Comparative toxicity and bioaccumulation of fenvalerate and esfenvalerate to earthworm Eisenia fetida. J Hazard Mater 310:82–88

Chapter 11
Application of Microbes in Remediation of Hazardous Wastes: A Review

Moni Kumari, Pooja Ghosh and Indu Shekhar Thakur

Abstract Currently, pollution control, environmental management, treatment and recycling of wastes have become critical issues. One of the major reasons behind the growing environmental pollution is illegal disposal of waste. Due to the toxicity of waste, establishing efficient and environmentally friendly method to degrade and detoxify these wastes represent an important research challenge. Various physio-chemical methods are applied all over the world for solid waste management. The application of microbes to degrade waste is gaining attention due to its environmental and economic benefits. The present review deals with application of microbes in bioremediation of hazardous wastes. This review also outlines the various factors that limit the use of microbial waste bioremediation technologies. Moreover, the prospects of waste valorization for the production of biopolymers, biofuels, biocompost and industrial enzymes are also discussed in the review article.

Keywords Microbes · Bioremediation · Valorization · Waste management Biofuel · Biocompost

M. Kumari · I. S. Thakur (✉)
School of Environmental Sciences, Jawaharlal Nehru University,
New Delhi 110067, India
e-mail: isthakur@hotmail.com

M. Kumari
e-mail: mona.jnu10@gmail.com

P. Ghosh
Centre for Rural Development and Technology, Indian Institute of Technology,
New Delhi 110016, India

S. J. Varjani et al. (eds.), *Bioremediation: Applications for Environmental Protection and Management*, Energy, Environment, and Sustainability,
https://doi.org/10.1007/978-981-10-7485-1_11

11.1 Introduction

Environmental pollution refers to the introduction of hazardous wastes into the environment. This is one of the most severe problems of the twenty-first century facing global attention and priority decision-making. As the year passes, hazardous waste causes grave and irreparable damage to the Earth and thus, it has become an issue of serious international concern (Orloff and Falk 2003).

All the hazardous toxic wastes that are improperly managed are potential threat to mankind and environment. The nature of these wastes could be either liquid, solid, sludge or gas according to the Resource Conservation and Recovery Act (RCRA), enacted in 1976. Based on the regulations set by environment protection agency (EPA), hazardous wastes are categorized into four major groups: (1) ignitability/highly flammable like alcohol, acetone and gasoline, (2) corrosive substance including hydrochloric acid, nitric acid and sulphuric acid, (3) reactivity/explosive and (4) highly toxicity/poisonous hazardous wastes are toxic in nature even at very low concentration.

Industrial hazardous wastes are produced either intentionally or unintentionally. According to the report of Chinese Nationwide General Investigation in 2007, 25.00 million tons of industrial hazardous waste is generated in China only (Sun and Wu 2007). Industries are major source of hazardous waste and are categorized into three main subcategories: (1) non-specific industrial source, for example halogenated solvents, (2) specific industrial source like wastewater produced in the industries during the course of 2, 4-dichlorophenoxyacetic acid (2, 4-d) production and (3) chemicals that have been used in the production of drugs, detergents, lubricants, dyes and pesticides like benzene. The best-known examples are hazardous chlorinated organic molecules such as polychlorinated naphthalenes (PCNs), polychlorinated dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs) and chlorophenols. Another group of inadvertently produced hazardous chemicals produced during the course of chemical combustive processes includes chemicals such as PAHs, hexachlorobenzene (HCB), dioxins like PCDDs and furans like PCDFs. Heavy metals such as chromium, nickel, copper, mercury and lead are also contaminants present in sludge, wastewater, vehicles and industrial activities above maximum permissible value. They too are considered as hazardous waste to due their environmental load and health effects.

Hazardous waste produced from agriculture and agro-industries includes fertilizers, pesticides and hazardous veterinary product wastes. Excess use of fertilizers, pesticides and other chemicals used in agriculture and the wastes formed from these cause land and water pollution. Chemicals/pesticides used in agriculture such as atrazine, endosulfan, DDT, toxaphene, aldrin, dieldrin, heptachlor, chlordane and lindane or gamma-hexachlorocyclohexane (γ-HCCH) are well-known hazardous waste. This group of toxic hazardous chemicals has been deliberately used in agriculture for enhanced production. Domestic-produced hazardous waste includes rat poisons, herbicides and pesticides, mosquito repellents, paints, disinfectants and fuels.

Hazardous waste produced in hospitals includes pathological waste, contaminated needle and pharmaceutical waste.

Among all the toxic hazardous wastes, some groups are specially designated as persistent contaminants due to their specific physicochemical properties as well as high toxicity levels. They are synthetic chemicals that have an intrinsic resistance to natural degradation processes and are therefore environmentally persistent. They have recalcitrant nature, long-term transportation, long half-life, adverse toxicological impact and bioaccumulation ability.

The uses of micro-organisms to destroy or reduce the concentration of hazardous wastes or any other contaminants present in the environment are called bioremediation. The two major success stories of bioremediation technologies of the past are the oil spill clean up of Prince William Sound and Gulf of Alaska (Atlas and Bartha 1998). Thereafter, bioremediation is used continuously as a good clean-up, cost-effective, energy efficient and eco-friendly alternative technique over the physicochemical methods. The two major processes to accomplish bioremediation are either by boosting the growth of indigenous microbial community present at the site called biostimulation or via introducing microbes having better bioremediation capabilities (Agnello et al. 2016). During bioremediation, microbes utilize toxic hazardous waste as a source of carbon and energy to destroy them into non-toxic or less toxic by-products. It is a low-cost technique, which generally has a high public acceptance and can often be carried out on site (Boopathy 2000). One of the major advantages of bioremediation is the generation of non-toxic elements such as carbon, nitrogen and hydrogen as the dead end by-products which simply get assimilated back into the environment. It is, however, not always be suitable as the range of contaminants on which it is effective is limited, the timescales involved are relatively long, and the residual contaminant levels achievable may not always be appropriate. Although the methodologies employed are not technically complex, considerable experience and expertise may be required to design and implement a successful bioremediation programme, due to the need to thoroughly assess a site for suitability and to optimize conditions to achieve a satisfactory result. It also has been observed that by utilizing the native microbial community present at the contaminated site, the bioremediation rate is quite slow. Hence, nowadays several advancements have been done in bioremediation strategies in order to increase its rate. This includes (i) adopting genetic engineered microbes/transgenic based bioremediation, (ii) application of exogenous biosurfactants producing microbes which increase the bioavailability of contaminants to the microbes thus rate of bioremediation, (iii) application of mixed microbial culture and (iv) by the application of rhizoremediation despite of bioremediation (Kumari et al. 2016; Sharma et al. 2017). The application of bacto-algal consortia leads to enhanced removal of several persistent organic contaminants present in landfill leachate over individual bacterial and algal treatment. It also has been observed that the bacto-algal consortia are more capable in reduction of cytotoxicity and genotoxicity over individual microbial culture after treatment of landfill leachate (Kumari et al. 2016).

Microbial degradation of hazardous waste involves the activities of several kinds of enzymes including oxidoreductase, oxygenase, dehydrogenase, Cytochrome

Table 11.1 Application of microbial enzymes involved in bioremediation of various hazardous wastes

Enzyme	Reaction	Applications
Oxidoreductases	Oxidative coupling of substrate	The detoxification of toxic organic compounds including phenolic, anilinic, chlorinated compounds
Oxygenases like monooxygenases and dioxygenases	Oxidation of reduced substrates by transferring oxygen from molecular oxygen (O_2) utilizing FAD/NADH/NADPH as a cosubstrate	Desulphurization, dehalogenation, denitrification, ammonification, hydroxylation, biotransformation, oxidation and biodegradation of various aromatic and aliphatic compounds
Laccases	Oxidation of a wide range of organic and inorganic compounds	Phenolic, aromatic, paradiphenols, aminophenols, polyphenols, polyamines, lignins and aryl diamine substrates
Peroxidases	Oxidation of substrate at the expense of hydrogen peroxide (H_2O_2)	Lignin and other phenolic compounds
Hydrolytic enzymes	Disrupt major chemical bonds in the toxic molecules	Reduction of toxicity of hazardous compounds
Lipases	Total hydrocarbon degradation	Bioremediation of oil spill

P450 enzyme, soluble methane monooxygenases (Table 11.1). The detoxification of hazardous contaminants is carried out through oxidative coupling by the action of oxidoreductases present in the microbial system. During such oxidation–reduction process, the hazardous toxic contaminants get transformed into harmless by-products.

11.2 Remediation Methods

11.2.1 Physicochemical Methods

Numerous physicochemical methods including coagulation–flocculation, filtration, precipitation, adsorption, ozonation, sedimentation, chemical precipitations, ion exchange, advanced oxidation and reverse osmosis have been employed for the removal of hazardous waste from the environment (Crini 2006). These methods have been found to be limited because these techniques are executed under controlled environmental conditions. Physicochemical techniques often involve high capital and operational costs, production of large volumes of sludge, high cost of equipments and require skilled manpower.

11.2.2 Biological Methods

Biological methods of treatment also called bioremediation are powered by micro-organisms for the degradation and detoxification of POPs. Bioremediation has distinct advantages over physicochemical remediation methods as it is cost-effective, eco-friendly and could be achieved without destructing indigenous flora and fauna (Timmis and Pieper 1999). During the course of bioremediation processes, micro-organisms use the contaminants as nutrient or energy sources (Tang et al. 2007). There are numerous benefits of using micro-organism for degradation of xenobiotic contaminants due to their ubiquitous presence, diversity and adaptation of variable metabolic pathways.

Since micro-organisms possess high biodegradation potential, they are of considerable biotechnological interest and their application in the degradation and detoxification of various hazardous wastes has been extensively investigated.

11.3 Bioremediation Processes: Two Main Categories

11.3.1 In situ Bioremediation

In situ bioremediation is the application of microbes for the on-site removal of pollutants. This technology of bioremediation depends on activity of contaminated site-specific indigenous microbial consortia (Agarwal 1998). However, this approach of bioremediation is less expensive and there is chance of permanent waste removal. Also, the chances of site disruption are very little, giving greater public acceptance to it. However, this method is often more suitable for remediation of soil with a low level of contaminants. For enhanced in situ bioremediation, effective microbial consortia can be established in the contaminated sites by providing proper temperature, moisture, nutrients and terminal electron acceptor (Hess et al. 1997; Agarwal 1998). During in situ bioremediation, anaerobic micro-organisms play better role over aerobic micro-organisms since they do not require expensive oxygen. It is very well studied that in situ bioremediation is successful in removing several monoaromatic organic pollutants including carbon tetrachloride (CT), tetrachloroethylene (TCA), trichloroethylene (TCE) or pentachlorophenol (PCP)-contaminated groundwater (Dyer et al. 2003; Widdowson 2004). Bioventing, biosparging and bioaugmentation are common in situ bioremediation processes.

11.3.2 Ex situ Bioremediation

Ex situ bioremediation is the major remediation technology and has been employed widely for the treatment of a wide range of xenobiotic hazardous wastes. In this technology, contaminated soil is taken out and treated elsewhere. It has been a topic of considerable research interest since last several decades. Ex situ bioremediation

technology has been executed by either bioaugmentation which means micro-organisms are added to the contaminants or biostimulation, i.e. by providing essential nutrients or biosurfactants to stimulate microbial degradation (Sayara et al. 2010). Landfarming, composting, biopiles and bioreactors are commonly applied ex situ bioremediation processes. It is usually observed that the ex situ bioremediation techniques lead to removal of a wide range of pollutants, easily controllable and a faster processes over in situ.

11.4 Microbial Application for the Bioremediation of Hazardous Wastes

Microbes reported for the bioremediation of several classes of hazardous waste are mainly bacteria, algae, actinomycetes and fungi as these groups of micro-organisms have the physiological and metabolic capabilities to degrade the pollutants (Strong and Burgess 2008).

11.4.1 Bacterial Treatment of Wastes

Numerous aerobic and anaerobic bacteria have unique catabolic pathways enabling them to degrade a number of hazardous contaminants, including pesticides, PHA, dioxins and furans present in the environment. These groups of bacterial strains have ability to use pollutants as a sole source of carbon and energy. Bacterial P450 oxygenase system, monooxygenase, dioxygenases, hydroxylases and dehalogenases are key enzymes that participate in biotransformation and mineralization of xenobiotic compounds. Some examples of bacteria possess the ability to degrade chemical belongs to persistent organic contaminants (POPs) are *Pseudomonas*, *Streptomyces*, *Paenibacillus*, *Bacillus* and *Pandoraea* (Arshad et al. 2008; Fuentes et al. 2011; Karigar and Rao 2011; Ali et al. 2014; Singh et al. 2014).

For the bioremediation of industrial wastes containing lignin in huge quantity, bioremediation is efficiently carried out by the bacteria possessing ligninolytic enzymes like laccase. This enzyme is a multicopper oxidase and is involved in degradation of wide-range industrial pollutants. The introduction of molecular oxygen into substrate is carried out by the dioxygenases, a multicomponent enzyme system. The ubiquitous presence of dioxygenases in the bacterial system participates in the transformation of more toxic aromatic hazardous wastes into less toxic aliphatic one.

11.4.2 Algal Treatment of Hazardous Wastes

Most of the published literature on degradation of hazardous contaminants has been focused on either bacteria or fungi, while the innate potential of algae and cyanobacteria has received relatively less attention and has not been fully realized. Microalgal cells possess a complex array of enzymatic antioxidant defence system,

which comprises mainly enzymes superoxide dismutase (SOD) and catalase (CAT) (Karigar and Rao 2011). Microalgae are one of the main diazotrophic components of the primary microbiota and significantly contribute to building-up soil fertility (Elizabeth and Harris 2008). Microalgae in their natural habitats are often exposed to various contaminants such as heavy metals, hexachlorobenzene, herbicides, insecticides, endocrine-disrupting chemicals and phenol (Hirooka et al. 2005; Dosnon-Olette et al. 2010), which impose toxic effects on the microalgae. Biodegradation of methyl parathion (MP) by microalgae and cyanobacteria was also reported by Megharaj et al. (1994). The authors have reported that microalgae or cyanobacteria utilized 1 ml of 1000 ppm commercial MP as a carbon and nitrogen source. There are reports on the role of algae in the biodegradation of lindane up to 48.8% by *Anabaena azotica* in 5 days (Zhang et al. 2012). Several algal species including *Scenedesmus* sp., *Chlamydomonas* sp. and *Chlorella* sp. have been identified for the degradation of fenamiphos, DDT and endosulfan (Megharaj et al. 2000; Sethunathan et al. 2004). Anthracenes (2.5 ppm), DDT (0.5 ppm) and Pyrene (0.1 ppm) are reported to be degraded by *Chlorella protothecoides*, *C. vulgaris* and *Scenedesmus quadricauda,* respectively (Lei et al. 2002; Yan et al. 2002).

11.4.3 Fungal Treatment of Hazardous Wastes

Mycoremediation is the technology where fungi are used for the degradation of pollutants. Since fungi are rapidly colonized, and more tolerant to pollutants, they are more efficient in bioremediation over other micro-organisms. Its hyphae can penetrate into soils and reach pollutant much faster than microbes (Reddy and Mathew 2002; Harms et al. 2011). Metabolic enzymes that catalyse xenobiotic biotransformation and detoxification reactions in eukaryotes are classified as phase I and phase II enzymes. Cytochrome P450 monooxygenase (P450s) and epoxide hydrolases constitute two important phase I oxidation enzyme groups. However, the application of various POPs as the source of carbon and energy by fungi has not been reported extensively. A diverse group of lignolytic and non-lignolytic fungi is able to oxidize POPs. Two main groups of enzymes are involved in the initial attack on PAHs by a fungus which includes cytochrome P-450 monooxygenase and lignin-degrading enzyme system. The mechanism of PAH metabolism by non-lignolytic fungi involves the oxidation of aromatic ring by P-450 monooxygenase (Chang et al. 2003). They generally incorporate one atom of oxygen into the aromatic nucleus and reduce the remaining atom to water, followed by enzymatic addition water to yield cis/trans-dihydrodiols (Sutherland et al. 1995). Arene oxide formed can then undergo non-enzymatic rearrangement to form phenol which can further be conjugated with glucose, xylose, gluconeric acid and sulphate. Various non-lignolytic fungi such as *A. niger*, *C. elegans* and *P. janthinellum* used cytochrome P 450 monooxygenase for the oxidative degradation of PAH. The most widely studied non-lignolytic fungus is *C. elegans*.

Many fungi have been tested for their ability to degrade endosulfan, including *Aspergillus terreus*, *Cladosporium oxysporum* (Mukherjee and Mittal 2005), *Mucor thermohyalospora* (Shetty et al. 2000) and *Fusarium ventricosum* (Siddique et al. 2003) (Table 11.2).

Table 11.2 Application of microbial species for the bioremediation of different classes of toxic hazardous wastes

Contaminants	Microbial pure culture	References
Mixture of PAHs	*Pseudomonas putida* (B1)	Chen et al. (2012)
High molecular weight PAHs	*Pseudomonas saccharophila* P15	Chen and Aitken (1999)
Cd, Cr (IV)	*Staphylococcus xylosus*	Ziagova et al. (2007)
Cr (IV)	*Bacillus licheniformis*	Zhou et al. (2007)
Chlorpyrifos	*Enterobacter* Strain B-14	Singh et al. (2004)
Tetrachlorvinphos	*Proteus vulgaris*	Ortiz-Hernández and Sánchez-Salinas (2010)
Diazinon	*Serratia marcescens* DI101	Abo-Amer (2011)
DDT, DDD and DDE	*Sphingobacterium* sp	Fang et al. (2010)
Pb, Ni, Cr	*Aspergillus niger, Aspergillus flavus*	Dwivedi (2012)
Cu, Mn, Zn	*Aspergillus brasiliensis, Penicillium citrinum*	Pereira et al. (2014)
Cd, Pb	*Chlorella vulgaris*	Aung et al. (2012), Edris et al. (2012)
Cd (II)	*Cladophora fascicularis*	Deng et al. (2008)
Cd, Hg, Pb, As, Co	*Spirogyra hyalina*	Kumar and Oommen (2012)
Sr	*Platymonas subcordiformis*	Mei et al. (2006)
Crude oil, a mixed hydrocarbon substrate, n-alkanes and isoalkanes as well as aromatic hydrocarbons	*Prototheca zopfii*	Walker et al. (1975)
Crude oil hydrocarbons	*Aspergillus*, *Cephalosporium* and *Penicillium*	Singh (2006)
Hazardous contaminants present in landfill leachate	*Pseudomonas* sp. ISTDF1	Ghosh et al. (2014)
Endosulfan	*Paenibacillus* sp. ISTP10	Kumari et al. (2014)
Bromophenol blue, fast blue RR, Congo red, crystal violet	*Trametes* sp. SQ01	Yang et al. (2009)

11.5 Degradation of Hazardous Waste Using Microbial Consortia

As the rate of pollution keeps increasing, the designing and constructing of synthetic microbial consortia has raised extensive attention for improving remediation technology. Microbial mixed cultures have been shown to be more suitable for bioremediation of recalcitrant compounds because of following reasons:

(1) The degradation of hazardous organic wastes by using microbial consortia is highly significant because microbial consortia together enhance the level of bioremediation.
(2) Microbial consortia together reduce treatment time of degradation.
(3) Pure culture of microbes can degrade only limited types of compounds, while some xenobiotic complex compounds can only degrade by cometabolism of more than one microbe present in a mixed culture.
(4) The application of bacto-algal microbial consortia leads to complete degradation of pollutants or toxic metabolites due to synergistic interactions among members of the associations (Kumari et al. 2016).
(5) Mixed microbial consortia easily adapt in the presence of different toxic wastes. The treatment using mixed culture is easy to handle and need minimal maintenance.
(6) Mixed cultures permit better utilization of the substrate.

11.6 Mechanism of Bioremediation

Chlorinated organic compounds such as chlorinated pesticides, solvents, polychlorinated naphthalenes (PCNs), polychlorinated dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs) and chlorophenols are common hazardous contaminants present in soil and groundwater. These classes of hazardous wastes are mainly bioremediated by micro-organisms by reductive dechlorination, dehydrochlorination, oxidation and isomerization of the parent molecule. Sulphur-containing hazardous wastes undergo bioremediation by sulphur-oxidizing micro-organisms. Such hazardous wastes are utilized as a source of sulphur for the growth of micro-organisms and concurrently produce soluble metal sulphate and sulphuric acids. Micro-organisms initiate degradation of another class of toxic compounds including dioxins and furans by hydroxylation of the aromatic ring with molecular oxygen. Several micro-organisms are known to grow on such class of hazardous compounds and oxidize it completely via salicylic acid to carbon dioxide.

11.7 Factors Affecting Bioremediation

(1) Bioavailability: This is an extremely important factor which determines the rate of biodegradation (Boopathy 2000). The physical nature and chemical composition of the waste determine their availability to the microbes. It is often observed that even though the hazardous wastes are biodegradable in nature, the rate of its degradation is quite low due to their unavailability. As for example, the microbial degradation of hydrophobic hazardous waste naphthalene and phenanthrene are utilized only when present in dissolve state.
(2) Soil nutrient: Nutrient bioavailability in the soil is one of the major factors that determine bioremediation rate. As the soil nutrient increases, it stimulates the indigenous microbial growth and activity in the soil ecosystem for enhanced bioremediation. The presence of adequate soil nutrient such as nitrogen, phosphorous and carbon also helps in building of various necessary enzymes that participate in bioremediation of hazardous waste. The balanced soil nutrients required for the microbial growths are carbon, nitrogen, phosphorous which should be in the ratio of 100:10:1 (Haghollahi et al. 2016).
(3) Soil oxygen: Microbial bioremediation of hazardous waste is too decided by the presence or absence of molecular oxygen (O_2) in the soil microbial environment. The very first enzymes that participate in aerobic are oxygenases, which rely on the molecular oxygen for their activity. Besides that, the presence of molecular oxygen also acts as an electron acceptor that is released on waste bioremediation. Since the solubility of molecular oxygen in the water is relatively low, thus, their presence is considered as a primary limiting factor for the aerobic microbial bioremediation.
(4) Temperature: As the microbial growth and activity are satisfactory only at optimum temperature, it measures the bioremediation of hazardous waste. At both extreme of temperature, microbial metabolism is inhibited which result in inhibition of bioremediation. At low temperature and high temperature, some psychrophilic and thermophilic microbial metabolism is active for the waste bioremediation. As the temperature increases from 4 to 35 °C, the rates of bioremediation increase by the mesophilic micro-organisms. The microbial growth doubles with every 10 °C rise in temperature leading to an increase in rate of bioremediation (Thibault and Elliott 1979).
(5) Soil moisture: All micro-organisms require soil moisture for their growth and function. It helps in circulation of water and nutrients in and out the micro-organisms. However, the presence of excess soil moisture leads to soil saturation creating resistance to oxygen transfer and ultimately diminish the amount of available oxygen required for microbial respiration (Haghollahi et al. 2016). Low levels of moisture content decrease microbial activity. The water holding capacity of the soil, its type, pore size distribution and texture decides the soil moisture content.

(6) Soil pH: The pH of soil gets altered due to both biotic and abiotic factors. By the process of fermentation and redox reactions, soil micro-organisms either consume or release H^+. Similarly due to metal leaching, pH of the soil gets decreased or its soil acidity increased under high rainfall conditions. Very few micro-organisms including acidophilic and basophilic can tolerate the extreme level of pH (very high or very low). As a consequence, pH of the soil influences soil microbial diversity. Soil pH is also important for enzyme activity because alteration in soil pH causes denaturation of microbial enzymes (Bonomo et al. 2001). It is mentioned in Singh et al. (2003) that at acidic pH, the rate of chlorpyrifos bioremediation is quite low; however, it increases significantly with an increase in soil pH.

(7) Type of soil: The texture of soil determines aeration, nutrient availability, water holding capacity and porosity. The sorption of nutrients by either ion exchange or precipitation to the soil is mainly facilitated by both inner surface and outer surface functional group. Clay is an important type of soil, and due to their very small size (<0.002 mm) and large surface area to volume ratio, it is considered as best for nearly all chemical, physical and biological activities (Buffle and De Vitre 1993). Besides that, soil having high clay content leads to more water adhesion or water holding capacity. Overall thus, very high microbial activities are found there (Brady and Weil 2000) (Fig. 11.1).

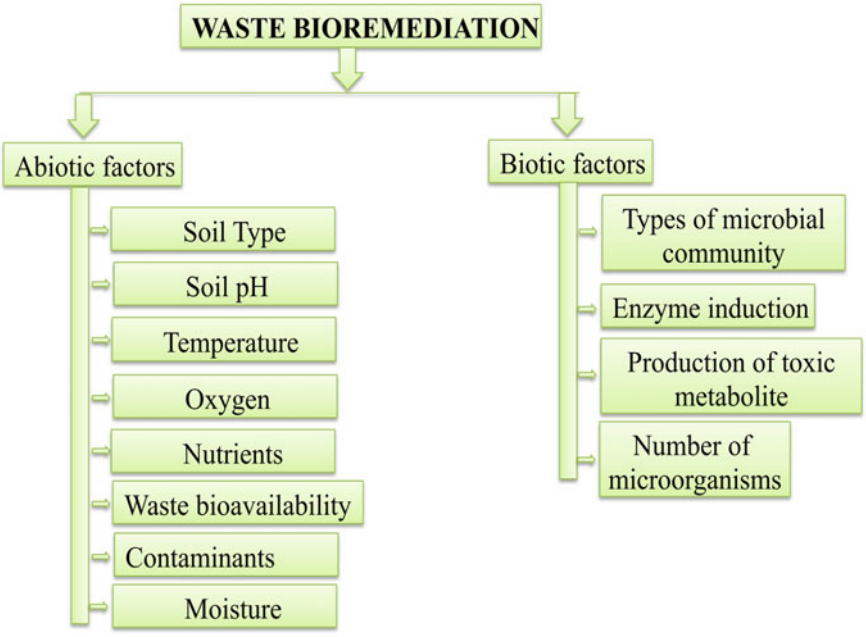


Fig. 11.1 Factors affecting waste bioremediation

11.8 Waste Valorization

Nowadays, the best way to reduce the environmental load of waste generated in such a huge amount is their application for the production of several value-added products such as materials, chemicals, fuels and other energy sources. The initiative of value-added by-products from waste is an approach that facilitates sustainable development.

Waste valorization is a new promising technique in which wastes so produced are treated or used as raw material for the production of a wide range of value-added products at very low capital investment. This emerging approach can not only reduce the environmental load of the waste but also the related negative impacts on the living system and environment. This waste utilization approach could also create new job opportunities and will also be helpful in the conservation of natural resources. The waste so produced from the industrial sector is further utilized for the production of renewable carbon in huge quantities and for the production of sustainable chemical (Koutinas et al. 2014). On the other hand, waste so produced from the agro-industrial could be used as a principal C5 and C6 sugar-based carbon source that can be either used alone or supplemented with various expensive nutrients like yeast extract for the production value-added products such as biodiesel, bioplastic and exopolysaccharides (EPS) at laboratory scale and pilot scale. Several value-added products that have been produced from wastes include biofuels like bioethanol and biohydrogen, short-chain organic acids, building-blocks, including 2, 3-butanediol, 1, 3-propanediol and succinic acid, polymers like bioplastics, i.e. polyhydroxyalkanoates (PHAs) (Koutinas et al. 2014). The major waste generated in pulp and paper industry is cellulose-based fibres which could be further processed for the production of useful products like textile and paper. Another waste generated in pulp and paper industry is spent sulphite liquor (SSL) which is utilized for the production of phenolic compounds mainly aromatics syringic, gallic and vanillic acids (Palmqvist and Hahn-Hägerdal 2000). For the production of several value-added products such as single-cell protein, bioethanol, bioplastics and bacterial cellulose, SSL is utilized as a raw material (Alexandri et al. 2016). Food waste is utilized for the production of another value-added product called hydroxymethylfurfural (HMF). It is utilized as the precursor of medicines, polymers, resins, solvents and biofuels (Mukherjee et al. 2015). Similarly, lignocellulosic waste biomass has been used for the production of phytosterols, polypropylene, acrylic acid and esters (Bardhan et al. 2015). 3.7×10^9 t of agricultural waste is produced worldwide annually. Agricultural waste mainly consists of 40% cellulose, 30% hemicellulose, 20% lignin, 5% proteins and 5% minerals (Pleissner and Venus 2014). This agricultural waste is utilized as a C5 and C6 carbon source after pretreatment for the production of several value-added products.

Another agriculture-based industry in India that generates a number of wastes in a very huge amount like bagasse and bagasse fly ash, sugarcane trash and press mud is the sugar industry. These wastes are used for the production of a number of value-added products like fuels and activated carbon. Either directly or biocomposting with distillery effluent, press mud is used as a fertilizer. Bagasse fly is utilized as an additive in cement and concrete (Balakrishnan and Batra 2011).

There are certain constrains of waste valorization that must be overcome which includes high water contents of wastes so produced, lack of highly efficient conversion techniques for processing of waste, infrastructure and expertise.

11.9 Advantages and Disadvantages of Bioremediation

Disadvantage	Advantage
(1) Not all the toxic hazardous waste is susceptible to rapid and complete microbial degradation	(1) Since bioremediation is a natural process and therefore has wide public acceptance for the treatment of toxic hazardous wastes
(2) Sometimes on microbial degradation, more toxic and persistent intermediate metabolite are formed than the parent compound	(2) It is a relatively economical, low-technology and less energy required technique for clean-up of hazardous waste
(3) Microbial degradation of toxic hazardous waste requires highly specific conditions such as microbial populations, suitable environmental growth conditions and appropriate levels of nutrients and contaminants	(3) On microbial degradation of hazardous wastes, the final residues are harmless products such as carbon dioxide, water and cell biomass
(4) It is very tough to extrapolate the microbial degradation from laboratory scale to pilot scale.	(4) Microbial degradation is useful for the complete destruction of a wide variety of toxic hazardous contaminants
(5) Since bioremediation is a very slow process, it requires months to year to clean-up the environment	(5) Microbes are able to degrade the contaminant, increase in numbers when the contaminant is present; when the contaminant is degraded, the biodegradative population declines
(6) Since microbial degradation of waste is a slow process, thus it needs longer time than the other physiochemical technologies for the complete degradation of toxic hazardous waste	(6) Many compounds that are legally considered to be hazardous can be transformed to harmless products. This eliminates the chance of future liability associated with treatment and disposal of contaminated material

(continued)

(continued)

Disadvantage	Advantage
(7) Regulatory uncertainty remains regarding acceptable performance criteria for bioremediation. There is no accepted definition of "clean", evaluating performance of bioremediation is difficult, and there are no acceptable endpoints for bioremediation treatments.	(7) Since microbial degradation is an environmentally friendly approach, thus it causes very less disruption of natural environment or minimal environmental impact
(8) Modern technologies like genetic engineering are required to develop highly efficient microbes for the degradation of toxic hazardous waste. A stronger scientific base is required for rational designing of process and success	(8) Bioremediation techniques are potentially ideal for the detoxification of hazardous waste present in the environment
(9) For the successful in situ degradation of toxic hazardous waste, the contaminated site must contain soil with high permeability	

11.10 Hazardous Waste Management

Since the hazardous waste is highly toxic in nature even at very low concentration, thus, the governments of different countries across the world have rules and regulations for the proper management of hazardous waste. It includes policy regarding collection, treatment and disposal of waste material that, when improperly handled, can cause substantial harm to human health and safety or to the environment. Waste management is required for both resource and environment protection and also to cut down the waste production. Some important points for hazardous waste management include:

(a) Identification of various groups of hazardous materials commonly used in industries, laboratory, agriculture, hospital and home.
(b) Proper characterization of wastes so that those wastes having similar composition are processed together.
(c) Guidelines must be provided for the proper storage, processing, transport and regulations of hazardous waste.
(d) Generate a policy regarding negative effects of disposable of hazardous waste.
(e) The movement of hazardous waste from one country to another should be under proper jurisdiction.
(f) There must be proper disposable site far from the city for the dumping of wastes.

(g) Industries must adopt proper approach regarding recycling and reuse of waste produced.
(h) Modernization of equipments in the industries so that the waste production is either stopped or minimized.
(i) Adopting new trends like waste valorization so that the waste so produced is further utilized for the production of useful materials and energy.

11.11 Conclusion

The aim of the present chapter is to raise awareness regarding the hazardous wastes produced from different sectors and how they can be managed using microbial remediation. Bioremediation of waste using microbial consortia has been found as a better option for the fast and efficient approach of waste removal over pure microbial culture. Focus has also been given on production of a number of value-added products from wastes for a bio-based economy in an environmentally friendly manner.

Acknowledgements We would like to express our sincere thanks to Department of Science and Technology, Govt. of India, for providing INSPIRE Faculty fellowship to Ghosh, P [DST/INSPIRE/04/2016/000362].

References

Abo-Amer AE (2011) Biodegradation of diazinon by *Serratia marcescens* DI101 and its use in bioremediation of contaminated environment. J Microbiol Biotechnol 21:71–80

Agarwal SK (1998) Environmental biotechnology, 1st edn. APH Publishing Corporation, New Delhi, India, pp 267–289

Agnello AC, Bagard M, van Hullebusch ED, Esposito G, Huguenot D (2016) Comparative bioremediation of heavy metals and petroleum hydrocarbons co-contaminated soil by natural attenuation, phytoremediation, bioaugmentation and bioaugmentation-assisted phytoremediation. Sci Total Environ 563–564:693–703

Alexandri M, Papapostolou H, Vlysidis A, Gardeli C, Komaitis M, Papanikolaou S, Koutinas AA (2016) Extraction of phenolic compounds and succinic acid production from spent sulphite liquor. J Chem Technol Biotechnol. https://doi.org/10.1002/jctb.4880

Ali U, Syed JH, Malik RN, Katsoyiannis A, Li J, Zhang G, Jones KC (2014) Organochlorine pesticides (OCPs) in South Asian region: a review. Sci Total Environ 476:705–717

Arshad M, Hussain S, Saleem M (2008) Optimization of environmental parameters for biodegradation of alpha and beta endosulfan in soil slurry by *Pseudomonas aeruginosa*. J Appl Microbiol 104:364–370

Atlas RM, Bartha R (1998) Fundamentals and applications. In: Microbial ecology, 4th edn. Benjamin/Cummings, San Francisco, Calif, USA, pp 523–530

Aung WL, Aye KN, Hlaing NN (2012) Biosorption of lead (Pb^{2+}) by using *Chlorella vulgaris*. In: International conference on chemical engineering and its applications, Bangkok, Thailand

Balakrishnan M, Batra VS (2011) Valorization of solid waste in sugar factories with possible applications in India: a review. J Environ Manag 92:2886–2891

Bardhan SK, Gupta S, Gorman ME, Haider MA (2015) Biorenewable chemicals: feedstocks, technologies and the conflict with food production. Renew Sust Energy Rev 51:506–520

Bonomo RP, Cennamo G, Purrello R, Santoro AM, Zappala R (2001) Comparison of three fungal laccases from *Rigidoporus lignosus* and *Pleurotus ostreatus*: correlation between conformation changes and catalytic activity. J Inorg Biochem 83:67–75

Boopathy R (2000) Factors limiting bioremediation technologies. Bioresour Technol 74:63–67

Brady NC, Weil RR (2000) Elements of the nature and properties of soils. Prentice Hall, Upper Saddle River, NJ, USA, pp 463–471

Buffle J, De Vitre RR (1993) Chemical and biological regulation of aquatic systems. Lewis Publishers, p 40

Chang BV, Chang W, Yuan SY (2003) Anaerobic degradation of polycyclic aromatic hydrocarbons in sludge. Adv Environ Res 7:623–628

Chen B, Yuan M, Qian L (2012) Enhanced bioremediation of PAH-contaminated soil by immobilized bacteria with plant residue and biochar as carriers. J Soils Sediments 12:1350–1359

Chen SH, Aitken MD (1999) Salicylate stimulates the degradation of high molecular weight polycyclic aromatic hydrocarbons by *Pseudomonas saccharophila* P15. Environ Sci Technol 33:435–439

Crini G (2006) Non-conventional low-cost adsorbents for dye removal: a review. Bioresour Technol 97:1061–1085

Deng L, Zhu X, Su Y (2008) Biosorption and desorption of Cd^{2+} from wastewater by dehydrated shreds of *Cladophora fascicularis*. Chin J Ocean Limnol 26:45–49

Dosnon-Olette R, Trotel-Aziz P, Couderchet M, Eullaffroy P (2010) Fungicides and herbicide removal in *Scenedesmus* cell suspensions. Chemosphere 79:117–123

Dyer M, Heiningen EV, Gerritse J (2003) A field trial for in-situ bioremediation of 1, 2-DCA. Eng Geol 70:315–320

Dwivedi S (2012) Bioremediation of heavy metal by algae: current and future perspective. J Adv Lab Res Bio 3:195–199

Edris G, Alhamed Y, Alzahrani A (2012) Cadmium and lead biosorption by *Chlorella vulgaris*. In: IWTA, 16th international water technology conference, Istanbul, Turkey

Elizabeth H, Harris (2008) Introduction to *Chlamydomonas* and its laboratory use. In: The chlamydomonas sourcebook, vol 1, 2nd edn. Academic Press. ISBN-10: 0123708745

Fang H, Dong B, Yan H, Tang F, Yu Y (2010) Characterization of a bacterial strain capable of degrading DDT congeners and its use in bioremediation of contaminated soil. J Hazard Mater 184:281–289

Fuentes MS, Sáez JM, Benimeli CS, Amoroso MJ (2011) Lindane biodegradation by defined consortia of indigenous *Streptomyces* strains. Water Air Soil Pollut 222:217–231

Ghosh P, Das MT, Thakur IS (2014) Mammalian cell line-based bioassays for toxicological evaluation of landfill leachate treated by Pseudomonas sp. ISTDF1. Environ Sci Pollut Res 21:8084–8094

Haghollahi A, Fazaelipoor MH, Schaffie M (2016) The effect of soil type on the bioremediation of petroleum contaminated soils. J Environ Manage 180:197–201

Harms H, Schlosser D, Wick LY (2011) Untapped potential: exploiting fungi in bioremediation of hazardous chemicals. Nat Rev Microbiol 9:177–192

Hess A, Zarda B, Hahn D (1997) In situ analysis of denitrifying toluene and m-xylene degrading bacteria in a diesel fuel contaminated laboratory aquifer column. Appl Environ Microbiol 63:2136–2141

Hirooka T, Nagase H, Uchida K, Hiroshige Y, Ehara Y, Nishikawa J, Nishihara T, Miyamoto K, Hirata Z (2005) Biodegradation of bisphenol A and disappearance of its estrogenic activity by the green alga *Chlorella fuscavar* vacuolata. Environ Toxicol Chem 24:1896–1901

Karigar CS, Rao SS (2011) Role of microbial enzymes in the bioremediation of pollutants: a review. Enzyme Res. https://doi.org/10.4061/2011/805187

Koutinas AA, Vlysidis A, Pleissner D, Kopsahelis N, Garcia IL, Kookos IK, Lin CSK (2014) Valorization of industrial waste and by-product streams via fermentation for the production of chemicals and biopolymers. Chem Soc Rev 43:2587–2627

Kumar JI, Oommen C (2012) Removal of heavy metals by biosorption using freshwater alga *Spirogyra hyalina*. J Env Bio 33:27–31

Kumari M, Ghosh P, Thakur IS (2014) Microcosmic study of endosulfan degradation by *Paenibacillus* sp. ISTP10 and its toxicological evaluation using mammalian cell line. Int Biodeter Biodegr 96:33–40

Kumari M, Ghosh P, Thakur IS (2016) Landfill leachate treatment using bacto-algal co-culture: an integrated approach using chemical analyses and toxicological assessment. Ecotoxicol Environ Saf 128:44–51

Lei A, Wong Y, Tam N (2002) Removal of pyrene by different microalgal species. Water Sci Technol 46:195–201

Megharaj M, Kantachote D, Singleton I, Naidu R (2000) Effects of long-term contamination of DDT on soil microflora with special reference to soil algae and algal metabolism of DDT. Environ Pollut 109:35–42

Megharaj M, Madhavi DR, Sreenivasulu C, Venkateswarlu UA (1994) Biodegradation of methyl-parathion by micro-algae and cyanobacteria. Bull Environ Contam Toxicol 53:292–297

Mei LI, Xitao X, Renhao XUE, Zhili LIU (2006) Effects of strontium-induced stress on marine algae *Platymonas subcordiformis* (Chlorophyta: *Volvocales*). Chinese J Oceanol Limnol 24:154–160

Mukherjee A, Dumont MJ, Raghavan V (2015) Sustainable production of hydroxymethylfurfural and levulinic acid: challenges and opportunities. Biomass Bioenergy 72:143–183

Mukherjee I, Mittal A (2005) Bioremediation of endosulfan using *Aspergillus terreus* and *Cladosporium oxysporum*. Bull Environ Contam Toxicol 75:1034–1040

Orloff K, Falk H (2003) An international perspective on hazardous waste practices. Int J Hyg Environ Health 206:291–302

Ortiz-Hernández ML, Sánchez-Salinas E (2010) Biodegradation of the organophosphate pesticide tetrachlorvinphos by bacteria isolated from agricultural soils in México. Rev Int Contam Ambient 26:27–38

Palmqvist E, Hahn-Hägerdal B (2000) Fermentation of lignocellulosic hydrolysates. I: inhibition and detoxification. Bioresource Technol 74:17–24

Pereira ARB, Soares CRFS, da Silva JF, da Silva M, Chalfoun SM (2014) Removal of trace element by isolates of *Aspergillus brasiliensis* EPAMIG 0084 and *Penicillium cirtinum* EPAMIG 0086 in biofilters. African J Biotech 13:3759–3773

Pleissner D, Venus J (2014) Agricultural residues as feedstocks for lactic acid fermentation. In: Obare SO, Luque R (eds) Green technologies for the environment, American Chemical Society, pp 247–263

Reddy C, Mathew Z (2002) Bioremediation potential of white rot fungi. In: Gadd G (eds) Cambridge University Press, Cambridge, UK

Sayara T, Sarra M, Sanchez A (2010) Effects of compost stability and contaminant concentration on the bioremediation of PAHs-contaminated soil through composting. J Hazard Mater 179:999–1006

Sethunathan N, Megharaj M, Chen ZL, Williams BD, Lewis G, Naidu R (2004) Algal degradation of a known endocrine disrupting insecticide, α-endosulfan, and its metabolite, endosulfan sulfate, in liquid medium and soil. J Agric Food Chem 52:3030–3035

Sharma JK, Gautam RK, Nanekar SV, Weber R, Singh BK, Singh SK, Juwarkar AA (2017) Advances and perspective in bioremediation of polychlorinated biphenyl-contaminated soils. Environm Sci Pollut Res 1–21. https://doi.org/10.1007/s11356-017-8995-4

Shetty PK, Mitra J, Murthy NBK, Namitha KK, Savitha KN, Raghu K (2000) Biodegradation of cyclodiene insecticide endosulfan by *Mucor thermohyalospora* MTCC 1384. Curr Sci 79:1381–1383

Siddique T, Benedict CO, Muhammad A, William TF (2003) Biodegradation kinetics of endosulfan by *Fusarium ventricosum* and a *Pandoraea* sp. J Agric Food Chem 51:8015–8019

Singh BK, Walker A, Morgan JAW, Wright DJ (2003) Effects of soil pH on the biodegradation of chlorpyrifos and isolation of a chlorpyrifos-degrading bacterium. Appl Environ Microbiol 69:5198–5206

Singh BK, Walker A, Morgan JAW, Wright DJ (2004) Biodegradation of chlorpyrifos by *Enterobacter* strain B-14 and its use in bioremediation of contaminated soils. Appl Environ Microbiol 70:4855–4863

Singh H (2006) Mycoremediation: fungal bioremediation. Wiley-Interscience, New York, NY, USA

Singh R, Singh P, Sharma R (2014) Microorganism as a tool of bioremediation technology for cleaning environment: a review. Proc Int Acad Ecol Environ Sci 4:1–6

Strong PJ, Burgess JE (2008) Fungal and enzymatic remediation of a wine lees and five wine-related distillery wastewaters. Bioresour Technol 99:6134–6142

Sun L, Wu Sh (2007) Analysis and thought about nationwide general investigation on hazardous waste disposal facilities. Nonferrous Met Eng Res 28:7–17

Sutherland JB, Rafii F, Khan AA, Cerniglia CE (1995) Mechanisms of polycyclic aromatic hydrocarbon degradation: microbial Transformation and degradation of toxic organic chemicals. Wiley-Liss, New York

Tang CY, Criddle QS, Fu CS, Leckie JO (2007) Effect of flux and technique. Biol Med 1:1–6

Thibault GT, Elliott NW (1979) Accelerating the biological cleanup of hazardous materials spills. In: Proceedings oil and hazards materials. Spills: prevention-control cleanup-recovery-disposal

Timmis KN, Pieper DH (1999) Bacteria designed for bioremediation. Trends Biotechnol 17:201–204

Walker JD, Colwell RR, Vaituzis Z, Meyer SA (1975) Petroleum degrading achlorophyllous alga *Prototheca zopfii*. Nature 254:423–424

Widdowson MA (2004) Modeling natural attenuation of chlorinated ethenes under spatially varying redox conditions. Biodegradation 15:435–451

Yan X, Yang Y, Li Y, Sheng G, Yan G (2002) Accumulation and biodegradation of anthracene by *Chlorella protothecoides* under different trophic conditions. Chin J Appl Ecol 13:145–150

Yang XQ, Zhao XX, Liu CY, Zheng Y, Qian SJ (2009) Decolorization of azo, triphenylmethane and anthraquinone dyes by a newly isolated *Trametes* sp. SQ01 and its laccase. Process Biochem 44:1185–1189

Zhang H, Hu C, Jia X, Xu Y, Wu C, Chen L, Wang F (2012) Characteristics of c-hexachlorocyclohexane biodegradation by a nitrogen-fixing cyanobacterium *Anabaena azotica*. J Appl Phycol 24:221–225

Zhou M, Liu Y, Zeng G, Li X, Xu W, Fan T (2007) Kinetic and equilibrium studies of Cr (VI) biosorption by dead *Bacillus licheniformis* biomass. World J Microbiol Biotech 23:43–48

Ziagova M, Dimitriadis G, Aslanidou D, Papaioannou X, Tzannetaki EL, Liakopoulou-Kyriakides M (2007) Comparative study of Cd(II) and Cr(VI) biosorption on *Staphylococcus xylosus* and *Pseudomonas* sp. in single and binary mixtures. Bioresour Tech 98:2859–2865

Chapter 12
Phytoremediation Techniques for the Removal of Dye in Wastewater

B. Bharathiraja, J. Jayamuthunagai, R. Praveenkumar and J. Iyyappan

Abstract Phytoremediation attempts to use plants and microbes associated with plant root systems to protect the environment by removal of pollutants in the form of inorganic and organic wastes. Phytoremediation is capable of treating pollutants of dye waste, which are derived from various sources. Adaptation in genetic levels is the basic attitude behind plants that able to manage the contaminants from polluted site. Various classifications of phytoremediation are discussed in this chapter. Among the various pollutants, textile dyes and effluents are identified to most predominant pollutants of our environment. Treatment of textile dyes using plant remains an unfamiliar area of research. Mechanisms of uptake of different dyes by plants have also been proposed. Selection criteria of plants for achieving high efficiency of treatment of dye contaminant wastewater have been projected. Use of *Lemna minor L.* plant with pond system in warmer regions has shown significant removal of Basic Red 46 dye. Different plants such as *Tecoma stans* var. *angustata*, *Scirpus grossus,* water hyacinth *Eichhornia crassipes,* aquatic plant *Spirodela polyrrhiza* have also been discussed for their potential of dye degradation. Consortium of *Petuniagrandiflora* and *Gailardia grandiflora* plants has been established for their role in dye degradation. Combined technology involving plant-associated micro-organisms with *Medicago sativa* L. and *Sesbania cannabina Pers* have also been proposed. Various impacts of azo dye on living organism have been discussed.

Keywords Phytoremediation · Cost-effective · Textile dyes Effluents · Azo dyes

B. Bharathiraja (✉) · J. Iyyappan
Vel Tech High Tech Dr. Rangarajan Dr. Sakunthala Engineering College, Avadi, Chennai 600062, Tamilnadu, India
e-mail: btrbio@gmail.com

J. Jayamuthunagai
Centre for Biotechnology, Anna University, Chennai 600025, Tamilnadu, India

R. Praveenkumar
Department of Biotechnology, Arunai Engineering College, Tiruvannamalai 606603, Tamilnadu, India

S. J. Varjani et al. (eds.), *Bioremediation: Applications for Environmental Protection and Management*, Energy, Environment, and Sustainability,
https://doi.org/10.1007/978-981-10-7485-1_12

12.1 Introduction

Nowadays, nearly 100,000 synthetic dyes are produced commercially and available in market, almost one million tons of dyes are produced annually in which about 10% of dyes are released as waste into environment and natural resources (Jadhav et al. 2010). The reason behind increased production is to meet the present needs of growing population, and also this scenario creates increased release of dye effluent, which is quite harmful to the environment. Commercially, azo dyes are the most important classes of dye stuffs owing to their superior tinctorial strength, easy preparation, cheap and easy availability of raw materials, abilities to cover the whole shade range and good fastness properties. Other classes of dyes include anthraquinones, phthalocyanines, aryl-carboniums and polymethines. Applications of dyes include in industries like textile, plastic, paper, concrete, medicine and rubber, with textile industry as the main consumer of dyes (Beenish et al. 2015). The disposal of these coloured substances results in one of the major problems for industry in wastewater treatment, due to the various aspects of discharged coloured wastes like damage to the aesthetic nature of the receiving channels, toxic to aquatic life and even carcinogenic or mutagenic in nature (Puvaneshware et al. 2006).

Removal of coloured substances from wastes is crucial, because the existence of very fine particle size of dyes (below 1 ppm) is clearly visible and influences water environment significantly. Reduction in the dissolved oxygen (DO) level and increased level of the biochemical oxygen demand (BOD) in dye-contaminated water body are the major adverse effects on aquatic life forms. Most of dyes are structurally complex, nonbiodegradable and stable to oxidation and light and hence cannot be treated by conventional methods. This makes serious threats with numerous problems for the treatment of dye waste. Several dyes are known to be carcinogenic. Particularly, azo dyes are highly toxic and produce aromatic amines, which are carcinogenic in nature.

Several physical and chemical methods have been recommended for the treatment of dye-contaminated wastewater such as adsorption on activated carbon, ion exchange, chemical precipitation, coagulation–flocculation, oxidation, electrochemical treatment and membrane filtration (Ahmad et al. 2015). But all these methods require high cost and low efficiency and fail in the presence of varying dyes in wastewater stream. Moreover, formation of toxic by-product is quite annoying of these conventional methods. However, these methods are measured as nondestructive since they transfer the dye from liquid to solid wastes. As a result, post-treatment of solid wastes is needed, which are more expensive in operations.

All kind of contaminated water with dyes cannot be treated with one technology. Sometimes combined technology is required based on nature of dyes, impurities and composition of the wastewater. Combined techniques have been applied towards treatment of dye-contaminated water, which include combined electrochemical oxidation, microbial oxidation, electrolysis and photocatalytic oxidation serially using micro-organism during microbial oxidation. Various techniques

involving combination of electrolytic oxidation and nanofiltration, ultrafiltration and reverse osmosis have also been employed on dye removal (Vergili et al. 2012).

Anionic, cationic and nonionic are the three types of dyes available, in which they have different auxochromic and chromophoric groups (Puvaneshware et al. 2006). Anionic dyes are extremely water soluble and difficult to remove by conventional method. Treatments are not sufficient for the complete removal of nonionic dyes, because they do not ionize in an aqueous form and need more concentrate to remove from the contaminated site. Instead of using physical and chemical methods, biological methods of treating such wastewater containing dyes are considered highly valuable owing to their eco-friendly nature, energy-saving nature, effectiveness and minimum usage of chemicals. The principle behind biological treatment is the conversion of dye wastes into simpler and harmless contents in the course of biological processes by various micro-organisms. Aerobic and anaerobic processes are conducted for wastewater treatment. The organisms exploited can be bacteria, fungi, algae or plants. Carbon dioxide, water and biomass are the final products of aerobic treatments, while anaerobic treatment produces carbon dioxide, methane and biomass.

Phytoremediation is one the biological treatments having more attention towards the treatment of dye-contaminated sites due to cost-effective technology, effectiveness and eco-friendly technology. Phytoremediation uses live plants for the removal of dye contaminants from the sources of water or soil. In this chapter, we will discuss about importance of various plants which serve for treatment of dye waste.

12.1.1 Phytoremediation

Phytoremediation (from Greek phyto, plant, and Latin remedium, cure) refers to the exploitation of plants to remove contaminants from polluted environments. It is one of the in situ methods, eco-friendly, dropping soil erosion, improving soil fertility by increasing organic matters in soil, and uses plants for degradation, extraction, transformation or detoxification of chemical contamination (Organum and Bacon 2006). Major contaminants such as metals, solvents, pesticides, explosives, crude oil, landfill leachates and hydrocarbons can be removed by phytoremediation methods. Genetic adaptation of the plants is the reason behind for managing the contaminants from the site (Alberto and Sigua 2013). During photosynthesis process, phytoremediation can also assist in eradication of carbon dioxide from the air. Structure and biological functions of the environments can be persevered by phytoremediation. Contaminants from the accumulated site can be efficiently removed by means of the exclusion of the plant that used in the process is the main advantage of phytoremediation (Balarak et al. 2016). Phytoremediation technology has been employed using growing aquatic plants like *Phragmites australis* (Hussein and Scholz 2017) and using free-floating plants like L. minor (Uysal et al. 2014). Researchers are finding suitable trees for effective bioremediation.

12.2 Mechanisms of Phytoremediation

Removal of contaminants from its sites using plants can be made by several possible ways. Plants can eliminate pollutants by acting as filters or traps from soil, sediment and/or water. Root system of the plants usually uptakes contaminants present in site and prevents the environment from the toxicity of contaminants (Mahar et al. 2016). Therefore, contaminants from various sources must be ready to be absorbed by roots, and this phenomenon makes suitable for the removal of dyes combined with bioavailability of dyes. The root systems of the plants absorb essential nutrients for their growth, as well as other contaminants due to adaptation.

More toxic complex organic pollutants can be degraded into simple complex, nontoxic in nature in the forms of carbon, oxygen and hydrogen. In contract, inorganic chemical substances can change their chemical structure or be transferred from one medium to another, which results in the disappearance of toxic chemical elements (Dickinson 2017). Supply of textile wastewater along with canal water mixing improved the growth and yield of field mustard (*Brassica campestris* L.) (Yaseen et al. 2017). An integrated system proved efficiency in treating coloured wastewaters, when *Prescaria barbata* plant inoculated with microbes and supplemented with rice agricultural waste showed effective removal of Reactive Black 5 dyes (Beenish et al. 2015).

12.3 Phytoremediation Process

Phytoremediation can be broadly classified based on fundamental processes, applicability and type of contaminant as (Wang et al. 2017):

- Phytodegradation: It includes uptake, storage and breakdown of pollutants with the help of secreted enzymes from plant tissue.
- Phytoextraction: It consists of absorption and store toxic contaminants in their root, stems and leaves.
- Phytostimulation or rhizodegradation: It uses symbiotic soil micro-organisms present in the rhizosphere and degrades contaminants.
- Phytovolatilization: It comprises the uptake of contaminants from the growth matrix of plants and succeeding discharge of volatilized contaminants into the atmosphere.
- Rhizofiltration: It occurs in the roots of plants to uptake also stored contaminants from an aqueous growth matrix.
- Phytostabilization: It involves plant-mediated immobilization of contaminant in soil by root absorption or precipitation of pollutants inside the roots of plants; thereby, reduction in bioavailability of contaminants occurs with controlled migration of contaminant from water erosion, soil dispersion and leaching.

The following table summarizes the treatment of various contaminants by using respective plants (Table 12.1) (Sureshvarr et al. 2010).

12.3.1 Selection of Plants for Remediation of Textile Dyes

Preliminary step involved in phytoremediation research is the selection of suitable plant with required uniqueness. Though many plants show ability to remediate

Table 12.1 Types of phytoremediation with media, contaminants and typical plants

Application	Media	Contaminants	Typical plants
Phytodegradation	Soil, groundwater, landfill leachate, land application of wastewater	Herbicides (atrazine, alachlor); aromatics (BTEX); chlorinated aliphatics (TCE); nutrients; ammunition wastes (TNT, RDX)	Phreatophyte trees (poplar, willow, cottonwood, aspen); grasses (rye, bermuda, sorghum, fescue); legumes (clover, alfalfa, cowpeas)
Phytoextraction	Soil, brownfields, sediments	Metals (Pb, Cd, Zn, As, Cu, Cr, Se, U) with EDTA addition for Pb, selenium	Sunflowers; Indian mustard; rapeseed plants; barley, hops; crucifers; serpentine plants; nettles, dandelions
Rhizodegradation	Soil, sediments, land application of wastewater	Organic contaminants (pesticides aromatic and polynuclear aromatic hydrocarbons	Phenolics releasers (mulberry, apple, osage orange); grasses with fibrous roots (rye, fescue, bermuda); aquatic plants for sediments
Phytovolatilization	Soil, groundwater, landfill leachate, land application of wastewater	Herbicides (atrazine, alachlor); aromatics (BTEX); chlorinated aliphatics (TCE); nutrients; ammunition wastes (TNT, RDX)	Phreatophyte trees (poplar, willow, cottonwood, aspen); grasses (rye, bermuda, sorghum, fescue); legumes (clover, alfalfa, cowpeas)
Phytostabilization	Soil, sediments, metals	(Pb, Cd, Zn, As, Cu, Cr, Se, U), hydrophobic organics (PAH, PCB, DDT, dieldrin)	Phreatophyte trees to transpire large amounts of water (hydraulic control); grasses to stabilize soil erosion; dense root systems are needed to sorb/bind contaminants

contaminated sites, nonedible plants are mostly preferred onto the dye-contaminated soils. Since phytoremediation technology of textile dyes involves removal of dyes by either degradation of the dye or the adsorption and/or accumulation of the dye. Compounds accrued in the plant roots can be further translocated to shoots and leaves. Nonedible plants are always preferred to avoid from entering dye compounds or their metabolites from entering the food chain.

We should aware of chemical structure and molecular weight of species, as well as the presence or absence of sulpho groups present in dye contaminants present in wastewater. Then only we can achieve high-efficient phytoremediation process. Since selection of plants for phytoremediation mainly depends on these factors of substances present in polluted environment (Mishra et al. 2015). Other operational parameters such as pH, contact time, dye concentration, biomass absorption and temperature also affect the efficiency of phytoremediation. Finally, better growth rate of plants in contaminated sites, bulky specific surface area in contact with water, potential of increased translocation, factors like bioconcentration factor (BCF) and translocation factor (TF) defines the success of the treatment methods (Nazir et al. 2011) of dye contaminants using phytoremediation.

12.3.2 Phytoremediation of Textile Dyes

Nowadays, many researchers are trying to demonstrate about unfamiliar part of phytoremediation particularly on the removal of textile dye. Phytoremediation research containing the removal of inorganic compounds like metals has accomplished the usage of transgenetic plants with improved phytoremediation capability of plants. The removal of dyes by using plant systems is still limited due to less knowledge about the basic mechanisms and processes involved in it. Phytoremediation research towards removal of dyes by plants provides a new dimension.

Textile industries are the major sources for dye wastewater and have significantly increased the values of biochemical oxygen demand, suspended solid particles and unfavourable pH (Yaseen and Scholz 2016). Different types of weeds, ferns, grasses or agricultural wastes have been recommended the removal of contaminated pollutants. Native populations of *Phragmites australis* have been widely studied for the remediation of textile effluents and mainly with respect to the removal of the dye, Acid Orange 7.

Yaseen and Scholz (2017) reported the ability of *Lemna minor L.* plant with pond system in warmer regions to remove the dye Basic Red 46 even at low concentrations from wastewater containing textile dyes under controlled and semi-natural conditions (Yaseen and Scholz 2017). Typical processes of wastewater treatment using plants may include degradation, accumulation, dissipation and immobilization processes of contaminants (Wang et al. 2017).

The tolerance of *Lemna minor* plant (Anamaria et al. 2015) on two synthetic dyes, namely crystal violet and malachite green, was reported by Anamaria et al. (2015)

Stress response of *L. minor* studies proved that uptake of about 80% of crystal violet and 90% malachite green dyes was due to phytoextraction and phytodegradation. Fourier transformation infrared spectroscopy, ultraviolet-visible spectroscopy, thin-layer chromatography and energy-dispersive X-ray spectroscopy are the analytical methods that used to find the mechanisms of phytoremediation.

Nisha and Emilia (2016) reported the significant effect on biodegradation of Brilliant green, a carcinogenic and teratogenic dye, by using callus cultures of the plant, *Tecoma stans* var. *angustata*. The ability of peroxidase enzymes in the presence of hydrogen peroxide to neutralize the adverse effect of the dye through the removal of pollutants from wastewaters was scrutinized (Nisha and Emilia 2016).

Degradation of a basic dye, methylene blue (MB), from synthetic wastewaters was reported by using *Scirpus grossus* plant by means of measuring biological oxygen demand (BOD), chemical oxygen demand (COD) and total organic carbon (TOC). Effective removals in the levels of BOD, COD and TOC have been reported with varying dye concentrations in constructed wetland systems (Abdulqader et al. 2017).

Kah et al. (2016) reported that removal of methylene blue (MB) and methyl orange (MO) can be achieved by roots of water hyacinth *Eichhornia crassipes* (Kah et al. 2016). Movafeghi et al. (2015) reported the potential of aquatic plant *Spirodela polyrrhiza* for the degradation of direct blue 129 (DB129) azo dye with an artificial neural network model. Removal of DB129 has been increased with rising temperature (20 °C) and initial dye concentration at pH4 (Movafeghia et al. 2015). The produced intermediate compounds due to cleavage of DB129 were reported by gas chromatography–mass spectroscopy (GC–MS) analysis. Plant consortium has also been utilized for the treatment of toxic pollutants from textile dye mixture using *Petunia grandiflora* and *Gailardia grandiflora* with reduced levels of BOD and COD (Anuprita and Jyoti 2014).

Artificial cell cultures of *Blumea malcolmii* were reported on decolourization of variety of dyes from textile industry effluent. *B. malcolmii* were able to decolourize the malachite green with 93.41%. This study showed the involvement of laccase, veratryl alcohol oxidase and dichlorophenol indophenol reductase enzymes on the degradation of the dye (Anuradha et al. 2011).

Some research studies showed improved phytoremediation in the presence of plant microbiome, plant-associated micro-organisms, like fungi and bacteria, due to capability of transformation of organic and inorganic compounds, bioweathering, element cycling, mycogenic mineral formation and fungal–clay interactions (Zujun and Lixiang 2016). A combined technique includes role of plants and micro-organism that may also assist in degradation of certain textile dyes. Both *Medicago sativa* L. and *Sesbania cannabina Pers* plants were reported to degrade wastewater containing acid red B or acid scarlet GR dyes when combined with *Gracilibacillus* sp.GTY, a salt-tolerant bacteria (Xiaobai and Xuemin 2013).

12.4 Removal of Azo Dyes

Azo dyes are one of the predominant dyes in textile industry with a huge volume of production every year. Azo dyes contain one or more nitrogen–nitrogen double bonds (–N==N–) and are primarily recalcitrant (Rawat et al. 2016). Azo dyes are mostly carcinogenic in nature by involving three different mechanisms. First mechanism depends on aromatic amines that are released due to cleavage of azo bond, which results in covalently binding DNA with metabolically oxidized state. Second mechanism involves free aromatic amine groups commonly present in azo dyes with structures, which results in metabolically oxidized form without azo reduction, and final mechanism involves activation of azo dyes through direct oxidation of the azo bond with electrophilic diazonium salts. These three different mechanisms make complexity in the prediction of carcinogenicity of azo dyes with absolute certainty.

Degradation process of azo dye begins with breakdown of azo bond, known as decolourization. Recently, biodecolourization of azo dye has received great attention among research scientists due to inability of micro-organisms to degrade azo dye results in low efficiency of biological treatment methods. Since, metabolic activity and growth of micro-organisms are suppressed by salinity and toxicity and of azo dye wastewater. Phytoremediation is recommended as one of the treatment method for removal of dye predominantly on azo dyes, which is based on the ability of plants to stimulate biodecolourization (Xiaobai and Xuemin 2013).

12.5 Advantages of Phytoremediation

When compared to other physical and chemical methods of remediation, phytoremediation for decontamination has many advantages due to its low cost. This is mainly because the need of less expensive equipments, easy to implement and do not require talented persons to handle. This phytoremediation technology can be engaged without disturbing the site of contaminants. Permanent solution can be achieved for the sites that are continuously being polluted through phytoremediation.

References

Abdulqader S, Rozaimah S, Hassimi A, Reehan A, Mushrifah I (2017) Treatment of methylene blue in wastewater using *Scirpus grossus*. Malays J Anal Sci 21(1):182–187

Ahmad A, Siti S, Sing C, Asma K, Waseem AW, Rajeev K, Mohd R (2015) Recent advances in new generation dye removal technologies: novel search of approaches to reprocess waste water. RSC Adv 39(2015):1–58

Alberto AM, Sigua GC (2013) Phytoremediation: a green technology to remove environmental pollutants. Am J Clim Change 2:71–86

Anamaria T, Erzsébet B, Cerasella I, Szende T, Luminita S, Cornelia M (2015) Biological removal of triphenylmethane dyes from aqueous solution by *Lemna minor*. Acta Chim Slov 62:452–461

Anuprita DW, Jyoti PJ (2014) Detoxification and decolorization of a simulated textile dye mixture by Phytoremediation using Petuniagrandi flora and Gailardia grandiflora: a plant–plant consortial strategy. Ecotoxicol Environ Saf 103:1–8

Anuradha NK, Mital UJ, Vishwas AB, Sanjay PG (2011) Phytodegradation of the triphenylmethane dye Malachite Green mediated by cell suspension cultures of Blumea malcolmii Hook. Biores Technol 102:10312–10318

Balarak D, Mahdavi Y, Ghorzin F, Sadeghi S (2016) Biosorption of acid blue 113 dyes using dried Lemnaminor biomass. Sci J Environ Sci 5:152–158

Beenish S, Madeeha J, Azeem K, Irfan A, Ann D (2015) Effectiveness of rice agricultural waste, microbes and wetland plants in the removal of reactive black-5 azo dye in microcosm constructed wetlands. Int J Phytorem 17:1060–1067

Dickinson N (2017) "Phytoremediation" plants and the environment 2:327–331

Hussein A, Scholz M (2017), Dye wastewater treatment by vertical-flow constructed wetlands. Ecolog Eng 101:28–38

Jadhav JP, Phugar SS, Dhanve RS, Jadhav SB (2010) Rapid biodegradation and decolorization of Direct Orange 39 (Orange TGLL) by an isolated bacterium Pseudomonas aeruginosa strain BCH. Biodegrdation 21(3):453–463

Kah A, Norhashimah M, Jie QO (2016) Phytoremediation of methylene blue and methyl orange using *Eichhornia crassipes*. Int J Environ Sci Dev 7(10):724–728

Mahar A, Wang P, Ali A, Awasthi MK, Lahori AH, Wang Q, Li R, Zhang Z (2016) Challenges and opportunities in the phytoremediation of heavy metals contaminated soils: a review. Ecotoxicol Environ Saf 126:111–121

Mishra S, Kumar A, Shukla P (2015) Study of water quality in Hindon River using pollution index and environmetrics. India Desal Water Treat. https://doi.org/10.1080/19443994.2015.1098570

Movafeghia A, Khataeeb AR, Moradia Z, Vafaei F (2015) Biodegradation of direct blue 129 diazo dye by Spirodela polyrrhiza: an artificial neural networks modeling. Int J Phytorem 145:165–179

Nazir A, Malik RN, Ajaib M, Khan N, Siddiqui MF (2011) Hyperacumulators of heavy metals of industrial areas of Islamabad and Rawalpindi. Pak J Bot 43(4):1926–1933

Nisha R, Emilia A (2016) A potential tissue culture approach for the phytoremediation of dyes in aquaculture industry. Biochem Eng J 115:23–29

Organum N, Bacon F (2006) Bioremediation technologies. In: Alvarez PJJ, Illman WA (eds) Bioremediation and natural attenuation. John Wiley & Sons, New Jersey, pp 351–455

Puvaneshware N, Muthukrishnan J, Gunasekran P (2006) Toxicity assessment and microbial degradation of azo dyes. Indian J Exp Biol 44:618–624

Rawat D, Mishra V, Sharma RS (2016) Detoxification of azo dyes in the context of environmental processes. Chemosphere 155:591–605

Sureshvarr K, Barathiraja B, Jayakumar M, Jayamuthunagai J, Balaji L (2010) Removal of azo dye compounds from paper industries wastes using phytoremediation methodology. Int J Chem Sci 8(1):687–700

Uysal Y, Aktas D, Caglar Y (2014) Determination of colour removal efficiency of Lemna minor L. from industrial effluents. J Environ Protect Ecol 15:1718–1726

Vergili I, Kaya Y, Sen U, Gonder B, Aydiner C (2012) Techno-economic analysis of textile dye bath wastewater treatment by integrated membrane processes under the zero liquid discharge approach. Resource Conserv Recycl 58:25–35

Wang L, Ji B, Hu Y, Liu R, Sun W (2017) A review on in situ phytoremediation of mine tailings. Chemosphere. https://doi.org/10.1016/j.chemosphere.2017.06.025

Xiaobai Z, Xuemin X (2013) Effect of different plants on azo-dye wastewater biodecolorization. Procedia Environ Sci 18:540–546

Yaseen D, Scholz M (2016) Shallow pond systems planted with Lemna minor treating azo dyes. Ecolog Eng 94:295–305

Yaseen DA, Scholz M (2017) Comparison of experimental ponds for the treatment of dye wastewater under controlled and semi-natural conditions. Environ Sci Pollut Res 24:16031–16040

Yaseen M, Muhammad Z, Aqleema K, Muhammad N (2017) Management of textile wastewater for improving growth and yield of field mustard (Brassica campestris L.). Int J Phytorem 145:256–275

Zujun D, Lixiang C (2016) Fungal endophytes and their interactions with plants in phytoremediation: a review. Chemosphere 225(2016):1751–1758

Chapter 13
Phenol Degradation from Industrial Wastewater by Engineered Microbes

Ravichandran Rathna and Ekambaram Nakkeeran

Abstract Over the centuries, a tremendous increase in human population has placed a demand for industrial growth, which enhanced the disposal of wastewater and resulted in developing a sustainable technology for wastewater treatment. Phenol and its derivatives are the most pondered substantial pollutants generated from pharmaceutical industries, basic organic chemical manufacturing industries, petroleum refineries, petrochemical industries, coal gasification operations, liquefaction process, tannery, pesticide manufacturing industries, and pulp and paper mills, which contain harmful pollutants that are toxic and carcinogenic in nature. Accumulation of phenol even at a lower concentration may be fatal to all living beings in the ecosystem. This chapter includes the overview of phenol pollution, deleterious effects of phenol in the ecosystem, biodegradation of phenols, and significance of engineered microbes for phenol degradation.

Keywords Phenolic compounds · Wastewater · Toxicity · Biodegradation Genetically engineered microbes

13.1 Introduction

The advent of the industrial revolution has manifested an incredible strain on Earth's ecology and dramatically improved the living standards of human life. Rapid urbanization and environment pollution are subjugating each other rather stay apace. The increasing environment pollution ultimately threatens the future quality of living standards. These complications urge the scientific communities to develop an efficient, reliable, eco-friendly, and viable remediation means to protect

R. Rathna · E. Nakkeeran (✉)
Research Laboratory, Department of Biotechnology, Sri Venkateswara College of Engineering (Autonomous), Sriperumbudur 602117, Tamil Nadu, India
e-mail: nakkeeran@svce.ac.in

R. Rathna
e-mail: rathnaravi.v@gmail.com

S. J. Varjani et al. (eds.), *Bioremediation: Applications for Environmental Protection and Management*, Energy, Environment, and Sustainability,
https://doi.org/10.1007/978-981-10-7485-1_13

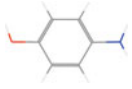
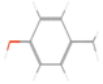
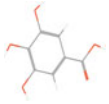
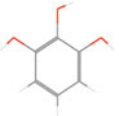
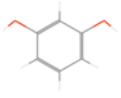
the ecosystem and benefit the society (El Fantroussi and Agathos 2005). Effluents generated from industries, municipalities, and agriculture contain varying concentration of noxious pollutants that exude into the water bodies (rivers, lakes, and sea) which gets bioaccumulated in the ecosystem (Abdel-Raouf et al. 2012; Abdel-Satar et al. 2017). As per UN World Water Development Report 2017, about 80% of world's wastewater is released into the environment without treatment and warns low-income countries to improve its wastewater management system and protect the public health and environment from damage. Industrial and mining activities generate a variety of chemical compounds that may pollute air and water which in turn has unintended negative consequences on living beings. Effluents generated from the municipality, industrial, and agriculture may contain harmful organic chemicals, such as hydrocarbons (El Nemr et al. 2016), heavy metals (Balkhair and Ashraf 2016), and phenolic compounds (Sihem and Lehocine 2012), and have the potential to enter the ecosystem and cause adverse effect.

Phenolic compounds are the widespread aromatic organic compound in the ecosystem and originate from the natural source and anthropogenic activities (Lakshmi and Sridevi 2015). Phenolic compounds (e.g., polyphenols, flavonoids, hydroxycinnamic acid) are known for its antioxidant activity, and they have a significant role in food industries such as antibrowning, flavoring, and nutraceutical agent (Ho 1992). Phenol and few of its derivatives are classified as Priority Pollutant under the Clean Water Act, the Environmental Protection Agency (Keith and Telliard 1979). The European Commission Directive on Environmental Quality Standards (Directive 2008/105/EC) also classified several phenolic compounds as priority contaminants (Salgueiro-González et al. 2012). The physiochemical properties of phenolic compounds are illustrated in Table 13.1 (PubChem Database).

Phenolic compounds are the most common and major pollutant generated in industrial effluents of pharmaceutical industries (Siripattanakul-Ratpukdi 2014), basic organic chemical manufacturing industries (Paisio et al. 2013), petroleum refineries (Wang et al. 2017), petrochemical industries (Nickheslat et al. 2013), coal gasification operations (Timourian et al. 1982), liquefaction process (Si et al. 2016), tannery (Paisio et al. 2014), pesticide manufacturing industries (Michałowicz and Duda 2007), and pulp and paper mills (Tripathi et al. 2014). According to International Market Analysis Research and Consulting (IMARC), phenol global market has increased at an average annual rate of 4% since 2008–2015 and reached a market value of 17.1 billion US dollars. Currently, Manufactures uses cumene peroxidation process for the simultaneous production of phenol and acetone (Zakoshansky 2007). Globally, almost 50% of the phenol produced is especially used in bisphenol-A (BPA) polycarbonate resins and phenol-formaldehyde resin industry. In the present scenario, Asia Pacific has fastest-growing phenol market, while Europe, the leading exporter and importer, and North American have a flat market.

In 1939, Nazis used phenol injection for mass human extermination during the Second World War. These phenolic compounds are highly toxic, teratogenic, and carcinogenic in nature (Michałowicz and Duda 2007), yet at permissible limit it can be used as a precursor in analgesic (aspirin) (Jack 1997), active ingredients in

Table 13.1 Physicochemical properties of phenolic compounds

Parameters	Compounds									
	Aminophenol	Butylated hydroxytoluene	Catechol	Coumaric acid	Cresol	Gallic acid	Nitrophenol	Phenol	Pyrogallol	Resorcinol
Molecular formula	C_6H_7NO	$C_{15}H_{24}O$	$C_6H_6O_2$	$C_9H_8O_3$	C_7H_8O	$C_7H_6O_5$	$C_6H_5NO_3$	C_6H_5OH	$C_6H_3(OH)_3$	$C_6H_6O_2$
Chemical structure										
Molecular weight (g/mol)	109.13	220.36	110.11	164.16	108.13	170.12	139.11	94.11	126.11	110.11
Physical state	Solid	Solid	Solid	Solid	Solid	Solid	Solid	Liquid	Crystalline powder	Solid
Odor	Amine-like weak odor	Slight powder	Sweet, fruity odor	Odorless	Phenolic like odor	Odorless	Sweet	Sweet, fruity odor	Odorless	Characteristics
pH	10	Not available	Not available	Not available	Not available	Not available	5.6	4.6–4.5	Not available	4–6
Solubility in water	Soluble	Insoluble	Soluble	Partially soluble	Partially soluble	Partially soluble	Partially soluble	Soluble	Soluble	Soluble
Specific gravity	1.328	1.03–1.04	1.344	Not available	1.047	0.6–0.7	1.48	1.057	1.453	1.292
Boiling point (°C)	284	265	245	Not available	191.5	Not available	279	182	309	281
Melting point (°C)	172	70	103–106	214	30	251	113.5	42	133–134	110

antipruritic compositions (Swinehart 1999), mixed with lidocaine to reduce pain (Shabana 2013), anesthetic (Chloraseptic) (Ho and Hollinrake 1989), chemotherapeutic agents (Kramer et al. 1955), antiseptic (Parachlorophenol) (Spangberg et al. 1973) and disinfectant (phenol) (Dico et al. 1989). Few approved non-prescription/over-the-counter (OTC) medicines as listed in Table 13.2. Phenolic compounds enter the living system through protein coagulation (Jain et al. 2006). When used at 5–10% concentrations, it has the ability to produce chemical neurolytic agent (neuh-ro-LIT-ick) which can have local anesthetic effects within 5–10 min (Jain et al. 2006). These phenolic compounds are also found to have drug interactions with hyaluronidase that acts as a radiopharmaceutical diagnostic imaging agent approved by Food and Drug Administration (FDA), USA, and technetium Tc-99 m tilmanocept that acts as a catalyst to promote absorption and distribution of other injected drugs.

The ubiquitous presence of phenolic compounds in the environment has been extremely reported and discussed in the works of literature. Exposure estimate by World Health Organization (WHO) for phenol intake in drinking water, smoked food, and air is 0.6 mg/day, 2 mg/day, and 4 mg/day, respectively (US Environmental Protection Agency 1992). As per European Commission Directive 2017/774, the admissible concentration for phenol in food materials is reduced from 1.5 mg/kg body weight per day to 0.5 mg/kg body weight/day. According to WHO and US Environmental Protection guidelines, the maximum admissible level of phenols in domestic and drinking water is 0.001 mg/L. According to water guidelines of Australia 2011, the permissible level of 2-Chlorophenol is 0.3 mg/L. In Japanese Water Pollution Control Law, phenolic compound level should be lower than 0.005 mg/L in drinking water. As per Canadian water quality guidelines, maximum acceptable level of phenol is 4 μg/ml. In the case of Bureau of Indian Standards (BIS), 105000 Standard for drinking water maximum desirable level of phenolic compounds is 0.001 mg/L.

Increasing awareness of phenol pollution in the environment by industrial effluents has attracted researchers for its effective removal. Phenol treatment methods are classified into physical, chemical, and biological based on the mode of action as shown in Fig. 13.1. Physical methods used for removing phenolic compounds are liquid–liquid extraction, pervaporation, filtration, adsorption, cloud point extraction method, etc. These methods are stumbling block because it is applicable to the low concentration of solutes, high capital is required, disposal of sludge after the process is difficult and membrane contamination is found (Lakshmi and Sridevi 2015). Using chemical methods, phenolic compounds are disrupted by the chemical processes such as electrocoagulation, photocatalysis, electrolytic oxidation. The main drawback of the above methods are high installation cost, incomplete removal of phenol, high-energy requirement, applicable to specific phenolic compounds, and generation of toxic sludge (Lakshmi and Sridevi 2015). These complications have led to the development of an indispensable method for effective removal of phenolic compounds from industrial wastewater. As an alternative, biological methods used for phenol treatment found to be favorable by completely removing the toxic chemicals economically. Waste management

Table 13.2 Few approved phenol-containing non-prescription/over-the-counter medicine

Drug name	Route of administration	Dosage	Purpose of use	Brand name	Marketed year	Chemical ingredient
Castellani	Topical	15 mg/ml	Infection	Pedinol Pharmacal, Inc	1972	Resorcinol, Phenol
Sore throat	Oral	1.4 g/100 ml	Anesthetic/ Analgesic	Hyvee	1992	Phenol
Good neighbor pharmacy sore throat	Oral	1.4 ml/100 ml	Analgesic	AmerisourceBergen	1994	Phenol
Throat relief	Oral	10.628 mg/ 100 ml	Anesthetic/ Analgesic	Western Family Foods	1999	Phenol
Equaline sore throat	Oral	1.4 g/100 ml	Anesthetic/ Analgesic	Supervalu	2004	Phenol
Healthy accent sore throat	Oral	–	Analgesic	DZA Brands	2008	Phenol
Cepastat	Oral	14.5 mg	Anesthetic	Insight Pharmaceutical	2009	Phenol
Phenol spray	Oral	1.4 mg/ml	Anesthetic/ Analgesic	Purine Pharma LLC	2015	Phenol
Sore throat relief cherry	Oral	1.4 g/100 ml	Anesthetic/ Analgesic	Cardinal Health Leads	2017	Acetyl aminophenol
Pain–A–Cherry	Tropical	1.4%	Anesthetic/ Analgesic	Lee Pharmaceuticals	Not known	Phenol and derivatives

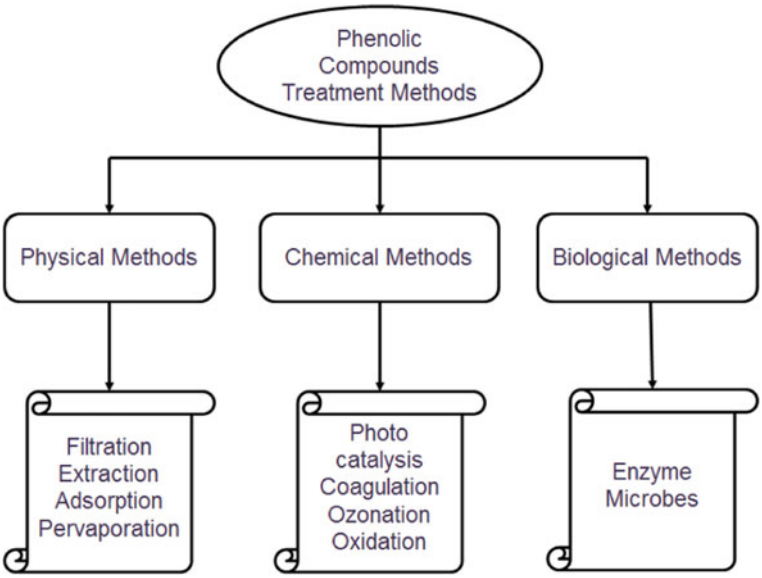


Fig. 13.1 Various methods used for phenol treatment

through microorganisms (bioremediation) found to be safe and cost-effective than the other methods. Microorganisms are used to break down the harmful contaminates through metabolic pathways and biochemical reactions. The advent of genetic engineering and the limitations of the conventional methods used for phenol treatment lead to the designing of genetically modified microorganisms (GMOs) possessing metabolic potential for cleanup process. This chapter fosters the overview of phenol pollution, deleterious effects of phenol in the ecosystem, biodegradation of phenols, and significance of engineered microbes for phenol degradation.

13.2 Manifestation of Phenol Pollution

Phenol is used vastly in various large-scale industries derived from chlorobenzene and sodium hydroxide. It is a colorless compound with feasible solubility range both in aqueous and in organic solvents. Phenol is mainly used in the production of cresols, xylenols, resins (phenolic), aniline, phenol-substituted alkyl products, in processing oil, coal, and metallurgy, as an intermediate compound in the production of bombs, weapons, explosives, and consumer product productions such as textile dyes, pesticides used for crop protections. Environmentally, phenol and its derivatives get dispersed in the atmosphere through exhaust expelled from vehicles

(Mulawa and Cadle 1981), and disinfectants and reagents used in chemical industries and laboratories.

Phenol and its intermediate derivatives have an anthropogenic origin and discharge as industrial effluents into the ecosystem as harmful exotoxins. In addition, phenols are produced by natural processes using effluents as their main source and are substituted with various chemical molecules that are present in the municipal sewage or water channels and reach the seawater. These free end phenols react with several accessible atoms that are released as waste in the living ecosystem. They are alkylated, methylated, nitrated, sulfonated, or chlorinated to form hazardous toxic derivatives. Hydroxybenzene-based phenolic derivatives produced even from natural resources through biosynthesis process that occurs in plants and its by-products of decomposed organic matters (Michałowicz and Duda 2007). These particular derivatives formed in plants that are rich in cellulose and hemicellulose, which produces amino acids with the influence of ultraviolet (UV) irradiation from sunlight. These substances cause deleterious effects to the flora and fauna present in the environment. The condensed water vapors that form clouds contain phenolic compound due to atmospheric chemical reactions (Falsig et al. 2006). These phenolic derivatives not only pollute the water systems and ocean resources through seawater channels but also affect the soil condition, dispersed in air. Therefore, it directly or indirectly affects the nature, living organisms, and human beings with deleterious effects.

13.2.1 Aromatic Alcohol

Chloro-aromatic compounds are introduced into the environment in large quantities through natural and industrial sources. Catechol (1,2-dihydroxybenzene) is an aromatic alcohol, which has the hydroxyl radical on the first and the second carbon positions. Catechols and chlorinated catechols formed because of degradation of chlorobenzenes (Spain and Nishino 1987) and chlorinated phenoxyacetics (Tiedje et al. 1969), respectively. Catechols and chlorocatechols are used as industrial reagents for photography, in the production of plastics, dyes, fur, rubber, cosmetics, and pharmaceuticals (Michałowicz and Duda 2007). They are also used in the production of synthetic material, insecticide, and drug synthesis. Chlorocatechols mostly occur in polluted water of evolutionary origin and in natural origin but present in low concentrations. The investigation of water samples from the polluted Ner River showed abundant toxic tetrachlorocatechol at a concentration of 0.002 ppm (Michałowicz and Duda 2007). Largest cities like Poland also showed low concentration of 4-chlorocatechol and 3,4,5-trichlorocatechol in drinking water.

Sulfonamides are used in the treatment of infections and also used as anti-inflammatory and analgesic drugs (Makhija et al. 2013). All dimers of aminophenols are used as a dye intermediate in coloring hair. The derivatives such as N and P aminophenols are used as a marker in the antibacterial drugs, lubricant, and oil (Michałowicz and Duda 2007). Particularly, humans working at a high level

of exposure to aniline are engaged in the production and processing of rubber industry.

The annual production of butylated hydroxytoluene (BHT) is approximately 62000 tons per year from the sources of industrial effluent and waste combustion. BHT and butylated hydroxyanisole (BHA) are usually used in the petroleum products and synthetic materials and act as an antioxidant which has the capability of preventing the materials from oxidation (Michałowicz and Duda 2007). The contents of BHT in the environment is low, and the samples for analysis of the content of BHT in the rivers of Germany reported that the concentration of compounds is 0.00002–0.00016 ppm.

13.3 Phenolic Compounds Toxicity Data

Phenol exposure largely found as a by-product effluent in industrial scale. Cumene-containing plants were widely used for extraction and production of coal tar. Due to high-quantity transformation of cumene phytoconstituents to produce coal tar, exposure to the phenolic compound was also increased. Phenol was the first compound that was checklisted out as the Priority Pollutant by the government of US Environment Protection Agency (US EPA). The exposure of phenol in the occupational group was found to be 580,000 in the USA due to phenol influence (Michałowicz and Duda 2007). The discharge of phenolic compound-based industrial effluents in the environment caused river water pollution in the Netherlands. The effluent discharged from petrol plant through sewage and drained in river water ended up with phenol pollution. The concentration reached more than 40 mg/L, whereas the amount of phenol content in natural water should range between 0.01 and 2.0 μg/L. The domestic water in the USA was found about 1 μg/L of phenol content. Phenol is exposed to living organisms not only through inhalation; the amount of phenol content is high in certain foods. Food such as grilled pork sausages has alarming concentrations of phenol such as 7–28.6 μg/kg; smoked meat has 37–70 mg/kg of phenol content in its outer layer. The daily exposure of phenol found to be 10–240 mg/person, which can lead to increased frequency of diarrhea, mouth ulcers, and darkened urination.

The exposure of phenolic compounds is more toward the phenol product occupation-based population. Phenolic constituents are emitted, when phenol resins subjected to high-temperature process. In Poland, about 8,000 workers are chronically exposed to phenol and phenol-derived products in the industry (Michałowicz and Duda 2007). However, compared to the industrial-based population, even general population of Poland are also affected due to high exposure and emission of phenol and other toxic compounds, e.g., Silesia. The effluents generated from medical, leather, and textile sectors contain increased concentrations of phenolic compounds to protect from various microbes. However, then other adverse effects are found due to chronic exposure of these compounds such as o-phenylphenol, trichlorophenol, and p-chloro-m-cresol and existence of benzene in vehicle

exhausts, gasoline, and cigarette fumes. When humans exposed to phenol vapors chronically, it leads to various symptoms and risks such as muscular weakness, anorexia, and icterus. Phenol, when absorbed into the human body through any mode, accumulated mainly in the liver, brain, and kidneys. Although these phenols are eliminated from the body after two days, they remain unchanged, but the accumulated phenols conjugate with sulfates and glucuronides. Insufficient data are available to predict the short-term and long-term effects of phenolic compounds on plants, birds, or land animals. Recent research revealed the accumulation of phenolic compound in the milk of grazing goats (Silanikove et al. 2010). Plants near the site of contaminants found to accumulate phenolic compounds, especially near coal tar. Phenolic compounds found to accumulate in the chicken flesh at 2–3 μg/kg (Michałowicz and Duda 2007).

Phenol toxicity is not only based on the dosage and concentration exposed, but it also depends on individual tolerance and their lethal dosage that slightly or significantly varies from one to the other. Reports have already revealed that even after administrating 30 g of phenol (60 mL, 50%), an individual adult was able to survive, but a simple exposure of a small portion of skin in forehand (60–90%) resulted in the death of an individual by initial penetration and necrosis (Hammam et al. 2015). Catechols, chlorophenols, pentachlorophenol, 2,4,5-tri chlorophenol, 2-chlorophenol, 2,4-dinitrophenol, and 4-methylphenol are some of the toxic phenolic compounds that have adverse toxic effects. Exposure of these phenolic compounds leads to acute poisoning with several signs and characteristics of skin and functional organ necrosis and decreases temperature and muscle weakness.

13.3.1 Deleterious Effects on Ecosystem

Phenolic compounds released from industrial wastewater first affect the aquatic ecosystem and slowly influence the soil. In general, phenolic acids are known to influence the nutrient cycling and organic matter degradation in soils (Min et al. 2015). Constituents of phenolic compounds accumulated in the soils due to industrial effluents are treated by biodegradation and leaching (Min et al. 2015). The leached phenols slowly percolate the soil and contaminate the hydrospheres, which in turns accumulates in aquatic organisms, when consumed by secondary and tertiary consumers it causes the negative impact in the living system. Phenolic compounds are toxic to fish and shellfish at parts-per-million and parts-per-billion level of concentration, respectively (Chen et al. 2010; Enguita and Leitão 2013). European Inland Fisheries Advisory Commission reported that phenol toxicity is intensified by the increase in salt concentration with decreased temperature and dissolved oxygen content. Few experimental fishes and its ecotoxicity are shown in Table 13.3. It is found that phenolic compounds enter aquatic organisms and disturbances its metabolic functions, impairs its growth and survival rate, damages the cells and tissues, and affects its reproduction (Saha et al. 1999).

Table 13.3 Ecotoxical information of phenol and its derivatives

Compounds	Organisms	Lethal concentration 50 (mg/L)	Exposure period (h)
Phenol	*Carassius auratus*	125	24
Phenol	*Pimephales promelas*	50	1
m-cresol	*Oncorhynchus mykiss*	8.9	96
m-cresol	*Salvelinus fontinalis*	7.6	96
m-cresol	*Daphnia magna*	18–8	48
BHA	*Oryzias latipes*	5	48
Resorcinol	*Pimephales promelas*	88.6	24
Nitrophenol	*Pimephales promelas*	27	96
Nitrophenol	*Daphnia magna*	35	24
Nitrophenol	*Oncorhynchus mykiss*	3.80–18.00	96
Nitrophenol	*Cyprinodon variegatus*	26.70–31.30	96
Aminophenol	*Oncorhynchus mykiss*	1.2	96
Aminophenol	*Daphnia magna*	0.2	48
Aminophenol	*Carassius auratus*	2	48

The chronic exposure of phenol *Anthocidaris crassispina* and *Perna viridis* showed impaired sperms mobility and accompanied with curtailed fertilization (Au et al. 2003). At a concentration of 0.1 mg/L, phenol affects the sperm quality in sea urchins, thereby affecting its survival rate. Research studies revealed that embryonic development of fishes was affected by phenol even less than 25 mg/L and, for prawns, it is 20–30 times sensitive (Law and Yeo 1997). Prawns found to bioaccumulate phenols that could accelerated by the presence of chromium in the seawater and vice versa (Qixing and Limei 1995). The histopathological effects of phenol on gastropods showed irreversible necrotic change (ultimately may lead to death) and reversible (Lajtner et al. 1996). The larval settlement of the barnacle *Balanus amphitrite darwin* was inhibited by phenolic compounds (Lau and Qian 2000). Phenolic compounds are also known for its antiseptics or disinfectants nature. Diatoms/microalgae are the primary producers in an ecosystem whose sensitivity is higher than the other primary and secondary producers in the aquatic ecosystem. The median effective concentration of phenol on *Dunaliella salina*, *Platymonas subcordiformis*, *Phaeodactylum tricornutum Bohlin*, and *Skeletonema costatum* was 72.29, 92.97 and 27.32 mg/L, respectively (Duan et al. 2017).

Concentration of phenolic compounds differs from one surface to another due to natural or man-made activities. The government of Poland evaluated phenolic pollution to analyze the presence of phenolic effluents and their rate of exposure. The industrial wastewater drained into sewage reached river Dzierzazna and revealed the presence of phenolic content as 0.01–2.0 μg/L. Ner River from central Poland was strongly polluted by the industrial effluents and reported that the amount of phenol content was low compared to its derivative compound. The

concentration of chlorophenol discharged in the ecosystem was found to be more than 2 μg/L, 6 μg/L of 2,4-dichlorophenol in Vistula River, 0.1–6.0 μg/L in Gulf of Gdansk, 2 μg/L of tetrachlorocatecol in Ner River, 0.028–1.22 μg/L in Odra River. It was further concluded that the landscape of Poland has very low and considerable amount of phenol. But it was found very high with strong occurrence in the industrialized areas and urbanized Silesia regions of Poland as 3.8–26.6 $\mu g/m^3$.

13.3.2 Impact and Fate of Phenolic Compounds on Humans

It is well known that phenol and its derivatives when released as industrial waste effluent are more toxic in nature, which can lead to deleterious effects in living organisms by systemic poisoning, especially to the human population. Phenols are generally corrosive and cause burns if get contacted; e.g., skin exposed to phenol causes necrosis to the cells. Further, these compounds affect the central nervous system (CNS) by systemic poisoning and the transient stimulation of CNS is in slow pace initially and rapidly increases CNS depression (Pope 2006). Depending on the concentration and amount of phenol exposed, it can cause coma and seizures in human within minutes or delayed but within 18 h of exposure (Agency for Toxic Substances and Disease Registry (ATSDR) 2014). In this particular duration, the subject can prone to several symptoms based on the concentration of phenol exposed. The symptoms express phenol presence in the body by causing preliminary signs of vomiting, diarrhea, profuse sweating, and hypotension and further lead to some complicated signs such as pulmonary edema, tachycardia, cardiac arrhythmia, and bradycardia.

Phenol is a low volatile compound, which limits the inhalation hazard compared to volatile toxins. If phenols exposed as effluent to the environment directly, the lungs absorb them rapidly in a short period. Exposure of phenol is more dangerous to children compared to adults because of the surface of the area of lungs, body weight, height, and diameter of the air way inside the lungs. Phenolic vapors are heavier than atmospheric air (ATSDR 2014). Therefore, children are more vulnerable to these corrosive agents because they are very close to the ground level than the adult due to their short stature.

All forms of phenol and its derivatives due to their corrosive in nature can cause toxic effects and irritation to the site of the skin that is in contact. However, a minimal diluted concentration of prolonged exposure also causes severe burns (1–2%). The absorption efficiency of phenol through skin contact and penetration is approximately equal to the absorption efficiency of phenol inhaled. Initial phenol exposure causes blood pressure elevation and suddenly puts down to shock with low blood pressure. Children are more susceptible to toxicants, and it gets absorbed easily and cause vulnerable effects to the skin exposed to phenol.

The bioavailability of phenolic compounds in humans is still a query and misery because few studies showed that phenolic compounds undergo extensive first-pass metabolism but in contrast. Dickinson and Taylor (1996) reported that phenol and 1-naphthol do not undergo pulmonary first-pass metabolism. There are only few literatures reported on the absorption and metabolism of phenolic compounds via lung and gastrointestinal tract. In some species, phenolic compounds are found to be conjugated with sulfate or glucuronic acid to form phenyl sulfate and phenyl glucuronide, respectively. The mechanism of phenol metabolism and its pathway in both in vitro and in vivo revealed that phenol and its metabolic derivatives bind covalently to the proteins present in plasma and tissues.

Major route of phenol elimination in animal as well in human is through renal excretion. The frequency and rate of phenol excreted through urine vary from one species to the other depending on the dosage exposed, rate of concentration, route of exposure. An investigation is carried out with three human male specimens to understand the rate of phenol and its derivatives elimination and excretion. It was found that when 0.01 mg/kg of phenol was orally administered, within 24 h about 90% of the administered dose was eliminated through urinary excretion as phenyl glucuronide and phenyl sulfate. In case of phenol vapor inhalation or topical administration, it is excreted with rate constant of 0.2/h. Phenol excreted as urine may have its color with green tint, which is due to the oxidation of quinones. About 52% eliminates of the phenol, and its derivatives remain unchanged in urine due to its half-life, which remains between 1 and 4.5 h.

13.4 Biodegradation of Phenols

Physicochemical methods such as advanced oxidation process, photocatalytic, electrolytic oxidation, solvent extraction, chemical oxidation, or integration of these methods to treat phenolic compounds in industrial effluents were effective, but it generates toxic sludge and is expensive and complex. Hence, there was a need to develop eco-friendly and economically effective technology to degrade phenolic constituents. Bioremediation mainly involves transformation, fragmentation, and conversion of the complex xenobiotics into simpler compounds, which are non-toxic (Krastanov et al. 2013). The literatures reported that the phenolic constitutes, carbon source, present in the environment are degraded by microbes. However, the survival rate of the microbes greatly depends on the concentration of xenobiotics. For the past few decades, scientific research mainly focused on microbial degradation of phenolic compounds. Researchers isolated potential microorganisms with adaptation mechanism that can biotransform the xenobiotics in the contaminated sources. Scientific studies have promulgated microbes such as bacteria, fungi, yeast, and algae with physiological and genetic basis to degrade phenols and its derivatives. Catabolic mechanism of phenol is illustrated in Fig. 13.2.

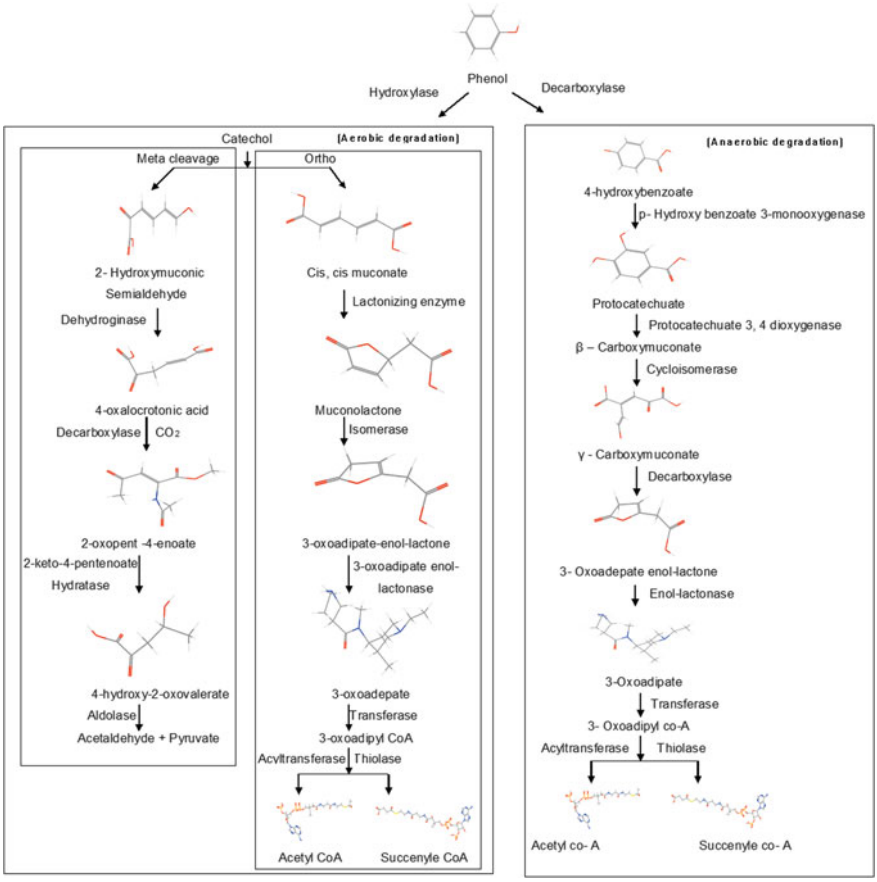


Fig. 13.2 Catabolic pathway for phenol degradation

Acinetobacter calcoaceticus strain isolated from petroleum wastewater eliminated 91.6% from 800 mg/L initial concentration of phenol in 48 h with a tolerance rate of 1700 mg/L (Liu et al. 2016). *Acinetobacter* sp. has the ability to degrade the phenolic compounds via meta-pathways (Ahmad et al. 2016). *Achromobacter xylosoxidans* Ns isolated from wetland settlement can tolerate p-nitrophenol up to 1.8 mM which could be degraded in 1.6 h (Wan et al. 2007). *A. xylosoxidans* was able to degrade 3-nitrophenol through 4-nitrocatechol and 1,2,4-benzenetriol as the key intermediates which was found similar to ortho-cleavage pathway (Wan et al. 2007). Autochthonous microorganism, *Bacillus pumilus,* isolated from contaminated groundwater of Germany was able to utilize cresol via oxidation of the methyl substituent, which cleaved through intradiol ring fission and led to 4-methyl mucono lactone as dead end product (Günther et al. 1995). The metabolize pathway was found similar to toluene metabolism in *Pseudomonas aeruginosa*. *Bacillus cereus* sp. degraded phenol via 2-hydroxymuconic semialdehyde as intermediate

compound through the meta-cleavage pathway (Banerjee and Ghoshal 2010). It was predicted that the experimental data followed Haldane inhibitory model (Banerjee and Ghoshal 2010). The metabolism of phenolic compounds is largely investigated in *Pseudomonas* sp., which is known for its ability to metabolize phenolic structure into a simple carbon source. Table 13.4 represents *Pseudomonas* strains capable of degrading phenolic compounds.

Fungi are known for its capacity to degrade complex products such as lignin, chitin, and cellulose. It is easily adaptable to extreme conditions and suitable as sorbent. The fungi degrade phenolic compounds through ortho- and para-hydroxylation pathways. *Aspergillus niger* was able to degrade 300 mg/L of phenol concentration at pH 7.2, 35 °C, and 120-h incubation period (Supriya and Neehar 2014). Fifteen strains such as *Aspergillus* sp., *Fusarium* sp., *Graphium* sp., *Penicillium* sp. isolated from Brazilian stainless-steel industrial effluents tolerate a concentration of 10 mM phenol (Santos and Linardi 2004). The genera Graphium found to be best among filamentous fungi isolated for phenol degradation via the β-ketoadipate pathway. *Aureobasidium* sp. has the potential to metabolize phenol using catechol 1,2-dioxygenase enzyme, and the tolerance limit was found to be 15–18 mM (dos Santos et al. 2009). *Candida maltosa* used phenol (1.7 g/L), catechol (1.5 g/L), and resorcinol (2 g/L) as carbon and energy source and had the ability to co-metabolize p-cresol, but benzoate or salicylate could not be metabolized (Fialova et al. 2004). *Ankistrodesmus braunii* and *Scenedesmus quadricauda i*solated from olive oil mill wastewater were able to remove 70% of 400 mg/L phenol (Pinto et al. 2002). *Ochromonas danica* was able to convert phenol to catechols by meta-cleavage pathway (Semple and Cain 1996).

13.4.1 Genetic Engineering in Biodegradation

The isolation of diverse microbes present in the polluted environment has the ability to degrade the pollutants. However, the degradation rate and adaptation to the pollutant of these microbes are relatively slow. The works of the literature reported that the accumulation of the pollutant in microbes during the process of bioremediation may be hazardous to nature. The novel structural entity of pollutants makes it complex for microorganisms to degradation by its own ability. Evolution of microbes with catabolic pathway is rather slow or inappropriate than the rate of contamination by pollutants in the environment (Urgun-Demirtas et al. 2006). Microbes evolved for degradation of these harmful pollutants are not vital to biotransform of these compounds by the existing pathways. The scientific advancement in molecular biology, genetic, and biochemical methods has led to the development of novel strain with desired metabolic pathway with modified substrate affinity, degradation of a range of substrates, cellular localization, and expression level using recombinant DNA technology (Urgun-Demirtas et al. 2006). The microbes engineered in their genetic material for its desired application using

Table 13.4 *Pseudomonas* strains capable of degrading phenolic compounds

Pseudomonas species	Isolated/Procured	Phenolic compound(s) depredated	Concentration (mg/L)	Time period for degradation (h)	References
Pseudomonas fluorescens PU1	Soil	Phenol	998	48	Mahiudddin and Fakhruddin (2012)
Pseudomonas aeruginosa MTCC 4997	Petrochemical industrial	Phenol	1400	144	Kotresha and Vidyasagar (2012)
Pseudomonas cepacia KK01	Termite intestines	Phenol and cresol	–	–	Kato et al. (2000)
Pseudomonas fluorescence	Oil-polluted area	Phenol	100–500	84–354	Agarry and Solomon (2008)
Pseudomonas putida EKII	Soil	Phenol	1000	38	Hinteregger et al. (1992)
Pseudomonas putida MTCC 1194	Microbial type culture collection and gene bank (MTCC)	Phenol	1000	162	Kumar et al. (2005)
		Catechol	500	94	
Pseudomonas sp. strain ADP	Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSM 11735)	Phenol	1,000	9	Neumann et al. (2004)

this technology are known as the genetically engineered microorganisms. The various techniques involved in biodegradation are illustrated in Fig. 13.3.

Bioremediation technique is more advantageous and economical than other convention methods. Figure 13.4 shows the steps involved in biodegradation using GMOs. Recalcitrant xenobiotics are non-biodegradable chemical compounds present in the environment due to pesticides, insecticides, herbicides, synthetic plastics, etc., that cannot be degraded by the microorganism and pollute the environment over a long period. Microorganisms do not have the capacity to produce enzymes for degradation of these large inert substances. In general, microbes can transform noxious compounds into non-toxic simple end products through co-metabolism process which is a non-beneficial biotransformation. Initially, genetically engineered microbes (GEM) are used for oil spill treatment in oceans by modifying its genetic material for utilizing the toxic organic chemicals.

In the present scenario, GEMs are applied in a variety of areas such as medicine, agriculture, food and nutrition, bioremediation, and other industries for the benefit of human society. GEMs have been developed based on the desired area of interest by the recognition of the certain genetic sequences responsible for the action and manipulating the gene for the desired function. This technology could improve the degradability of wide range of xenobiotics that are hazardous to the environment.

The first step is involved in the design and construction of the GEM based on the wild microbe ability to degrade the xenobiotics through its genetic material with the biochemical mechanisms, pathways, and enzymes. GEMs are the highly efficient

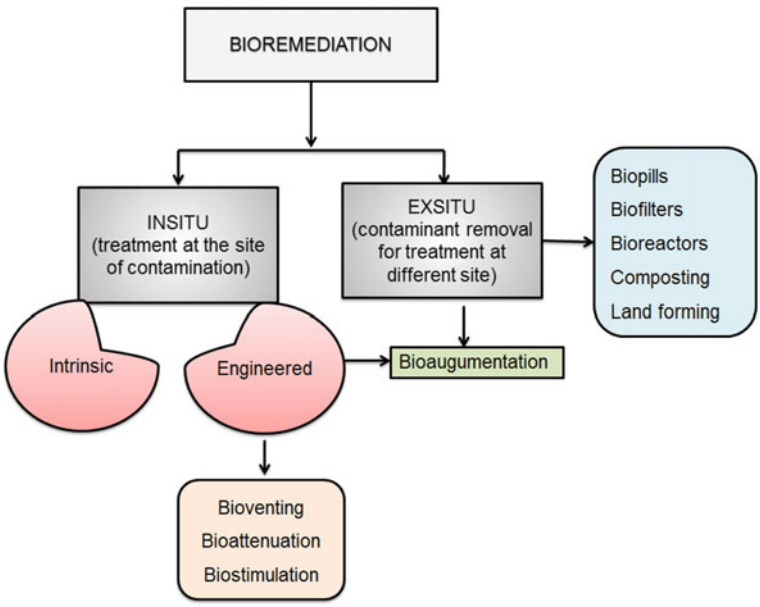


Fig. 13.3 Bioremediation techniques

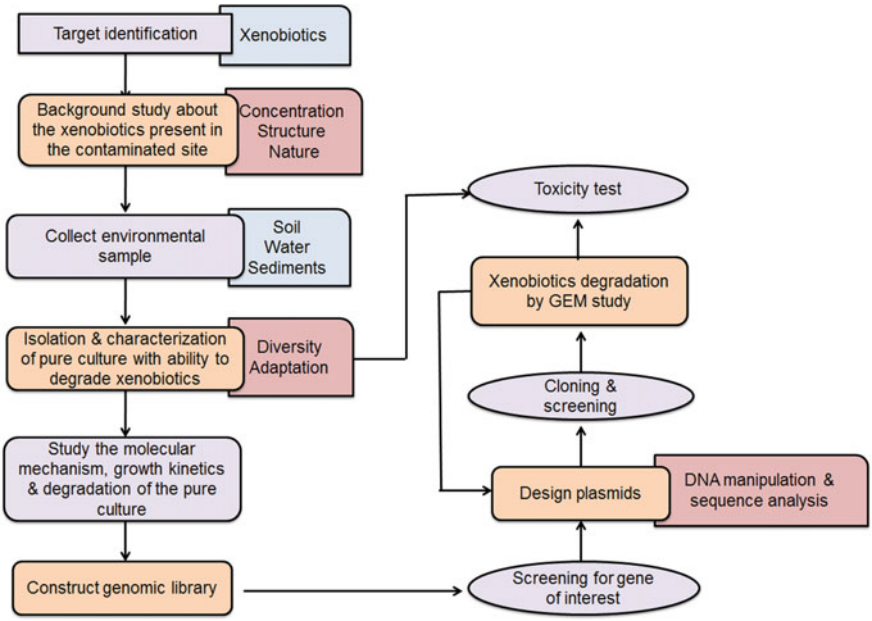


Fig. 13.4 Steps involved in biodegradation using GMOs

successor or alternative to wild microbes that slowly degrade the xenobiotics. Genes associated with degradation of xenobiotics are available through genome library. Figure 13.5 shows the various approaches using GMOs for bioremediation applications.

13.5 Engineered Plasmids for Phenol Treatment

Phenol and its derivatives are first hydroxylated to di-hydroxylated benzene ring catechol by phenol hydroxylase which is metabolized into acetyl-CoA and succinate (ortho-cleavage pathway—intradiol cleavage) or acetaldehyde and pyruvate (meta-cleavage pathway—extradiol cleavage). *Pseudomonas* sp. strain is largely studied since it is capable of oxidizing aliphatic and aromatic compounds. Soil microorganism *Pseudomonas* sp. strain CF600 is known for its ability to catabolize phenol and methyl substituted phenols (cresols, and 3,4-dimethylphenol) by the set of enzymes encoded by the plasmid located *dmp* operon (Nordlund et al. 1990). A conjugative catabolic plasmid pVI150 encodes all the genetic information related to biodegradation of aromatic compounds, which is similar to OCT (degrades octane, hexane, and decane) and CAM (decomposes camphor) plasmid. Phenolic compounds are dissimilated by multicomponent enzyme phenol hydroxylase followed by extradiol cleavage. Plasmid pVI150 belongs to Inc P-2 incompatibility

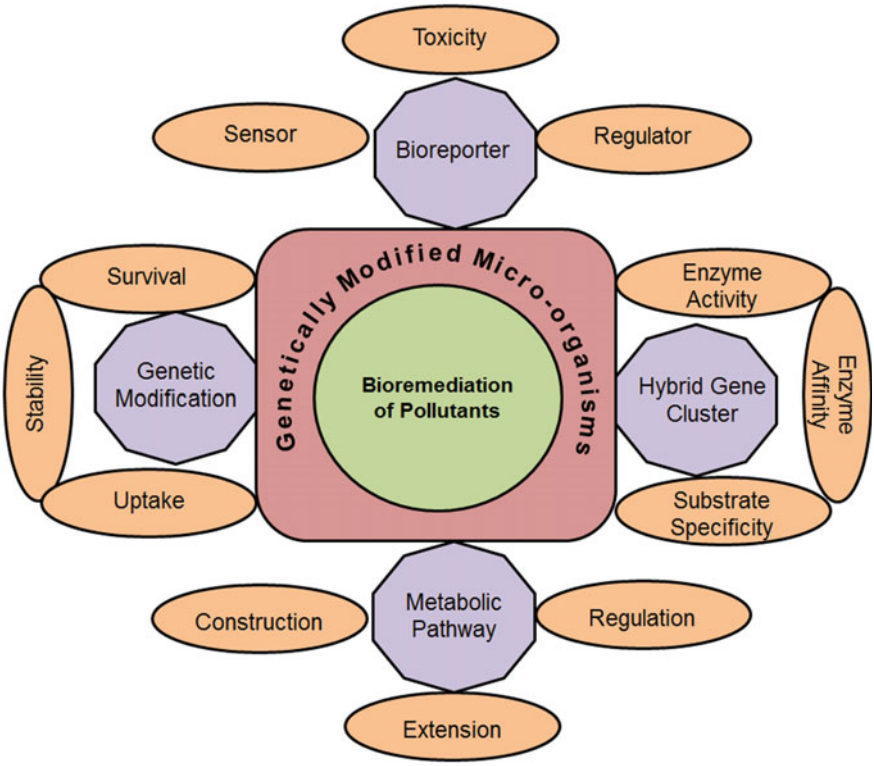


Fig. 13.5 Approaches for the development of GMOs for bioremediation applications

group, which has the potential resistant (drugs, mercurial, metal complexes, and ultraviolet light), inhibition (phage growth and fertility of other plasmids), and degradation (degradation of camphor and alkanes) capacity (Fennewald et al. 1978). *Pseudomonas* sp. strain ADP bacteria help in metabolizing toxic phenolic compounds and a xenobiotic atrazine, which was regulated by RpoN (sigma factor 54) (Neumann et al. 2004). *Pseudomonas* strain PB2701 found to express phenol hydroxylase activity (5.5-kb SacI-NruI fragment) and further metabolized by the enzymes of the chromosomally encoded ortho-cleavage pathway (Neumann et al. 2004). Knowledge on the phenol catabolic genes in *Rhodococcus erythropolis* CCM2595 helped in developing GEM with improved phenol degrading capacity (Zídková et al. 2013).

Ralstonia eutropha strain E2 isolated from oil-refinery sludge, which has the ability to degrade phenol by meta-cleavage pathway, but *P. aeruginosa* PAOlc do not have the ability to degrade phenol but could degrade catechol (Hino et al. 1998). Cloning of catechol meta-cleavage activity of *Escherichia coli* JMl09 was able to transform phenol to 2-hydroxymuconic semialdehyde via catechol and reported that phenol hydroxylase cloned from strain E2 exhibits the novel kinetic properties as in

intact cells of strain E2. Expression of phenol degradative function in *P. putida* Paw strains was controlled by two regions in the 17-kb transposon from the host chromosome into the plasmid carrying these genes (Kivisaar et al. 1990). *Pseudomonas* sp. are reported with two key enzymes, phenol monooxygenase and catechol 1,2 dioxygenase genes, that play a central role in the metabolism of aromatic compounds through ortho-cleavage pathways. *Pseudomonas putida* strain H with pPGH1 of 200–220 kb molecular mass plasmid carried three genes coded for metabolizing enzymes (phenol hydroxylase, metapyrocatechase, 2-hydroxymuconic semialdehyde) responsible for initiation of phenol catabolism via the meta-cleavage pathway (Herrmann et al. 1987). *Bacillus stearothermophilus* BR219 with catabolic genes pheA (conversion of phenol to catechol) and pheB (catechol to 2-hydroxymuconic semialdehyde) was cloned in *E. coil* and found that the expression of phenol hydroxylase present in the recombinant pPH229 influenced strongly by cresols than phenol (Kim and Oriel 1995). *P. pickettii* PKO1 isolated from a soil microcosm has the ability to utilize phenol as a carbon and energy sources, whose genomic DNA bank was constructed in *P. aeruginosa* PAO1c by using vector plasmid pRO1727 and identified phlA and phiR gene-encoding phenol hydroxylase and transcription of phlA (Kukor and Olsen 1990). Substrate-specific (phenol and m-cresol) *P. aeruginosa* PAO1c was engineered with plasmid pRO1957 for phenolic compound degradation. Genetically engineered *Pseudomonas* sp. JS150 had the enhanced capacity to bioaugment the phenol present in the soil and found to be stable throughout the experiments; hence, it is considered as a future potential strain to bioaugment the phenol-contaminated areas (Mrozik et al. 2011).

13.6 Risk Assessment in Genetic Engineering

GEMs could be used for successful degradation of hazardous xenobiotics released into the environment by the industries. Findings from the basic sciences to degrade xenobiotics using engineered microbes are known for practical translation. The major issue with GEM is survival and gene transfer of recombinant microbe (Wasilkowski et al. 2012). Mrozik et al. (2015) reported that the stability of GEM is higher than the wild strain. The risk is omnipresent, and it needs to be managed and assessed for effective use of technology. Therefore, prior to the release of GEM into the surrounding, its chromosomal analysis, gene number, functional properties of genes, gene flow, interactions, the viability of GEM, the technology used for transformation, allergenicity, and toxicity studies have to be assessed and studied. Based on these studies, scientific communities develop a novel and safe strategy. One such example is the design of suicidal genetically engineered microorganisms developed by exploring the antisense RNA that has the ability to do programmed cell death after degradation of xenobiotics (Joutey et al. 2013). The possibility of gene transfer for GEM to other microorganisms present in the surrounding could be avoided by using transposons (Wasilkowski et al. 2012). Recently, field study was carried out using bioluminescence-producing GEM for bioremediation, which helps in assessing the spread of microbes (Urgun-Demirtas et al. 2006).

13.7 Regulatory Affairs

The US EPA regulates certain genetically modified microorganisms that are intended for pesticidal purposes as bioremediation agents. Toxic Substances Control Act (TSCA), 1977, also helps in reviewing the researches conduct using GMOs under the Biotechnology Rule 40 in the field of bioremediation (Wozniak et al. 2012). In 1993, the Canadian government established the Federal Regulatory Framework for Biotechnology, which defines biotechnology and regulates all of its applications including microbes used for bioremediation. In India, Regulatory Framework notified under the Environment Protection Act, 1986, referred as Rules 1989, regulates GMOs and related products. In particular, Industrial and Environmental Applications Branch (IEAB) regulates GMO used for the industrial purpose from production to bioremediation. United Nations Environmental Programme (UNEP) developed Protocol to the Convention of Biological Diversity to regulate GMOs, or living modified organisms (LMO), or products of modern biotechnology. In European Union (EU), the release of GMO into the environment is regulated by European Economic Community (EEC) directives 90/220 (deliberate release directive) and EEC directives 90/219 (contained use directive).

13.8 Conclusions

In this contemporary world, treatment and management of waste are still an issue with all kinds of sophisticated technology. Wastewater from industries, municipalities, and agriculture has noxious pollutants which are released into the environment even after the treatment. Of many pollutants, phenolic compounds are found to be the common hazard pollutant in the wastewater. Extensive researches have been carried out for removing phenol and its derivatives using physical and chemical methods, but bioremediation by microbes found to be the best method. Since they have the ability to rapidly cleanse and purify the environment and protect the ecosystem balance, microorganism isolated from the site of contamination found to degrade the phenolic compound slowly and its survival rate at high concentration phenolic compounds is low or negligible. Based on these complications and development to superbug to treat oil spills, it led to the development of GEMs that degrade phenolic compounds efficiently and economically. However, the applications of these GEMs for phenolic compounds degradation are still in laboratory scale because of the instability of GEMs and gene transfer. These critical issues can be rectified in the near future by enhancing the knowledge on the use of GEM. Further studies on GEM based on genomic structure, gene functions, biochemistry mechanism, metabolic pathway, and toxicity would help to transform from laboratory to translational research. However, there are stringent regulations and laws globally for regulating the release of the engineered microbes into the environment. This technique has the ability to reduce toxic pollutants in wastewater generated from industries and helps toward the resilient and sustainable future.

References

Abdel-Raouf N, Al-Homaidan AA, Ibraheem IBM (2012) Microalgae and wastewater treatment. Saudi J Biol Sci 19(3):257–275

Abdel-Satar AM, Ali MH, Goher ME (2017) Indices of water quality and metal pollution of Nile River, Egypt. Egypt J Aquat Res 43(1):21–29

Agarry SE, Solomon BO (2008) Kinetics of batch microbial degradation of phenols by indigenous *Pseudomonas fluorescence*. Int J Environ Sci Technol 5(2):223–232

Agency for Toxic Substances and Disease Registry (ATSDR) (2014) Medical management guidelines for phenol. https://www.atsdr.cdc.gov/mmg/mmg.asp?id=144&tid=27

Ahmad SA, Shamaan NA, Syed MA, Khalid A, Ab Rahman NA, Khalil KA, Dahalan FA, Shukor MY (2016) Meta-cleavage pathway of phenol degradation by *Acinetobacter* sp. strain AQ5NOL 1. Rendiconti Lincei 1–9

Au DW, Yurchenko OV, Reunov AA (2003) Sublethal effects of phenol on spermatogenesis in sea urchins (*Anthocidaris crassispina*). Environ Res 93(1):92–98

Balkhair KS, Ashraf MA (2016) Field accumulation risks of heavy metals in soil and vegetable crop irrigated with sewage water in western region of Saudi Arabia. Saudi J Biol Sci 23(1): S32–S44

Banerjee A, Ghoshal AK (2010) Phenol degradation by Bacillus cereus: pathway and kinetic modeling. Biores Technol 101(14):5501–5507

Chen H, Yao J, Wang F, Zhou Y, Chen K, Zhuang R, Choi MM, Zaray G (2010) Toxicity of three phenolic compounds and their mixtures on the gram-positive bacteria *Bacillus subtilis* in the aquatic environment. Sci Total Environ 408(5):1043–1049

Dickinson PA, Taylor G (1996) Pulmonary first-pass and steady-state metabolism of phenols. Pharm Res 13(5):744–748

Dico CL, Caplan YH, Levine B, Smyth DF, Smialek JE (1989) Phenol: tissue distribution in a fatality. J Forensic Sci 34(4):1013–1015

dos Santos VL, de Souza Monteiro A, Braga DT, Santoro MM (2009) Phenol degradation by *Aureobasidium pullulans* FE13 isolated from industrial effluents. J Hazard Mater 161(2):1413–1420

Duan W, Meng F, Lin Y, Wang G (2017) Toxicological effects of phenol on four marine microalgae. Environ Toxicol Pharmacol 52:170–176

El Fantroussi S, Agathos SN (2005) Is bioaugmentation a feasible strategy for pollutant removal and site remediation. Curr Opin Microbiol 8(3):268–275

El Nemr A, Moneer AA, Ragab S, El Sikaily A (2016) Distribution and sources of n-alkanes and polycyclic aromatic hydrocarbons in shellfish of the Egyptian Red Sea coast. Egypt J Aquat Res 42(2):121–131

Enguita FJ, Leitão AL (2013) Hydroquinone: environmental pollution, toxicity, and microbial answers. BioMed Res Int 2013

Environmental Health Criteria 161: Phenol, US Environmental Protection Agency, Cincinnati, OH (1992)

Falsig H, Gross A, Kongsted J, Osted A, Sloth M, Mikkelsen KV, Christiansen O (2006) Uptake of phenol on aerosol particles. J Phys Chem A 110(2):660–670

Fennewald M, Prevatt W, Meyer R, Shapiro J (1978) Isolation of Inc P-2 plasmid DNA from *Pseudomonas aeruginosa*. Plasmid 1(2):164–173

Fialova A, Boschke E, Bley T (2004) Rapid monitoring of the biodegradation of phenol-like compounds by the yeast *Candida maltosa* using BOD measurements. Int Biodeterior Biodegradation 54(1):69–76

Günther K, Schlosser D, Fritsche W (1995) Phenol and cresol metabolism in *Bacillus pumilus* isolated from contaminated groundwater. J Basic Microbiol 35(2):83–92

Hammam AM, Zaki MS, Yousef RA, Fawzi O (2015) Toxicity, mutagenicity and carcinogenicity of phenols and phenolic compounds on human and living organisms [A review]. Adv Environ Biol 9(8):38–49

Herrmann H, Janke D, Krejsa S, Kunze I (1987) Involvement of the plasmid pPGH1 in the phenol degradation of *Pseudomonas putida* strain H. FEMS Microbiol Lett 43(2):133–137

Hino S, Watanabe K, Takahashi N (1998) Phenol hydroxylase cloned from strain E2 exhibits novel kinetic properties. Microbiology 144(7):1765–1772

Hinteregger C, Leitner R, Loidl M, Ferschl A, Streichsbier F (1992) Degradation of phenol and phenolic compounds by *Pseudomonas putida* EKII. Appl Microbiol Biotechnol 37(2):252–259

Ho CT (1992) Phenolic compounds in food. ACS Symp Ser 506:2–7

Ho SL, Hollinrake K (1989) Acute epiglottitis and Chloraseptic. BMJ: Brit Med J 298(6687):1584

Jack DB (1997) One hundred years of aspirin. Lancet 350(9075):437

Jain P, Dutta A, Sood J (2006) Coeliac plexus blockade and neurolysis: an overview. Indian J Anaesth 50(3):169–177

Joutey NT, Bahafid W, Sayel H, El Ghachtouli N (2013) Biodegradation: involved microorganisms and genetically engineered microorganisms. Biodegradation-life of Science. InTech, Rijeka, pp 289–320

Kato K, Kozaki S, Imamura T, Sakuranaga M (2000) U.S. patent No. 6,096,530. U.S. Patent and Trademark Office, Washington, DC

Keith L, Telliard W (1979) ES&T special report: priority pollutants: I—A perspective view. Environ Sci Technol 13(4):416–423

Kim IC, Oriel PJ (1995) Characterization of the *Bacillus stearothermophilus* BR219 phenol hydroxylase gene. Appl Environ Microbiol 61(4):1252–1256

Kivisaar M, Ho R, Kasak L, Heinaru A, Habicht J (1990) Selection of independent plasmids determining phenol degradation in *Pseudomonas putida* and the cloning and expression of genes encoding phenol monooxygenase and catechol 1,2-dioxygenase. Plasmid 24(1):25–36

Kotresha D, Vidyasagar GM (2012) Degradation of phenol by novel strain *Pseudomonas aeruginosa* MTCC 4997 isolated from petrochemical industrial effluent. Int J Microbial Resource Technol 1–8

Kramer PE, Robbins ML, Smith PK (1955) Phenolic compounds as chemotherapeutic agents against poliomyelitis virus in tissue culture. J Pharmacol Exp Ther 113(3):262–271

Krastanov A, Alexieva Z, Yemendzhiev H (2013) Microbial degradation of phenol and phenolic derivatives. Eng Life Sci 13(1):76–87

Kukor JJ, Olsen RH (1990) Molecular cloning, characterization, and regulation of a *Pseudomonas pickettii* PKO1 gene encoding phenol hydroxylase and expression of the gene in *Pseudomonas aeruginosa* PAO1c. J Bacteriol 172(8):4624–4630

Kumar A, Kumar S, Kumar S (2005) Biodegradation kinetics of phenol and catechol using *Pseudomonas putida* MTCC 1194. Biochem Eng J 22(2):151–159

Lajtner J, Erben R, Klobučar GIV (1996) Histopathological effects of phenol on the digestive gland of *Amphimelania holandri* Fér. (Gastropoda, Prosobranchia). Bull Environ Contam Toxicol 57(3):458–464

Lakshmi MC, Sridevi V (2015) A review on biodegradation of phenol from industrial effluents. J Ind Pollut Control 25(1):1–15

Lau SC, Qian PY (2000) Inhibitory effect of phenolic compounds and marine bacteria on larval settlement of the barnacle *Balanus amphitrite amphitrite* Darwin. Biofouling 16(1):47–58

Law AT, Yeo ME (1997) Toxicity of phenol on *Macrobrachium rosenbergii* (de Man) eggs, larvae, and post-larvae. Bull Environ Contam Toxicol 58(3):469–474

Liu Z, Xie W, Li D, Peng Y, Li Z, Liu S (2016) Biodegradation of phenol by bacteria strain *Acinetobacter Calcoaceticus* PA isolated from phenolic wastewater. Int J Environ Res Public Health 13(3):300

Mahiudddin M, Fakhruddin ANM (2012) Degradation of phenol via meta cleavage pathway by Pseudomonas fluorescens PU1. ISRN Microbiol 2012

Makhija DT, Somani RR, Chavan AV (2013) Synthesis and pharmacological evaluation of anti-inflammatory mutual amide prodrugs. Indian J Pharm Sci 75(3):353

Michałowicz J, Duda W (2007) Phenols—sources and toxicity. Polish J Environ Stud 16(3):347–362

Min K, Freeman C, Kang H, Choi SU (2015) The regulation by phenolic compounds of soil organic matter dynamics under a changing environment. Biomed Res Int 2015:1–11

Mrozik AGNIESZKA, Swędzioł ŻANETA, Miga SEWERYN (2015) Comparative study of phenol degradation with a wild-type and genetically modified P. vesicularis (pBR322). plasmid stability and fame profiling. Environ Prot Eng 41(1)

Mrozik A, Miga S, Piotrowska Seget Z (2011) Enhancement of phenol degradation by soil bioaugmentation with *Pseudomonas* sp. JS150. J Appl Microbiol 111(6):1357–1370

Mulawa PA, Cadle SH (1981) Measurement of phenols in automobile exhaust. Anal Lett 14 (9):671–687

Neumann G, Teras R, Monson L, Kivisaar M, Schauer F, Heipieper HJ (2004) Simultaneous degradation of atrazine and phenol by *Pseudomonas* sp. strain ADP: effects of toxicity and adaptation. Appl Environ Microbiol 70(4):1907–1912

Nickheslat A, Amin MM, Izanloo H, Fatehizadeh A, Mousavi SM (2013) Phenol photocatalytic degradation by advanced oxidation process under ultraviolet radiation using titanium dioxide. J Environ Public Health 1–9

Nordlund INGRID, Powlowski JUSTIN, Shingler VICTORIA (1990) Complete nucleotide sequence and polypeptide analysis of multicomponent phenol hydroxylase from *Pseudomonas* sp. strain CF600. J Bacteriol 172(12):6826–6833

Paisio CE, Quevedo MR, Talano MA, González PS, Agostini E (2014) Application of two bacterial strains for wastewater bioremediation and assessment of phenolics biodegradation. Environ Technol 35(14):1802–1810

Paisio CE, Talano MA, González PS, Pajuelo-Domínguez E, Agostini E (2013) Characterization of a phenol-degrading bacterium isolated from an industrial effluent and its potential application for bioremediation. Environ Technol 34(4):485–493

Pinto G, Pollio A, Previtera L, Temussi F (2002) Biodegradation of phenols by microalgae. Biotech Lett 24(24):2047–2051

Pope CN (2006) Central nervous system effects and neurotoxicity. In: Toxicol of organophosphate and carbamate compounds, pp 271–291

Qixing Z, Limei D (1995) Joint effects of chromium and phenol on marine prawns (*Penaeus japonicus*). Mar Pollut Bull 31(4–12):387–389

Saha NC, Bhunia F, Kaviraj A (1999) Toxicity of phenol to fish and aquatic ecosystems. Bull Environ Contam Toxicol 63(2):195–202

Salgueiro-González N, Concha-Graña E, Turnes-Carou I, Muniategui-Lorenzo S, López-Mahía P, Prada-Rodríguez D (2012) Determination of alkylphenols and bisphenol A in seawater samples by dispersive liquid–liquid microextraction and liquid chromatography tandem mass spectrometry for compliance with environmental quality standards (Directive 2008/105/EC). J Chromatogr A 1223:1–8

Santos VL, Linardi VR (2004) Biodegradation of phenol by a filamentous fungi isolated from industrial effluents-identification and degradation potential. Process Biochem 39(8):1001–1006

Semple KT, Cain RB (1996) Biodegradation of phenols by the alga *Ochromonas danica*. Appl Environ Microbiol 62(4):1265–1273

Shabana AM (2013) Prevention of propofol injection pain, using lidocaine in a large volume does it make a difference. A prospective randomized controlled double blinded study. Egypt J Anaesth 29(4):291–294

Si BC, Li JM, Zhu ZB, Zhang YH, Lu JW, Shen RX, Zhang C, Xing XH, Liu Z (2016) Continuous production of biohythane from hydrothermal liquefied cornstalk biomass via two-stage high-rate anaerobic reactors. Biotechnol Biofuels 9(1):254

Sihem A, Lehocine MB (2012) Batch adsorption of phenol from industrial waste water using cereal by-products as a new adsrbent. Energy Proc 18:1135–1144

Silanikove N, Leitner G, Merin U, Prosser CG (2010) Recent advances in exploiting goat's milk: quality, safety and production aspects. Small Ruminant Res 89(2):110–124

Siripattanakul-Ratpukdi S (2014) Phenolic based pharmaceutical contaminated wastewater treatment kinetics by activated sludge process. J Clean Energy Technol 2(2):150–153

Spain JC, Nishino SF (1987) Degradation of 1, 4-dichlorobenzene by a *Pseudomonas* sp. Appl Environ Microbiol 53(5):1010–1019

Spangberg L, Engström B, Langeland K (1973) Biologic effects of dental materials: 3. Toxicity and antimicrobial effect of endodontic antiseptics in vitro. Oral Surg Oral Medi Oral Pathol 36 (6):856–871

Supriya C, Neehar D (2014) Biodegradation of phenol by *Aspergillus niger*. (International Organization of Scientific Research) J Pharm 4(7):11–17

Swinehart JM (1999) U.S. patent no. 5,961,997. U.S. Patent and Trademark Office, Washington, DC

Tiedje JM, Duxbury JM, Alexander M, Dawson JE (1969) 2,4-D metabolism: pathway of degradation of chlorocatechols by *Arthrobacter* sp. J Agric Food Chem 17(5):1021–1026

Timourian H, Felton JS, Stuermer DH, Healy S, Berry P, Tompkins M, Battaglia G, Hatch FT, Thompson LH, Carrano AV, Minkler J (1982) Mutagenic and toxic activity of environmental effluents from underground coal gasification experiments. J Toxicol Environ Health Part A 9 (5–6):975–994

Tripathi BM, Kumari P, Weber KP, Saxena AK, Arora DK, Kaushik R (2014) Influence of long term irrigation with pulp and paper mill effluent on the bacterial community structure and catabolic function in soil. Indian J Microbiol 54(1):65–73

Urgun-Demirtas M, Stark B, Pagilla K (2006) Use of genetically engineered microorganisms (GEMs) for the bioremediation of contaminants. Crit Rev Biotechnol 26(3):145–164

Wan N, Gu JD, Yan Y (2007) Degradation of p-nitrophenol by *Achromobacter xylosoxidans* Ns isolated from wetland sediment. Int Biodeterior Biodegradation 59(2):90–96

Wang B, Shui Y, Ren H, He M (2017) Research of combined adsorption-coagulation process in treating petroleum refinery effluent. Environ Technol 38(4):456–466

Wasilkowski D, Swędzioł Ż, Mrozik A (2012) The applicability of genetically modified microorganisms in bioremediation of contaminated environments. CHEMIK nauka-technika-rynek 1(66):817–826

Wozniak CA, McClung G, Gagliardi J, Segal M, Matthews K (2012) Regulation of genetically engineered microorganisms under FIFRA, FFDCA and TSCA. In: Regulation of agricultural biotechnology: The United States and Canada. Springer Netherlands, pp 57–94

Zakoshansky VM (2007) The cumene process for phenol-acetone production. Pet Chem 47 (4):273–284

Zídková L, Szőköl J, Rucká L, Pátek M, Nešvera J (2013) Biodegradation of phenol using recombinant plasmid-carrying *Rhodococcus erythropolis* strains. Int Biodeterior Biodegradation 84:179–184

Chapter 14
Insect Gut Bacteria and Their Potential Application in Degradation of Lignocellulosic Biomass: A Review

Rajesh Kumar Prasad, Soumya Chatterjee, Sonika Sharma, Pranab Behari Mazumder, Mohan G. Vairale and P. Srinavas Raju

Abstract Lignocellulosic biomass is most abundant in the environment. Enzymatic breakdown of lignocellulose, an important component of common waste materials, can be an essential step toward mitigating the wastes and generating biofuel. The diverse microbial community is maintained within the insect gut as per their food habit, ecological niche. Certain insects have shown tremendous enzymatic potential as a feed on lignocellulosic materials for their nutrition. In this context, scientific community has become interested to explore different insect gut microbial diversity through the advent of new technologies. The present manuscript encompasses the potential role of insect gut bacteria, aspects of colonization, and role in degradation of lignocellulosic biomass. Further, the significance of potential bacteria for harnessing the enzymes and appropriateness of application in lignocellulosic wastes degradation is also discussed in this review.

Keywords Insect gut microbiota · Cellulase enzyme · Cellulosome Lignocellulosic waste biomass

14.1 Introduction

The six-legged critters called insects belonging to the invertebrate group of animals are the most abundant multicellular organisms on the planet. They play incredible role to maintain the milieu, as Prof. EO Wilson, famous biologist, rightly said "if insects were to vanish, the environment would collapse into chaos." Class Insecta of phylum Arthropoda of the Kingdom Animalia is a widely diverse brunch that

R. K. Prasad · S. Chatterjee (✉) · S. Sharma · M. G. Vairale · P. S. Raju
Defence Research Laboratory, DRDO, Tezpur 784001, Assam, India
e-mail: drlsoumya@gmail.com

R. K. Prasad · P. B. Mazumder
Department of Biotechnology, Assam University, Silchar 788011, Assam, India

S. J. Varjani et al. (eds.), *Bioremediation: Applications for Environmental Protection and Management*, Energy, Environment, and Sustainability,
https://doi.org/10.1007/978-981-10-7485-1_14

includes a wide range species. The number of existing insect species is estimated from six to ten million (Chapman 2006) and potentially corresponds to over 90% of the diverse animal life forms on Earth (Erwin 1982). Insects, present in almost all inhabited condition, typically possess the basic body plan composed of three segments: head, thorax, and abdomen and having three pairs of jointed appendages (legs). Major groups of insect species belong to orders like Coleoptera (sheet winged, e.g., Beetles), Lepidoptera (paired wings, e.g., Butterflies), Diptera (e.g., Flies), and Hymenoptera (e.g., Bees, Ants, etc.) (Wheeler et al. 2001).

Due to diverse habitation, the food habits of insects vary widely. A number of diverse microorganisms play a major role in digestion, metabolism, and nutrition in insect gut (Russell and Moran 2005; Douglas 2011; Krishnan et al. 2014; Sudakaran et al. 2015). Typically, insect's digestive tract consists of foregut, midgut (or ventriculus), and hindgut (Chapman et al. 2013) although diversified modifications of the tract are eminently related to the adaptation to different feeding modes (Chapman et al. 2013; Engel and Moran 2013a, b). Many insects utilize the lignocellulosic material as their primary food source by degrading the complex polysaccharide using inhabiting gut bacteria and subsequently converting it into glucose monomer molecule (Sun and Scharf 2010). Gut bacteria of such insects are capable of producing various cellulolytic and ligininolytic enzymes that can break down the most abundant biological macromolecule. Lignocellulosic compounds are the basic structural components of plants. Cellulose is known as the world's most abundant organic polymer, and plants produce approximately 4×10^9 tons of cellulose per year (Irfan et al. 2012; Chatterjee et al. 2015). As they are aplenty and producing a huge biomass, having molecular complexity, are difficult to degrade (Sari et al. 2016). Due to this reason, the lignocellulosic biomass also creates environmental nuisance and pollution. However, proper utilization of the material may help in generating different products like ethanol, biogas (methane). Generally, expensive, instrument intensive various chemical and physical treatment processes are followed to convert this natural component into energy resources (Vandenbossche et al. 2014). Enzyme-based biodegradation can be a choice to develop appropriate method for proper utilization of the biomass into a productive formulation. In this context, lignocellulolytic enzymes produced by the gut bacteria of insects have been drawing interests to scientific community recently, due to its enormous scope of exploration of potential species.

The present review work encompasses the potential role of insect gut bacteria in degradation of lignocellulosic biomass. Detailed literature survey was carried out to incorporate aspects of microbial colonization in insect gut, cellulolytic enzyme production, genomic evolution, and role of insect diet in cellulase production. Further, the importance of prospective bacteria for harnessing the cellulase enzyme and appropriateness of application in lignocellulosic wastes degradation is also discussed in this review.

14.2 Insect Gut Environment

Physiochemical conditions of insect gut are important factor that affects the microbial colonization. The actively regulated lumen of different gut compartments varies in pH and oxygen availability (Dow 1992; Hyun et al. 2014). The pH of gut lumen may vary extensively in insects. Gut microbiota variation within the insect depends upon various factors like the insect order, morphological state (metamorphosis stages), gut condition that varies over life cycle of the particular insect. The aerobic or anaerobic conditions of insect gut depends upon the shape of the gut, size of insect (more anaerobic conditions in bigger insects), availability and partial pressure of oxygen in gut etc. (Elbert and Brune 1997; Hyun et al. 2014). Microbial colonization and metabolism also dynamically shape within the insect gut as per the state of different compartments.

Interestingly, the pH of lepidopteran guts is extremely alkaline (with pH around 11–12) in nature that helps them to digest tannin-rich leaves enhancing their nutrient availability within gut; however, the microbial population is very less as a consequence (Berenbaum 1980; Appel and Martin 1990; Dow 1992; Harrison 2001; Engel and Moran 2013a, b). This alkalinity is maintained through recycling of H+ into the cytoplasm by midgut electrogenic K+ pump which is energized by a H+-pumping V-ATPase and net transport of alkali metal is attained by linking it to a nH+/alkali metal exchanger; the electrical field generated by the V-type ATPase that confers high luminal pH in lepidopteran insects is explained as a model of passive (Nernstian) distribution of proton (Dow 1992). Midgut of the lepidopteran insect has the potentiality to generate pH gradient using metabolic energy and pH profiles observed along the gut is basically due to morphological difference in gut sub regions and differential acid–base transportability of midgut (Dow 1992). Similarly, Boudko et al. (2001) stated that alkaline environment in the midgut (anterior region) is dependent on V-ATPase pumps that maintain strong gradients in hydrogen ion concentrations in mosquito larvae. Less extreme pH gradients reported by Appel and Martin (1990) found in the lumens of a number of non-holometabolous insects. However, guts of few soil-feeding termite species show extreme variation with pH ranging from 5 to >12, having selective alkaline-tolerant symbiotic bacteria from Firmicutes, Clostridium, and Planctomycetes (Brune and Ohkuma 2010; Bignell 2010; Kohler et al. 2012; Engel and Moran 2013a, b). Termite guts have several hindgut compartments or paunches harboring distinct sets of microbial communities acting as bioreactors with high rates of turnover of hydrogen pools (Pester and Brune 2007; Engel and Moran 2013a, b). Microbial fermentation producing acetate, lactate, and formate are abundant in the hindgut and midgut regions in the larvae scarab beetle (*Pachnoda ephippiata*) (Lemke et al. 2003; Cazemier et al. 2003).

14.3 Microbial Colonization Within Insect Gut

In holometabolous insects, four phases (egg, larvae, pupa, and adult) are eminent in the life cycle, which takes place through metamorphosis of molting and demolting (Moll et al. 2001; Minard et al. 2013). During the phases of metamorphosis, the microbial habitat of insect gut is affected considerably. While, during molting, insect midgut, constantly renewing peritrophic matrix along with microbial population; however, due to peeling of exoskeleton at foregut and hindgut, they are subjected to significant changes in terms of microbial population (Fukatsu and Hosokawa 2002; Minard et al. 2013; Engel and Moran 2013a, b). Moll et al. (2001) reported that during metamorphosis in some insects (as, e.g., mosquito) total to near total eradication of the gut bacteria takes place (Moll et al. 2001; Engel and Moran 2013a, b). Habitat of insects and the source of food consumed by them also affect the gut microflora colonization (Oliver 2003; Douglas 2015). Again, many insects, in their adult stages, represent specialized crypts or paunches that support microbial habitat (Fukatsu and Hosokawa 2002; Engel and Moran 2013a, b).

Gut epithelium of insect consists of folded membrane with either epithelial enterocyte or endocrine cells (Marianes et al. 2013). Enterocytes having microvilli secretes varied enzyme and help in assimilation of nutrients, while endocrine cells, devoid of microvilli, produce peptide and hormones (Beehler-Evans and Micchelli 2015). Other cell types such as goblet cells (in Lepidoptera), cuprophilic cells (in Dipterans) also play an important role in ion transport, with H+ pumps, that results in either alkaline or acidic conditions inside the insect gut (Huang et al. 2015). The gut epithelium functions as a selective barrier which helps in the uptake of nutrients and exchange of ions and water (Simpson et al. 2015). The transport is facilitated by two routes: transcellular route (across the epithelial cells) and paracellular route (between the epithelial cells). Beside this, water content of the body fluids is also regulated by the gut epithelium through channels known as aquaporins (Spring et al. 2009; Huang et al. 2015) and during water and heat stress condition this water moved down the osmotic gradient (from high concentration to low compartment) across biological membranes, hence, help the insects to survive in severe condition (Fig. 14.1).

14.4 Insect Gut Microbial Composition

The gut of insects has a varied group of microorganisms, which are usually mutualistic in nature and help in digestion of intractable plant polymers, supplying nutrients, stimulus of midgut self-renewal providing resistance to parasite invasion, and host fitness with different environmental conditions (Hosokawa et al. 2006; Oliver 2003; Douglas 2015). In this manner, a mutualistic association is formed between insect and intracellular microbes to play diverse metabolic roles to their host, even, working with new metabolic pathways to utilize nutrients which may

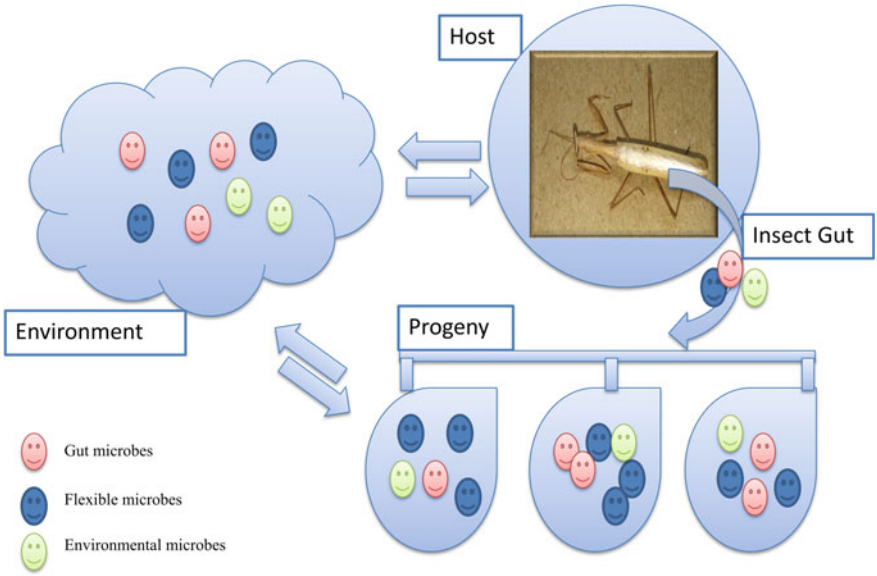


Fig. 14.1 Schematic representation of exchange of microbes between insect and environment

otherwise be missing from their conventional food sources (Baumann 2005; Carrasco et al. 2014). Reports suggest that the extracellular symbiotic microbes associated with the alimentary tract of stinkbugs insect (*Pentatomidae* sp.) sustain in the uneven environment due to their potential adaptation as evident in γ-proteobacteria (Nikoh et al. 2011; Hosokawa et al. 2006; Kikuchi et al. 2009). Fukatsu and Hosokawa (2002) reported that vertical transmission of microorganisms to newly hatched nymph takes place through a symbiont capsule being ingested deposited in the midgut by the nymphs of insect. Similar observations have been reported by Kikuchi et al. (2009). During the development of midgut, the anterior portion becomes free from symbiont microorganisms, while the posterior part transformed into a baggy organ having diverse groups of symbiont cells (Hosokawa et al. 2006; Nikoh et al. 2011). However, regarding structural, functional, and evolutionary studies and their correlation on symbiont microorganisms and insect gut, the available scientific information needs to be augmented (Douglas 2015).

The insect gut bacteria also acts as an iron reservoir for the host that helps as iron sink and source for physiological activities. Pesek et al. (2011) reported that *Microbacterium arborescens* in the larval gut of *Spodoptera exigua* (Beet armyworm) possess iron reservoirs that help bacterial enzyme (Peroxidase) to inhibit the occurrence of cell-damaging oxygen radicals. Several other structurally similar enzymes (also known as DNA protecting proteins) also help the host insect during starvation (Pesek et al. 2011). Gut bacteria also contribute in maintaining biogeochemical cycle by recycling nitrogen, as members of enterobacteriace species

Klebsiella, *Roseateles aquatilis* found in larvae of southern pine beetle accumulate nitrogen in the environment (Krishnan et al. 2014). Insect feeding on wood (lignocellulose) sources, as a biochemical catalyst, has enormous impact in carbon cycling in nature (Sun and Scharf 2010; Taggar 2015). These lignocellulosic enzymes have a wide range of potential applications including industries for various application purposes.

14.4.1 According to Diet

Anatomy and physiology of insect's intestines differ greatly and act as the host a variety of microorganisms as per their food habit. The insect gut microbial diversity represents a large source of unexplored microbes that participate in various activities from utilization of different organic polymers, nitrogen fixation, methanogenesis, pesticide degradation, pheromone production to pathogen prevention (Nardi et al. 2002; Reeson et al. 2003; Mrázek et al. 2008). As per the report, the estimated number of bacteria ranges from 109 in honey bees, to 105 in a fruit fly, to negligible numbers in the plant sap-feeders (downloaded from http://schaechter.asmblog.org/schaechter/2013/06/). Different food habits trigger more diversity in gut bacteria. Hernández et al. (2013) reported that polyphagous Iberian geotrupid dung beetles *Thorectes lusitanicus* that feeds on wide range of food material from dung, acorns, fungi, fruits, to carrion, harbors assorted group of aerobic, facultative anaerobic, and aerotolerant gut bacteria. However, environment plays a critical role in acquisition of gut microbial population, which are more or less constant with respect to the habitats it shares (Wang et al. 2011). The relationships between the insect and its gut microbiome are dynamic one, and resident bacteria play a major role in colonization in the gut even by non-indigenous species. The insect gut is thus a "hot spot" for gene transfer (transfer of plasmids and transconjugation) between bacterial strains that inevitably contribute toward insect's food habit and nutrition (Dillon and Dillon 2004). Anderson et al. (2013) reported that six of seven bacterial phylogenetic groups present in the hindgut of honey bee (*Apis mellifera*) play a critical role to manage important functions related to the health of host. Engel and Moran (2013a, b) suggested that high levels of genetic diversity and functional differences within gut possibly due to niche partitioning within the species during evolution. Gut bacteria also play a role in pathogen protection in insects. Reports on *Wolbachia*, a maternally inherited intracellular bacterium which is found in 40% of insects and other arthropod species, suggest that, it augments pathogen protection, survival against viral-infection in many insect species (Moreira et al. 2009; Bian et al. 2010; Friberg et al. 2011; Zug and Hammerstein 2012; Kuraishi et al. 2013a, b; Ye et al. 2013).

Insect gut microbial composition depends upon several biological and ecological factors (Nikoh et al. 2011; Colman et al. 2012). However, host diet plays a major role in gut bacterial diversity in insect species. Xylophagus insects feeding on decaying woods possess the abundance gut flora (102.8 ± 71.7 species-level

OTUs/sample, 11.8 ± 5.9 phylogenetic diversity (PD)/sample) while the insects like bees feeding on relatively simpler food materials have low abundance in bacterial flora (11.0 species-level OTUs/sample ± 5.4, 2.6 ± 0.8 PD/sample) (Colman et al. 2012). Although insect guts can also harbor protists, fungi, archaea; however, bacterial population plays dominant role in insect physiology, food habit, nutrition, etc.; however, their maintenance depends upon the social transmission (Hongoh 2010). Wood or detritus eaters have fungi in their guts, while methanogenic archaea are present in the guts of beetles and termites that feed on dung, detritus, or wood (Brune 2010; Engel and Moran 2013a, b).

On the other hand, food components play a dominating role in microbial population having little impact on host species characterization. Pernice et al. (2014) reported that phylogenetically distantly related insect species with different gut microbial composition when feed and cultured on similar food substrates exhibited similar microbial communities in their gut. Further, Mikaelyan et al. (2015) found that diverse gut bacteria help termite to effectively digest the different wood sources through a synergistic and symbiotic effort. However, apart from bacteria, termite gut also contains other intestinal flora, like cellulolytic flagellates, prokaryotic communities, and archaeal populations (Lozupone et al. 2012; Mikaelyan et al. 2015; Brune 2010). Distinct phylogenetic pattern occurs in the termite gut microflora from different subfamilies that show the diet is the main factor which formulates the bacterial community structure having distinctive microenvironmental conditions (Mikaelyan et al. 2017).

14.4.2 Role in Partner Selection

As discussed above, gut microbiome manipulates a number of aspects including fitness of organism that otherwise influences its mating preferences. Dodd (1989) reported the insect *Drosophila pseudoobscura* preferred positive assortative mating where it favored mating partners reared for more than 25 generations in the same media; whereas, the flies reared on other media (either starch-based or maltose-based media) preferred mating partner came out from their own rearing media, becoming a population of either "starch flies" or "maltose flies." Similarly, Sharon et al. (2010) examined the mating preference of *Drosophila melanogaster* using molasses and starch as rearing media. It was found that the mating preference of these flies appeared only after one generation and was maintained for at least 37 generations; however, mating preference was eliminated after treating with antibiotic, which signifying the vital role of microbiota of fly gut for the phenomenon (Sharon et al. 2010). These observations triggered the scientific community to study further on the role of gut microbial population based on dietary substances. As reported, gut microbiomes have an effect on longevity and reproduction capacity to an organism. A study on antibiotic-treated termites *Zootermopsis angusticollis* and *Reticulitermes flavipes* showed the reduced diversity and decrease of useful microbes in their gut flora and subsequent malnourishment that

led to the production of significantly less number of eggs (Rosengaus et al. 2011). Brucker and Bordenstein (2013) showed, in their study on the parasitic wasp *Nasonia* sp., that the bacteria in this insect gut of wasp species (*Nasonia giraulti* and *Nasonia vitripennis*) execute themselves as a living barrier that prevents their evolutionary trail from mating with each other and precisely preserve a different sets of gut microbiomes. However, after their forceful crossbreed, the hybrids develop an indistinct microbial population in the gut that causes their premature deaths (Brucker and Bordenstein 2013). Further, in this study, it was found that bacterial constituents and abundance are unequal in hybrids in comparison with the parental species. While *Providencia* sp. is the major gut bacteria in parental species, *Proteus mirabilis* became dominant in the hybrid one, which signifies that interbreeding between two species caused damaging modification to the gut flora; therefore, the microbiome of *Nasonia* helps to remain the two species separate (Brucker and Bordenstein 2013).

14.4.3 Genome Evolution

Symbiotic association with the gut microbes is believed to be one of the key factors that assist the largest phylum of the animal kingdom to be so successful over the centuries (Warnecke et al. 2007; Lize et al. 2014; Brown and Wernegreen 2016). Through the mechanism of differentiation during the process of vertical transmission, among environmentally acquired varied groups of bacteria, symbiotic microorganisms can selectively be preserved by the insects (Kiers et al. 2008). Gut environment has profound impact toward evolution of gut associates either obligate or facultative (Kikuchi et al. 2009; Nikoh et al. 2011). Transfer of gut-associated bacteria through vertical (queen to daughter) or horizontal transmission (between workers) assists the host by serving in the developmental phases (Kwong and Moran 2015). Kikuchi et al. (2009) reported the transmission of symbiotic mutualistic bacteria like *Buchnera aphidicola* sp. (in aphids), *Wigglesworthia glossinidia* sp. (in tsetse flies) take place packed in mycetocytes. This vertical transmission plays an important role in nutrition (Kikuchi et al. 2009). Further, accelerated molecular evolution, AT-biased nucleotide composition, and reduced genome size changes have been noticed conferring to major evolutionary pattern in these symbionts (Wernegreen 2002; Brown and Wernegreen 2016). The study of microorganism diversity in insect gut can be done individually or metagenomic approaches. Culture-dependent classical methods have many inadequacies in terms of species diversity study, as many of the insect gut bacteria may not be cultured in laboratory conditions. However, metagenomic approaches can provide an insight to all the microbial community presents in the gut, along with scope to ascertain inter- and intra-specific role (Brune 2010; Ellegaard and Engel 2016).

14.5 Lignocellulose as a Component: Physiological Property

"Lignocellulose" is the combination of three biopolymers: cellulose, hemicellulose, and lignin which make the natural, rigid structural component of plants. Cellulose is a polysaccharide linear chain of thousands of β(1 → 4) linked D-glucose units, with chemical formula $(C_6H_{10}O_5)_n$ (Fig. 14.2). Molecular weight of cellulose ranges from 200,000 to 2,000,000, corresponding to 1250–12,500 glucose molecules per residues (Bashir et al. 2013; Chatterjee et al. 2015) molecule. The cellulose polymer is subdivided into four different categories (Cellulose I, II, III, and IV) which vary in physical and chemical properties (Zhang and Zhang 2013). Lignocellulosic biomass makes a large portion of the plant biomass, approximately 50% in the world (Sanchez and Cardona 2008). It is the most abundant component of terrestrial ecosystem and thus represents a massive source of food and energy for diverse group of microorganisms (Shaikh et al. 2013). Cellulose, a tasteless, odorless, hydrophilic, homopolysaccharide, is more crystalline in nature than starch. Cellulose requires temperature beyond 320 °C to attain the amorphous state in contrast to temperature above 60–70 °C, which converts the crystalline starch into amorphous state. Application of strong acid can break amorphous state of cellulose and produces nano-crystalline cellulose (Peng et al. 2011).

In lignocellulosic biomass, lignin comprises around 10–30% and is the second most abundant natural organic polymer that enables plants to generate rigid structures and gives protection against hydrolysis of cellulose and hemicellulose (de Gonzalo et al. 2016). Lignin is a highly cross-linked polymer, formation of which is activated by plant laccases and/or peroxidases. A range of ether and carbon–carbon bonds (β–β, β–O–4, and β–5 bonds) polymerizes the 4-hydroxyphenylpropanoid monomers (monolignols having phenolic moieties, like p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) groups), and the composition of which varies

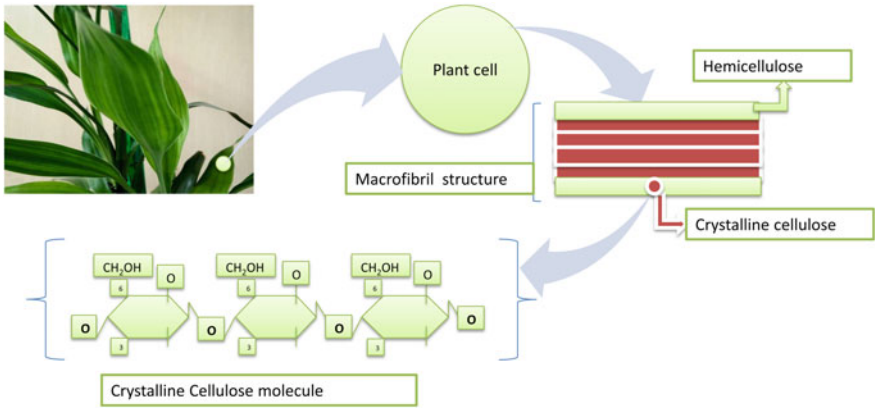


Fig. 14.2 Schematic representation of cellulose molecule in plant cell

depending on the plant tissue and species (Vanholme al. 2010; de Gonzalo et al. 2016). The lignin degradation usually takes place in two stages. The first stage involves extracellular, non-specific depolymerization, forming aryl and biaryl compounds (like β-aryl ethers) following mineralization of these compounds through specific catabolic enzymes and pathways (Sainsbury et al. 2013). Most of the lignin degradation studies show that enzymes such as manganese peroxidase (MnP), lignin peroxidase (LiP), and laccase from fungi (white rot fungi) are the best enzyme that degrades the lignin component (Sainsbury et al. 2013; de Gonzalo et al. 2016).

Bacterial ligninolytic enzymes are peroxidase in nature, which belong to the family of heme-containing peroxidases, better known as dye-decolorizing peroxidases (DyPs, EC 1.11.1.19), contains a non-covalently bound heme *b* cofactor (Van Bloois et al. 2010; Colpa et al. 2014; Yoshida and Sugano 2015; Singh and Eltis 2015). In the year 1999, the first member of this family, DyP from *Bjerkandera adusta* (order: Polyporales), was isolated and characterized (Kim and Shoda 1999). Till then, different bacterial DyPs have been studied and have been reported, which suggest that putative DyP-encoding genes are amply present in bacterial genomes (Lambertz et al. 2016). Other Dyp enzymes that have been reported in bacteria are PpDyP from *Pseudomonas putida* MET94 (Santos et al. 2014), BsDyP from *Bacillus subtilis* KCTC2023 (Min et al. 2015), SviDyP from *Saccharomonospora viridis* DSM 43017 (Yu et al. 2014), and TfuDyP from *Thermobifida fusca* (Van Bloois et al. 2010). Interestingly, in the year 1988, in laboratory studies, bacterial "lignin peroxidase" has been reported from *Streptomyces viridosporus* (Wang et al. 1990; Thomas and Crawford 1998; de Gonzalo et al. 2016). Recently, Davis et al. (2013) reported gene encoding a putative Tat-secreted DyP in *Streptomyces* isolate. However, in bacteria, due to the complexity of the proteins having several disulfide bonds, integrate calcium ions and a heme cofactor, DyP is usually glycosylated, which is in contrast to the regular peroxidases present in fungi (Lambertz et al. 2016). There are some special conditions which the bacterial machinery requires for folding and processing in manufacturing protein. Although genetic engineering technique has been used to express various DyPs in *E. coli*, which is comparable to that of fungal peroxidases (de Gonzalo et al. 2016; Lambertz et al. 2016). Bacterial peroxidases and laccases are recently being used for large-scale recombinant enzyme development. Studies have been reported that bacterial laccases can be produced in *E. coli* (Ihssen et al. 2015; de Gonzalo et al. 2016). Therefore, bacterial enzymes are comparatively easier to produce and have potential application in lignin biodegradation.

14.6 Enzymatic Breakdown of Lignocellulose

Natural degradation lignocellulosic biomass occurs through coordinated action of a set of enzymes. The conversion of lignocellulose into simpler molecule, including glucose, is essential to utilize the biomass in a productive manner. Plant

biomass-derived products (like aromatic products, carbohydrates, ethanol) can be used as food and flavor compounds, polymer precursors, pharmaceutical building blocks, fuel, etc. (Asgher et al. 2014; Ragauskas et al. 2014; Kawaguchi et al. 2016; de Gonzalo et al. 2016). However, biochemical reactions for various processes use to operate at favorable pH, temperature, pressure, and other biotic and abiotic conditions. Lignocellulosic biomass hydrolysis requires a set of coordinated action of multiple enzymes. Cellulase enzyme is a complex package of three different classes of enzymes: (1) Endo-1,4-β-endoglucanase binds to non-crystalline part of cellulose, cleaves glucosidic linkages, (2) Exo-1,4-β-exoglucanase binds to crystalline part of the cellulose, and cleaves the molecule while, (3) β-glucosidase enzyme cleaves the cellobiose (a disaccharide molecule) releasing glucose monomers. These three classes of cellulase enzyme are necessary to breakdown the crystalline cellulose into simpler forms such as glucose (Willis et al. 2010; Chatterjee et al. 2015). Nature of cellulose, source of cellulolytic enzymes, optimal condition for catalytic activity, and production of enzymes also play critical role in bioconversion of cellulose (Chatterjee et al. 2015).

14.7 Cellulosomes Complex

Due to its high recalcitrant crystal structure, cellulose degradation is limited to few microorganisms and is a complicated chore. In anaerobic bacteria, cellulases are bound to scaffoldin, forming multicomponent, multienzyme cellulosome complexes that efficiently can degrade cellulosic substrates (Béguin and Lemaire 1996; Bayer et al. 2004; Bae et al. 2013). Cohesin–dockerin interaction helps the non-catalytic subunit called scaffoldin, to bind the various enzyme subunits into the complex. The interaction is highly specific between the scaffoldin-based cohesin modules and the enzyme-borne dockerin domains, which forms the assembly of the cellulosome (Bayer et al. 2004; Bae et al. 2013; Haitjema et al. 2017). This multienzyme complex facilitated by cohesin–dockerin interaction, which is the basis for newly emerging field of synthetic biology (Haitjema et al. 2017). Investigation of the growth substrate-dependent variations in cellulosomal systems has been studied with the advances in proteomics study approach. Further, deigned minicellulosomes have contributed to investigate the immediacy and targeting effects of synergistic action of cellulosomal complex. The arrangement of genes in multiple-scaffoldin or enzyme-linked group on the genome contributes toward the diversity in cellulosome structural design (Bayer et al. 2004; Haitjema et al. 2017). Chimeric cohesin-bearing scaffoldins have been used for amalgamation of recombinant dockerin-containing enzymes, for assembling the designer cellulosomes (Stern et al. 2016). Interestingly, chimeric scaffoldin, having six cohesins, has been reported to form the largest designer cellulosome. However, this has resulted in the instability of the scaffoldin polypeptide, limited numbers of available cohesin–dockerin specificities, and low expression levels (Stern et al. 2016). Again, study related to the regulation of cellulosome-related genes through genetic engineering tools and

approach and promising genomics of cellulosome-producing bacteria has facilitated in examining the assembly and consequences of the multienzyme complex (Bayer et al. 2004; Bae et al. 2013). Stern et al. (2016) reported that a designer cellulosome complex having a hexavalent scaffoldin attached to adaptor scaffoldin, having a type-II cohesin forms an effective enzyme complex which is having potential capacity up to 70% as compared to that of native cellulosomes for solubilization of natural lignocellulosic substrates.

14.8 Biotechnological Application of Cellulase Enzyme

Importance and application of microbial enzymes increased rapidly in mid-1980s, where different industrial applications have also been identified. Research on insect gut micobiome has indicated the potential use of bacterial enzymes, especially cellulase for biotechnological application (Kuhad et al. 2011; Su et al. 2017). The list of insects producing cellulase has been reviewed elsewhere (Chatterjee et al. 2015). It is obvious that inside the gut of insect these cellulase enzymes help in nutrition by deconstruction of food materials. As for example, heterotermitidae and rhinotermitidae groups of termite possess highly potential cellulolytic enzymes that help in digestion of complex polysaccharide food materials including wood and wood-based products (Martin et al. 1983; Chatterjee et al. 2015; de Gonzalo et al. 2016). It has been reported that the cellulosome complex presents in the bacteria residing at hindgut area of termites that have the capacity to degrade lignocellulosic material with their cell wall by surrounding the food substrates (Bayer et al. 2004; Tokuda et al. 2005; Scharf et al. 2011; Bae et al. 2013; Chatterjee et al. 2015; Stern et al. 2016; Haitjema et al. 2017). The catalyst of this complex system together is more effective than a single enzyme unit for lignocellulosic degradation (Stern et al. 2016). However, the termite gut has a complex niche of community of bacterial, archaeal, and eukaryotic gut symbionts that synergistically break down the plant fibers into the products like acetate, hydrogen, and methane (Brune 2014; Brune and Dietrich 2015; Mikaelyan et al. 2017). It has been estimated that termite gut can digest 74–99% of cellulose and 65–87% of hemicelluloses within hours (Li et al. 2017). More than 4700 bacterial phylotypes have been detected in the lower termite *Reticulitermes*, where Bacteriodetes, Proteobacteria, Spirochetes, Firmicutes, and Eubacteria are prominent members of this microbiota that help in biomass degradation (Cragg et al. 2015). Some Archaeal species present in the termite gut can degrade lignocellulose at higher temperature. Reports suggest that endoglucanase GH12 gene presents in archaeon Pyrococcus, and genes encoding laccase enzymes from Halobacteriales, and Thermoproteales are potential element that caters lignocellulosic degradation in archaea (Graham et al. 2011; de Gannes et al. 2013; Tian et al. 2014; Cragg et al. 2015).

14.8.1 In Waste Management

Most of the agricultural and household wastes contain lignocellulose as major components. The waste amelioration process can easily be achieved by treating wastes using bacterial cellulases and lignin-degrading enzymes (Kuhad et al. 2011; Gupta et al. 2011; Brune and Dietrich 2015; Chatterjee et al. 2015). Composition and dynamics of microflora play a major role during this process of enzymatic degradation; however, a detailed study of microbial succession and selection to accelerate the process is important for effective and appropriate management of biowastes (Kuhad et al. 2011; Gupta et al. 2011).

14.8.2 Food and Brewage Industry

Increase demand of fruit and vegetable juice has drawn the attention of food and brewage industries toward macerating enzymes like cellulase, pectinase, and related enzymes to ease in processing of fruit and vegetable juice. The conventional system involves multistep processing like maceration, extraction, clarification, and stabilization. Using cellulase for macerating fruit pulp can yield better in starch and protein extraction (Ventorino et al. 2015). In addition, the better maceration helps in color and carotenoid extraction of fruits and vegetables which in turn help in improved texture, quality, flavor, aroma, and viscosity of fruit purees (Kuhad et al. 2011; Chatterjee et al. 2015). Further using enzymes' mixture or in combination like pectinases, cellulases, and hemicellulases improves extraction, malaxation, and quality of olive-based oil and paste (Wongputtisin et al. 2014). It has also been observed that infusion of enzymes such as pectinases and β-glucosidases reduces bitterness of citrus fruits by some extent (Sharada et al. 2014). Similarly, these microbial enzymes have a key role in alcoholic beverages production also. Brewing of beer initiates with barley malt or malted sorghum which contains raw starch and protein material which require enzymes' to convert it into simpler form like sugars, amino acids, and peptides. Enzymes help in improving skin maceration, color extraction, clarification, filtration, and stability (Singh et al. 2007). Further, to produce liquor controlled fermenting conditions, along with microbial enzymes play a major role in deciding the quality and yields of the fermented products.

14.8.3 Ethanol Production from Lignocellulosic Biomasses

Nowadays, through the use of starch or sucrose as provided by agricultural crops such as corn, wheat, or sugarcane, fermentation of ethanol is being carried out on a larger scale. The biological conversion of the lignocellulosic wastes produces either

ethanol, methanol, or hydrogen, which depends upon the process (biochemical or thermochemical) and ideal microorganism (Dutta et al. 2014). In nature, degradation of organic matter, i.e., lignocelluloses leads to methane generation, whereas ethanol and hydrogen are the intermediates by-products in anaerobic degradation (Ahring and Westermann 2007). Ethanol is an environmentally safe liquid transportation fuel as it does not contribute toward greenhouse gas emissions because ethanol produced from the renewable plant materials and CO_2 generated from the ethanol burning is recycled by the plant body in their photosynthesis process (Limayem and Ricke 2012; Saini et al. 2015). The fermentation of lignocellulosic substrates is an eminent and collective process. This tough process of conversion of lignocellulose to ethanol has several steps, such as (i) detaching (or delignification) lignin from other molecules to release free cellulose and hemicellulose from the lignocellulosic material; (ii) depolymerization of carbohydrate to release sugars from cellulose and hemicelluloses; (iii) fermentation of hexose and pentose sugars to produce ethanol (Lee 1997; De Souza 2013) (Tables 14.1 and 14.2).

Table 14.1 Microbes from different insects gut having potential cellulolytic activity

Insect (common name)	Order/ Family	Gut microorganism	References
Termites	Isoptera	*Methanobrevibacter ruminantium, Trichonympha, Clostridium, Actinomycetes*	Gupta et al. (2011)
Butterfly	Lepidoptera	*Enterobacter cloacae*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae, Pseudomonas fluorescens,* and *Proteus mirabilis*	Fischer et al. (2013)
Bookworm	Lepidoptera	*Trichonympha, Clostridium, Actinomycetes*	Gupta et al. (2011)
Crickets	Orthoptera	*Caldicellulosiruptor* spp.	
Paddy grasshopper	Orthoptera	*Enterococcus faecalis, Enterococcus durans, Flavobacterium odoratum, Serretia marcescens*	Shil et al. (2014)
Scarab beetles	Scarabaeidae	*Firmicutes, Clostridium*	Engel and Moran (2013a, b)
Yellow stem borer	Lepidoptera	*Bacillus* spp., *Mycobacterium* spp.	Bashir et al. (2013)

Table 14.2 Cellulose digestion in different insects with respect to enzyme produced (adopted from Martin et al. 1983; Fischer et al., 2013; Engel and Moran (2013a, b); Chatterjee et al. 2015)

Order/Family	Enzyme	Mechanism	Substrates
Coleoptera Isoptera Heterotermitidae Rhinotermitidae Diptera	Cellobiohydrolases	Action on β-1,4-glycosidic bonds on the linear chain of cellulose by removing cellobiose units from the non-reducing ends	Microcrystalline cellulose powder, Avicel (a micro-crystalline cellulose powder), cotton, swollen cellulose, cellodextrins. Limited activity toward CMC
Coleoptera Cerambycidae Blattodea Blaberidae	β-1,4-endoglucanase	Random attack on and β-1,4-glucosidic bonds, releasing transient cellodextrins, cellobiose, and glucose	Soluble derivatives of cellulose, phosphoric acid swollen cellulose, CMC, and cellodextrins. No activity toward crystalline cellulose. Hardly any activity toward cellobiose
Isoptera Termitidae Blattodea Lepidoptera Nematocera Blaberidae	β-D-glucosidase	Hydrolysis of the β-1,4-glucosidic bond of cellobiose to generate glucose	Cellobiose, other β-linked disaccharides of glucose, and cellodextrins. No activity toward cellulose

14.8.4 Pulp and Paper Industry

Annually, massive amount lignocellulosic biomass is being processed in pulp and paper industry. Use of cellulase enzyme for efficient conversion of lignocellulosic waste into quality paper is an eco-friendly and appropriate approach. Enzymes-based process includes pre-bleaching of pulp and deinking process that helps in pulp freeness and cleanliness, as a result, improves fiber brightness and strength properties (Kuhad et al. 2011; Chatterjee et al. 2015).

14.8.5 Textile Industry

Cellulase has been employed widely in textile industries for biostoning of jeans, biopolishing of cotton, and other cellulosic fabrics. Earlier biostoning was performed mechanically by pumice stone which use to cause damage to the fiber; however, after the introduction of cellulase enzyme, this process becomes easy with less damage to fiber (Kuhad et al. 2011; Chatterjee et al. 2015). Further, acidic cellulase takes care of biopolishing process as a result soft, smooth, and bright color fabric obtain.

14.9 Conclusion

Insect gut microbiota varies widely. As for examples, complex gut microbial communities can be found in termite gut, while, little or no gut microbiota are present in sap-feeding insects (Colman et al. 2012; Chapman et al. 2013). As a whole, less complexity of microbial community structure in insect gut can be found, which may be due to varied reasons like, the simple gut structure (that can afford fewer ecological niches), smaller retention time (due to their tiny structure), lacking a "classical" adaptive immune system etc. (Colman et al. 2012; Chapman et al. 2013; Engel and Moran 2013a, b). However, bacterial consortia in insect gut specifically help the host in digestion of food and various other activities (Engel and Moran 2013a, b). Lignocellulose eaters (scarab beetles and termite), however, retain food longer having fermentative guts with diverse gut microbial communities (Colman et al.2012). Recent developments of omics technologies have led the researchers to know more about the insect gut bacteria and their potential enzymatic activities upon lignocelluloses degradation. Further, several applications like production of biofuels from such wastes have pushed the researchers to understand different aspects related to biotechnological applications, like lignocellulose-active genes, substrate binding paradigms, oxidation of polysaccharides, architecture of enzyme domain, enzymatic synergies, and lignin bond breakdown (Brunecky et al. 2013; Agger et al. 2014; Cragg et al. 2015). However, there are several aspects like comprehensive individual enzyme-based sequence–structure–function relationships or synergistic action of cocktails of enzymes that work together within the insect gut are yet to be ascertained (Cragg et al. 2015). It is essential to explore these unknown aspects to optimize the enzyme activities in different industrial applications. In coming years, the relevant findings will enormously help to understand about the diversity of insect gut microbial community, in one hand, and various applications to develop sustainable eco-friendly technologies for generation of wealth (e.g., biofuel) from lignocellulosic wastes.

Acknowledgements Authors thankfully acknowledge Director, DRL, and DRDO for fellowship to RKP for kind support to carry out the work. Authors' sincerely apologies many colleagues and their scientific publications that could not been referred here due to space limitation. Further, authors declare there is no conflict of interest present.

References

Agger JW, Isaksen T, Varnai A, Vidal-Melgosa S, Willats WGT, Ludwig R, Horn SJ, Eijsink VGH, Westereng B (2014) Discovery of LPMO activity on hemicelluloses shows the importance of oxidative processes in plant cell wall degradation. Proc Natl Acad Sci USA 111:6287–6292

Ahring BK, Westermann P (2007) Coproduction of bioethanol with other biofuels. Adv Biochem Eng Biotechnol 108:289–302

Anderson KE, Sheehan TH, Mott BM, Maes P, Snyder L, Schwan MR, Walton A, Jones BM, Corby-Harris V (2013) Microbial ecology of the hive and pollination landscape: bacterial associates from floral nectar, the alimentary tract and stored food of honey bees (*Apis mellifera*). PLoS ONE8:e83125. https://doi.org/10.1371/journal.pone.0083125

Appel HM, Martin MM (1990) Gut redox conditions in herbivorous lepidopteran larvae. J Chem Ecol 16 (12):3277–3290. https://doi.org/10.1007/BF00982098

Asgher M, Bashir F, Iqbal HMN (2014) A comprehensive ligninolytic pre-treatment approach from lignocellulose green biotechnology to produce bio-ethanol. Chem Eng Res Des 92:1571–1578

Bae J, Morisaka H, Kuroda K, Ueda M (2013) Cellulosome complexes: natural biocatalysts as arming microcompartments of enzymes. J Mol Microbiol Biotechnol 2:370–378

Bashir Z, Kondapalli VK, Adlakha N, Sharma A, Bhatnagar RK, Chandel G, Yazdani SS (2013) Diversity and functional significance of cellulolytic microbes living in termite, pill-bug and stem-borer guts. Sci Rep 3:2558. https://doi.org/10.1038/srep02558

Baumann P (2005) Biology bacteriocyte-associated endosymbionts of plant sap-sucking insects. Annu Rev Microbiol 59:155–189

Bayer EA, Belaich JP, Shoham Y, Lamed R (2004) The cellulosomes: multienzyme machines for degradation of plant cell wall polysaccharides. Annu Rev Microbiol 58:521–554

Beehler-Evans R, Micchelli CA (2015) Generation of enteroendocrine cell diversity in midgut stem cell lineages. Development 142:654–664 [PubMed: 25670792]

Béguin P, Lemaire M (1996) The cellulosome: an exocellular, multiprotein complex specialized in cellulose degradation. Crit Rev Biochem Mol Biol 31:201–236

Berenbaum M (1980) Adaptive significance of midgut pH in larval Lepidoptera. Am Nat 115:38–146

Bian G, Xu Y, Lu P, Xie Y, Xi Z (2010) The endosymbiotic bacterium Wolbachia induces resistance to dengue virus in Aedes aegypti. PLoS Pathog 6:e1000833. https://doi.org/10.1371/journal.ppat.1000833

Bignell DE (2010) Morphology, physiology, biochemistry and functional design of the termite gut: an evolutionary wonderland. In: Bignell DE, Roisin Y, Lo N (eds) Biology of termites: a modern synthesis. Springer, Dordrecht, pp 375–412

Boudko DY, Moroz LL, Linser PJ, Trimarchi JR, Smith PJ, Harvey WR (2001) In situ nalysis of pH gradients in mosquito larvae using non-invasive, self-referencing, pH-sensitive microelectrodes. J Exp Biol 204:691–699

Brown BP, Wernegreen JJ (2016) Deep divergence and rapid evolutionary rates in gut associated Acetobacteraceae of ants. BMC Microbiol 16:140. https://doi.org/10.1186/s12866-016-0721-8

Brucker RM, Bordenstein SR (2013) The hologenomic basis of speciation: gut bacteria cause hybrid lethality in the genus Nasonia. Science 9(341):667–669

Brune A (2010) Methanogens in the digestive tract of termites. (Endo)symbiotic methanogenic archaea. In: Hackstein JHP (ed) Microbiology monographs. Springer, pp 81–100

Brune A (2014) Symbiotic digestion of lignocellulose in termite guts. Nat Rev Microbiol 12:168–180. https://doi.org/10.1038/nrmicro3182

Brune A, Dietrich C (2015) The gut microbiota of termites: digesting the diversity in the light of ecology and evolution. Annu Rev Microbiol 69:145–166

Brune A, Ohkuma M (2010) Role of the termite gut microbiota in symbiotic digestion. In: Bignell DE, Roisin Y, Lo N (eds) Biology of termites: a modern synthesis. Springer, Dordrecht, pp 439–475

Brunecky R, Alahuhta M, Xu Q, Donohoe BS, Crowley MF, Kataeva IA, Yang S-J, Resch MG, Adams MWW, Lunin VV et al (2013) Revealing nature's cellulase diversity: the digestion mechanism of *Caldicellulosiruptor bescii* CelA. Science 342:1513–1516

Carrasco P, Perez-Cobas AE, van de PC, Baixeras J, Moya A, Latorre A (2014) Succession of the gut microbiota in the cockroach *Blattella germanica*. Int Microbio 17:99–109. https://doi.org/10.2436/20.1501.01.212

Cazemier AE, Verdoes JC, Reubsaet FAG, J. Hackstein HP, Drift C van der, Camp Op den HJM (2003) *Promicromonospora pachnodae* sp. nov., a member of the (hemi) cellulolytic hindgut flora of larvae of the scarab beetle *Pachnoda marginata*. Antonie Leeuwenhoek 83:135–148

Chapman AD (2006) Numbers of living species in Australia and the World. Canberra: Aus Biol Res Study. ISBN 978-0-642-56850-2

Chapman RF, Simpson SJ, Douglas AE (2013) The insects: structure and function, 5th edn. Cambridge University Press, Cambridge

Chatterjee S, Sharma S, Prasad RK, Datta S, Dubey D, Meghvansi MK, Vairale MG, Veer V (2015) Cellulase enzyme based biodegradation of cellulosic materials: an overview. South Asian J Exp Biol 5:271–282

Colman DR, Toolson EC, Takacs-Vesbach CD (2012) Do diet and taxonomy influence insect gut bacterial communities? Mol Ecol 21:5124–5137

Colpa DI, Fraaije MW, Van BE (2014) DyP-type peroxidases: a promising and versatile class of enzymes. J Ind Microbiol Biotechnol 41:1–7

Cragg SM, Beckham GT, Bruce NC, Bugg TDH, Distel DL, Dupree P, Etxabe AG, Goodell BS, Jellison J, McGeehan JE, McQueen-Mason SJ, Schnorr K, Walton PH, Watts JEM, Zimmer M (2015) Lignocellulose degradation mechanisms across the tree of life. Curr Opin Chem Biol 29:108–119

Davis JR, Goodwin L, Teshima H, Detter C, Tapia R, Han C, Huntemann M, Wei CL, Han J, Chen A, Kyrpides N, Mavrommatis K, Szeto E, Markowitz V, Ivanova N, Mikhailova N, Ovchinnikova G, Pagani I, Pati A, Woyke T, Pitluck S, Peters L, Nolan M, Land M, Sello JK (2013) Genome sequence of *Streptomyces viridosporus* strain T7A ATCC 39115, a lignin-degrading actinomycete. Genome Announc.1:pii:e00416-13

de Gannes V, Eudoxie G, Hickey WJ (2013) Prokaryotic successions and diversity in composts as revealed by 454-pyrosequencing. Bioresour Technol 133:573–580

de Gonzalo G, Colpa DI, Habib MHM, Fraaije MW (2016) Bacterial enzymes involved in lignin degradation. J Biotechnol 236:110–119

De Souza WR (2013) Microbial degradation of lignocellulosic biomass. https://doi.org/10.5772/54325

Dillon RJ, Dillon VM (2004). The gut bacteria of insects: nonpathogenic interactions. Ann Rev Entomol 49:71–92. https://doi.org/10.1146/annurev.ento.49.061802.123416

Dodd DMB (1989) Reproductive isolation as a consequence of adaptive divergence in *Drosophila pseudoobscura*. Evolution 43:1308–1311

Douglas AE (2011) Lessons from studying insect symbioses. Cell Host Microbe 10(4):359–367. https://doi.org/10.1016/j.chom.2011.09.001

Douglas AE (2015) Multiorganismal insects: diversity and function of resident microorganisms. Annu Rev Entomol 60:17–34. https://doi.org/10.1146/annurev-ento-010814-020822

Dow JA (1992) pH gradients in lepidopteran midgut. J Exp Biol 172:355–375

Dutta K, Daverey A, Lin JG (2014) Evolution retrospective for alternative fuels: first to fourth generation. Renew Energy 69:114–122

Elbert A, Brune A (1997) Hydrogen concentration profiles at the oxicanoxic interface: a microsensor study of the hindgut of the wood-feeding lower termite *Reticulitermes flavipes* (Kollar). Appl Environ Microbiol 63:4039–4046

Ellegaard KM, Engel P (2016) Beyond 16S rRNA community profiling: intra-species diversity in the gut microbiota. Front Microbiol. https://doi.org/10.3389/fmicb.2016.01475

Engel P, Moran NA (2013a) The gut microbiota of insects diversity in structure and function. FEMS Microbiol Rev 37:699–735

Engel P, Moran NA (2013b) Functional and evolutionary insights into the simple yet specific gut microbiota of the honey bee from metagenomic analysis. Gut Microbes 4:60–65

Erwin TL (1982) Tropical forests: their richness in Coleoptera and other arthropod species. Coleopt Bull 36:74–75

Fischer R, Ostafe R, Twyman RM (2013) Cellulases from insects. Adv Biochem Eng Biotechnol. https://doi.org/10.1007/10_2013_206

Friberg U, Miller PM, Stewart AD, Rice WR (2011) Mechanisms promoting the long-term persistence of a Wolbachia infection in a laboratory-adapted population of *Drosophila melanogaster*. PloS One6:e16448. https://doi.org/10.1371/journal.pone.0016448

Fukatsu T, Hosokawa T (2002) Capsule-transmitted gut symbiotic bacterium of the Japanese common plataspid stinkbug, *Megacopta punctatissima*. Appl Environ Microbiol 68:389–396. https://doi.org/10.1128/AEM.68.1

Graham JE, Clark ME, Nadler DC, Huffer S, Chokhawala HA, Rowland SE, Blanch HW, Clark DS, Robb FT (2011) Identification and characterization of a multidomain hyperthermophilic cellulase from an archaeal enrichment. Nat Commun 2:375

Gupta R, Khasa YP, Kuhad RC (2011) Evaluation of pretreatment methods in improving the enzymatic saccharification of cellulosic materials. Carbohyd Polym 84:1103–1109

Haitjema CH, Gilmore SP, Henske JK, Solomon KV, de Groot R, Kuo A, Mondo SJ, Salamov AA, LaButti K, Zhao Z, Chiniquy J, Barry K, Brewer HM, Purvine SO, Wright AT, Hainaut M, Boxma B, van Alen T, Hackstein JHP, Henrissat B, Baker SE, Grigoriev IV, O'Malley MA (2017) A parts list for fungal cellulosomes revealed by comparative genomics. Nat Microbiol 2:17087. https://doi.org/10.1038/nmicrobiol.2017.87

Harrison JF (2001) Insect acid-base physiology. Annu Rev Entomol 46:221–250 [PubMed:11112169]

Hernández N, Escudero JA, Millán AS, González-Zorn B, Lobo JM, Verdú JR, Suárez M (2013) Culturable aerobic and facultative bacteria from the gut of the polyphagic dung beetle *Thorectes lusitanicus* Jeckel. Insect Sci. https://doi.org/10.1111/1744-7917.12094

Hongoh Y (2010) Diversity and genomes of uncultured microbial symbionts in the termite gut. Biosci Biotechnol Biochem 74:1145–1151

Hosokawa T, Kikuchi Y, Nikoh N, Shimada M, Fukatsu T (2006) Strict host-symbiont cospeciation and reductive genome evolution in insect gut bacteria. PLoS Biol 4(10):e337

Huang JH, Jinga X, Douglas AE (2015) The multi-tasking gut epithelium of insects. Insect Biochem Mol Biol 67:15–20. https://doi.org/10.1016/j.ibmb.2015.05.004

Hyun JH, Woon RS, Woong WT, Ja JM, Soo KM, Sang PD, Changmann Y, Do NY, Ji KH, Hye CJ, Yong KJ, Ri SN, Hee KS, Jae LW, Woo BJ (2014) Insect gut bacterial diversity determined by environmental habitat, diet, developmental stage, and phylogeny of host. Appl Env Microbiol 80(17):5254–5264

Ihssen J, Reiss R, Luchsinger R, Thöny-Meyer L, Richter M (2015) Biochemical properties and yields of diverse bacterial laccase-like multicopper oxidases expressed in *Escherichia coli*. Sci Rep 5:10465

Irfan M, Safdar A, Syed Q, Nadeem M (2012) Isolation and screening of cellulolytic bacteria from soil and optimization of cellulase production and activity. Turk J Biochem 37:287–293

Kohler T, Dietrich C, Scheffrahn RH, Brune A (2012) High resolution analysis of gut environment and bacterial microbiota reveals functional compartmentation of the gut in wood-feeding higher termites (Nasutitermes spp.). Appl Environ Microbiol 78:4691–4701

Kawaguchi H, Hasunuma T, Ogino C, Kondo A (2016) Bioprocessing of bio-based chemicals produced from lignocellulosic feedstocks. Curr Opin Biotechnol 42:30–39

Kiers ET, Denison RF (2008) Sanctions, cooperation, and the stability of plant-rhizosphere mutualisms. Annu Rev Ecol Evol Syst 39:215–236

Kikuchi Y, Hosokawa T, Nikoh N, Meng XY, Kamagata Y, Fukatsu T (2009) Host-symbiont co-speciation and reductive genome evolution in gut symbiotic bacteria of acanthosomatid stinkbugs. BMC Biol 7:2. http://www.biomedcentral.com/1741-7007/7/2

Kim SJ, Shoda M (1999) Purification and characterization of a novel peroxidase from Geotrichum candidum Dec 1 involved in decolorization of dyes. Appl Environ Microbiol 65:1029–1035

Krishnan M, Bharathiraja C, Pandiarajan J, Prasanna VA, Rajendhran J, Gunasekaran P (2014) Insect gut microbiome—an unexploited reserve for biotechnological application. Asian Pacific J Trop Biomed 4(Suppl 1):S16–S21

Kuhad RC, Gupta R, Singh A (2011) Microbial cellulases and their industrial applications. Enzyme Res Article ID 280696. https://doi.org/10.4061/2011/20696

Kuraishi T, Hori A, Kurata S (2013a) Host-microbe interactions in the gut of Drosophila melanogaster. Front Physiol 4:375

Kuraishi T, Hori A, Kurata S (2013b) Host-microbe interactions in the gut of *Drosophila melanogaster*. Front Physiol 17. https://doi.org/10.3389/fphys.2013.00375

Kwong WK, Moran NA (2015) Evolution of host specialization in gut microbes: the bee gut as a model. Gut Microbes 6(3):214–220

Lambertz C, Ece S, Fischer R, Commandeur U (2016) Progress and obstacles in the production and application of recombinant lignin-degrading peroxidases. Bioengineered 7:145–154

Lee J (1997) Biological conversion of lignocellulosic biomass to ethanol. J Biotechnol 56:1–24 PubMed PMID: 9246788

Lemke T, Stingl U, Egert M, Friedrich MW, Brune A (2003) Physicochemical conditions and microbial activities in the highly alkaline gut of the humus-feeding larva of *Pachnoda ephippiata* (Coleoptera: Scarabaeidae). Appl Environ Microbiol 69(11):6650–6658

Li H, Yelled DJ, Lie C, Yang M, Ke J, Zhang R, Liu Y, Zhu N, Mo X, Ralph J, Currie CR, Mo J, Liang S (2017). www.pnas.org/lookup/suppl. https://doi.org/10.1073/pnas.1618360114/-/DCSupplemental

Limayem A, Ricke SC (2012) Lignocellulosic biomass for bioethanol production: current perspectives, potential issues and future prospects. Prog Energy Combust Sci 38:449–467

Lize A, McKay R, Lewis Z (2014) Kin recognition in Drosophila: the importance of ecology and gut microbiota. ISME J 8:469–477

Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R (2012) Diversity, stability and resilience of the human gut microbiota. Nature 489:220–230

Marianes A, Spradling AC, Brand A (2013) Physiological and stem cell compartmentalization within the Drosophila midgut. Elife 2:e00886 [PubMed: 23991285]

Martin M (1983) Cellulose digestion in insects. Comp Biochem Physiol 75 A:313–324

Mikaelyan A, Dietrich C, Kohler T, Poulsen M, Sillam DD, Brune A (2015) Diet is the primary determinant of bacterial community structure inthe guts of higher termites. Mol Ecol 24 (20):5284–5295. https://doi.org/10.1111/mec.13376

Mikaelyan A, Meuser K and Brune A (2017) Microenvironmental heterogeneity of gut compartments drives bacterial community structure in wood- and humus-feeding higher termites. FEMS Microbiol Ecol 93:fiw210. https://doi.org/10.1093/femsec/fiw210

Min K, Gong G, Woo HM, Kim Y, Um Y (2015) A dye-decolorizing peroxidase from *Bacillus subtilis* exhibiting substrate-dependent optimum temperature for dyes and β-ether lignin dimer. Sci Rep 5:8245

Minard G, Mavingui P, Moro CV (2013) Diversity and function of bacterial microbiota in the mosquito holobiont. Parasites Vectors 6:146

Moll RM, Romoser WS, Modrzakowski MC, Moncayo AC, Lerdthusnee K (2001) Meconial peritrophic membranes and the fate of midgut bacteria during mosquito (Diptera: Culicidae) metamorphosis. J Med Entomol 38:29–32

Moreira LA, Iturbe-Ormaetxe I, Jeffery JA, Lu G, Pyke AT, Hedges LM, Rocha BC, Hall-Mendelin S, Day A, Riegler M, Hugo LE, Johnson KN, Kay BH, McGraw EA, van den HAF, Ryan PA, O'Neill SL (2009) A Wolbachia symbiont in Aedes aegypti limits infection with dengue; Chikungunya; and Plasmodium. Cell 139:1268–1278

Mrázek J, Strosová L, Fliegerová K, Kott T, Kopecný J (2008) Diversity of insect intestinal microflora. Folia Microbiol (Praha) 53:229–233. https://doi.org/10.1007/s12223-008-0032-z

Nardi JB, Mackie RI, Dawson JO (2002) Could microbial symbionts of arthropod guts contribute significantly to nitrogen fixation in terrestrial ecosystems? J Ins Physiol 48:751–763. https://doi.org/10.1016/S0022-1910(02)00105-1

Nikoh N, Hosokawa T, Oshima K, Hattori M, Fukatsu T (2011) Reductive evolution of bacterial genome in insect gut environment. Genome Biol Evol 3:702–714. https://doi.org/10.1093/gbe/evr064

Oliver KM, Russell JA, Moran NA, Hunter MS (2003) Facultative bacterial symbionts in aphids confer resistance to parasitic wasps. Proc Natl Acad Sci USA 100:1803–1807

Peng BL, Dhar N, Liu HL, Tam KC (2011) Chemistry and applications of nannocrystalline cellulose and its derivatives: a nanotechnology prospective. Can J Chem Eng 89(5):1191–1206

Pernice M, Simpson JS, Ponton F (2014) Towards an integrated understanding of gut microbiota using insects as model systems. J Insect Physiol 69:12–18

Pesek J, Buchler R, Albrecht R, Boland W, Zeth K (2011) Structure and mechanism of iron translocation by a Dps protein from *Microbacterium arborescens*. J Biol Chem 286. https://doi.org/10.1074/jbc.M111.246108

Pester M, Brune A (2007) Hydrogen is the central free intermediate during lignocellulose degradation by termite gut symbionts. ISME J 1(6):551–565

Ragauskas AJ, Beckham GT, Biddiy MJ, Chandra R, Chen F, Davis MF, Davison BH, Dixon RA, Gilna P, Keller P, Langan P, Naskar AK, Saddler JN, Tschaplinski TJ, Tuskan GA, Wyan CE (2014) Lignin valorization: improving lignin processing in the biorefinery. Science 344:1246843

Reeson AF, Jankovic T, Kasper ML, Rogers S, Austin AD (2003) Application of 16S rDNA-DGGE to examine the microbial ecology associated with a social wasp Vespula germanica. Insect Mol Biol 12:85–91. https://doi.org/10.1046/j.1365-2583.2003.00390.x

Rosengaus RB, Zecher CN, Schultheis KF, Brucker RM, Bordenstein SR (2011) Disruption of the termite gut microbiota and its prolonged consequences for fitness. Appl Environ Microbiol 77:4303–4312

Russell JA, Moran NA (2005) Horizontal transfer of bacterial symbionts: heritability and fitness effects in a novel aphid host. Appl Environ Microbiol 71:7987–7994

Saini JK, Tewari L, Saini R (2015) Lignocellulosic agriculture wastes as biomass feedstocks for second-generation bioethanol production: concepts and recent developments. 3 Biotech 5:337–353. https://doi.org/10.1007/s13205-014-0246-5

Sainsbury PD, Hardiman EM, Ahmad M, Otani H, Seghezzi N, Eltis LD, Bugg TDH (2013) Breaking down lignin to high-value chemicals: the conversion of lignocellulose to vanillin in a gene deletion mutant of *Rhodococcus jostii* RHA1. ACS Chem Biol 8:2151–2156

Sanchez OJ, Cardona CA (2008) Trends in biotechnological production of fuel ethanol from different feedstocks. Bioresour Technol 99(13):5270–5295

Santos A, Mendes S, Brissos B, Martins LO (2014) New dye-decolorizing peroxidases from *Bacillus subtilis* and *Pseudomonas putida* MET94: towards biotechnological applications. Appl Microbiol Biotechnol 98:2053–2065

Sari SLA, Pangastuti A, Susilowati A, Purwoko T, Mahajoeno E, Hidayat W, Mardhena I, Panuntun DF, Kurniawati D, Anitasari R (2016) Cellulolytic and hemicellulolytic bacteria from the gut of Oryctes rhinoceros larvae. Biodiversitas 17:78–83

Scharf ME, Karl ZJ, Sethi A, Boucias DG (2011) Multiple levels of synergistic collaboration in termite lignocellulose digestion. PloS One 6:e21709. https://doi.org/10.1371/journal.pone.0021709

Shaikh NM, Patel AA, Mehta SA, Patel ND (2013) Isolation and screening of cellulolytic bacteria in habiting different environment and optimization of cellulase production. Univ J Environ Res Technol 3(1):39–49

Sharada R, Venkateswarlu G, Venkateswar S, Anand RM (2014) Applications of cellulases—review. Int J Pharmac Chem Biolog Sci 4(2):424–437

Sharon G, Segal D, Ringo JM, Hefetz A, Zilber-Rosenberg I, Rosenberg E (2010) Commensal bacteria play a role in mating preference of *Drosophila melanogaster*. Proc Natl Acad Sci USA 107:20051–20056

Shil RK, Mojumder S, Sadida FF, Uddin M, Sikdar D (2014) Isolation and identification of cellulolytic bacteria from the gut of three Phytophagus insect species. Braz Arch Biol Tech 57 (6):927–932. https://doi.org/10.1590/S1516-8913201402620

Simpson SJ, Clissold FJ, Lihoreau M, Ponton F, Wilder SM, Raubenheimer D (2015) Recent advances in the integrative nutrition of arthropods. Annu Rev Entomol 60:293–311 [PubMed: 25341097]

Singh R, Eltis LD (2015) The multihued palette of dye-decolorizing peroxidases. Arch Biochem Biophys 574:56–65

Singh A, Kuhad RC, Ward OP (2007) Industrial application of microbial cellulases. In: Kuhad RC, Singh A (eds) Lignocellulose biotechnology: future prospects. I.K. International Publishing House, New Delhi, India, pp 345–358

Spring JH, Robichaux SR, Hamlin JA (2009) The role of aquaporins in excretion in insects. J Exp Biol 212:358–362 [PubMed: 19151210]

Stern J, Moraïs S, Lamed R, Bayer EA (2016) Adaptor scaffoldins: an original strategy for extended designer cellulosomes, inspired from nature. M Bio 7(2):e00083. https://doi.org/10.1128/mBio.00083-16

Su LH, Zhao S, Jiang SX, Liao XZ, Duan CJ, Feng JX (2017) Cellulase with high β-glucosidase activity by *Penicillium oxalicum* under solid state fermentation and its use in hydrolysis of cassava residue. World J Microbiol Biotechnol 33(2):37

Sudakaran S, Retz F, Kikuchi Y, Kost C, Kaltenpoth M (2015) Evolutionary transition in symbiotic syndromes enabled diversification of phytophagous insects on an imbalanced diet. ISME J 9:2587–2604

Sun JZ, Scharf ME (2010) Exploring and integrating cellulolytic systems of insects to advance biofuel technology. Insect Sci 17:163–165

Taggar MS (2015) Insect cellulolytic enzymes: novel sources for degradation of lignocellulosic biomass. J Appl and Nat Sci 7(2):625–630

Thomas L, Crawford DL (1998) Cloning of clustered *Streptomyces viridosporus* T7A lignocellulose catabolism genes encoding peroxidase and endoglucanase and their extracellular expression in Pichia pastoris. Can J Microbiol 44:364–372

Tian JH, Pourcher AM, Bouchez T, Gelhaye E, Peu P (2014) Occurrence of lignin degradation genotypes and phenotypes among prokaryotes. Appl Microbiol Biotechnol 98:9527–9544

Tokuda G, Lo N, Watanabe H (2005) Marked variations in patterns of cellulase activity against crystalline- vs. carboxymethyl-cellulose in the digestive systems of diverse, wood-feeding termites. Physiol Entomol 30:372–380

Van Bloois E, DE Torres Pazmi, Winter RT, Fraaije MW (2010) A robust and extracellular heme-containing peroxidase from *Thermobifida fusca* as prototype of a bacterial peroxidase superfamily. Appl Microbiol Biotechnol 86:1419–1430

Vandenbossche V, Brault J, Vilarem Gérard, Hernández-Meléndez Oscar, Vivaldo-Lima Eduardo, Hernández-Luna Martín, Barzana Eduardo, Duque Aleta, Manzanares Paloma, Ballesteros Mercedes, Mata Julio, Castellón Erick, Rigal Luc (2014) A new lignocellulosic biomass deconstruction process combining thermo-mechano chemical action and bio-catalytic enzymatic hydrolysis in a twin-screw extruder. Ind Crops Prod 55:258–266

Vanholme R, Demedts B, Morreel K, Ralph J, Boerjan W (2010) Lignin biosynthesis and structure. Plant Physiol 153:895–905

Ventorino V, Aliberti A, Faraco V, Robertiello A, Giacobbe S, Ercolini D, Amore A, Fagnano M, Pepe O (2015) Exploring the microbiota dynamics related to vegetable biomasses degradation and study of lignocellulose-degrading bacteria for industrial biotechnological application. Sci Rep 5:8161. https://doi.org/10.1038/srep08161

Wang ZM, Bleakley BH, Crawford DL, Hertel G, Rafii F (1990) Cloning and overexpression of a lignin peroxidase gene from *Streptomyces viridosporus* in *Streptomyces lividans*. J Biotechnol 13:131–144

Wang Y, Gilbreath TM, Kukutla P, Yan G, Xu J (2011) Dynamic gut microbiome across life history of the malaria mosquito Anopheles gambiae in Kenya. PLoS One 6:e24767. https://doi.org/10.1371/journal.pone.0024767

Warnecke F, Luginbuhl P, Ivanova N, Ghassemian M, Richardson TH, Stege JT, Cayouette M, McHardy AC, Djordjevic G, Aboushadi N (2007) Metagenomic and functional analysis of hindgut microbiota of a wood feedinghigher termite. Nature 450:560–565

Wernegreen JJ (2002) Genome evolution in bacterial endosymbionts of insects. Nat Rev Genet 3:850–861

Wheeler WC, Whiting M, Wheeler QD, Carpenter JM (2001) The phylogeny of the extant hexapod orders. Cladistics 17:113–169

Willis JD, Oppert C, Jurat-Fuentes JL (2010) Methods for discovery and characterization of cellulolytic enzymes from insects. Insect Sci 17:184–198

Wongputtisin P, Khanongnuch C, Kongbuntad W, Niamsup P, Lumyong S, Sarkar PK (2014) Use of *Bacillus subtilis* isolates from Tua-nao towards nutritional improvement of soya bean hull for monogastric feed application. Lett Appl Microbiol. 59(3):328–333. https://doi.org/10.1111/lam.12279

Ye YH, Woolfit M, Rancès E, O'Neill SL, McGraw EA (2013) Wolbachia-associated bacterial protection in the mosquito Aedes aegypti. PLoS Negl Trop Dis 7:e2362

Yoshida T, Sugano Y (2015) A structural and functional perspective of DyP-type peroxidase family. Arch Biochem Biophys 574:49–55

Yu W, Liu W, Huang H, Zheng F, Wang X, Wu Y, Li K, Xie X, Jin Y (2014) Application of a novel alkali-tolerant thermostable DyP-type peroxidase from *Saccharomonospora viridis* DSM 43017 in biobleaching of eucalyptus kraft pulp. Plos One 9:e110319-e

Zhang XZ, Zhang YHP (2013) Cellulases: characteristics, sources, production, and applications. Bioprocessing technologies in biorefinery for sustainable production of fuels, chemicals, and polymers. Wiley

Zug R, Hammerstein P (2012) Still a host of hosts for Wolbachia: analysis of recent data suggests that 40% of terrestrial arthropod species are infected. PLoS ONE 7:e38544

Chapter 15
Bioremediation of Volatile Organic Compounds in Biofilters

Margavelu Gopinath, Rose Havilah Pulla, K. S. Rajmohan, Parthasarthy Vijay, Chandrasekaran Muthukumaran and Baskar Gurunathan

Abstract In India, 12 lakhs deaths per annum take place due to air pollution according to a report by Greenpeace organization. Volatile organic compounds are major air pollutants which are released into the environment through mobile sources, stationary sources, area sources, and natural sources. Stationary sources such as petrochemical and pharmaceutical industries release VOCs like toluene which is known to cause several health hazards including lung cancer. In addition to it, VOCs pollute air, soil, and water which are a growing environmental concern. Based on the concentration level of the VOCs, several removal techniques have been employed to combat VOCs. Non-biological methods such as ozonation, absorption, adsorption, incineration, catalytic oxidation, condensation, membrane separation are being employed. Several biological methods ranging from biotrickling filters to biofilters have been demonstrated, and they are found to be economical. The biofilters are simple to construct, easy to operate, and cost effective. Major advantage of this method is the pollutant is converted into biodegradable waste which can decompose within a moderate time frame, thus producing no secondary pollutants. In this chapter, biofilters, microorganisms, biofilter preparation and reaction mechanism are discussed. More emphasis was given on operation, processes, conditions, and stability of biofilters. The recent advancements in biofilters including application of foam for enhanced separation

M. Gopinath (✉) · R. H. Pulla · K. S. Rajmohan · P. Vijay
Department of Chemical Engineering, College of Engineering Studies, University of Petroleum and Energy Studies, P.O. Bidholi Via-Prem Nagar, Dehradun 248007, India
e-mail: mgopinath@ddn.upes.ac.in

C. Muthukumaran
Department of Industrial Biotechnology, Government College of Technology, Coimbatore 641 013, India

B. Gurunathan
Department of Biotechnology, St. Joseph's College of Engineering, Chennai 600119, Tamil Nadu, India

S. J. Varjani et al. (eds.), *Bioremediation: Applications for Environmental Protection and Management*, Energy, Environment, and Sustainability,
https://doi.org/10.1007/978-981-10-7485-1_15

and the limitations of the biofiltration methods are also discussed. Future scope and summary of the chapter are given at the end of the chapter to provide an insight into biofilters research.

Keywords Biodegradation · Biofilters · Volatile organic compounds Reactors · Fungi · Pollution · Lung cancer

15.1 Introduction

15.1.1 Air Pollution

Release of untreated industrial and domestic waste into natural resources like water, land, or air which can disturb the ecological balance is called environment pollution. Depending on the component of the earth which is polluted, pollution is classified as air, water, soil, and noise. Further, they are classified into biodegradable and non-biodegradable pollutants (Sharma et al. 2009). Furthermore, they are classified based on the nature of emission into the atmosphere. Airborne pollutants are classified as primary and secondary pollutants. Pollutants may cause severe health concerns, ecosystem disruptions, or both.

One of the primary air pollutants which mainly contribute to indoor air pollution is volatile organic compounds (VOCs). Volatility is the tendency of a compound to get vaporized based on its vapor pressure. Compounds that can volatize under ambient conditions without any aid of energy input are termed as volatile organic compounds (VOCs). The exposure to VOCs under indoor environment possesses health threats. Based on the difference in their degree of boiling point, VOCs are classified into very volatile organic compounds (VVOCs), volatile organic compounds (VOCs), and semi-volatile organic compounds (SVOCs). Common examples of each type of VOCs are listed in Table 15.1 (Forbes 2015; Tham et al. 2011).

Table 15.1 Classification of volatile organic compounds

Classification	Temperature range	Examples	Methods of air sampling
VVOC	<0–50 °C	Dichlorodifluoro methane (R12), propylene, formaldehyde carbons	Tedlar bags, canisters
VOC	50–100 to 240–260 °C	Benzene, toluene, ethylbenzene, xylenes (BTEX)	Canisters impingers/ bubblers
SVOC/ particulate matters	240–260 to 380–400 °C	Phthalate esters, nitrosamines, haloethers, and trihalomethanes. Diphenyl ethers. Polybrominated diphenyl furans, polychlorinated biphenyls	Impingers/ bubblers, solid sorbents fractionators

VOCs are released from coating, paint, plastics, electronics, automobile manufacturing industries, and transport vehicles. Because of their toxic and carcinogenic nature, VOCs cause severe health issues. Also, they disrupt the ecosystem by causing odor problems, ozone depletion, and global warming (Yoon and Park 2002; Morgado et al. 2004; Moe and Irvine 2001). VOCs released from the above-mentioned industries into the atmosphere can lead to smog formation. Environmental Protection Agency (USEPA) has listed VOCs (such as toluene) in the list of priority pollutants.

India ranks 141st position in the Environmental Performance Index (2016), indicating lack of air quality which is mainly inline for the release of chemicals, particulates, and biological materials into the environment. This is caused by chemical, petrochemical, fertilizer, coal mining, leather, textile, tannery, pharmaceutical, food processing, paint, paper and pulp, and printing industrial emissions which are shown in Fig. 15.1. The control of gaseous emission in the environment is becoming increasingly important and cost expensive to many industries. Acute increase in the concentrations of air pollutants in the environment warrants the implementation of more stringent national and international regulations. In spite of several regulations implemented for the control of gaseous emissions, the increasing level of VOCs in the environment of ground-level ozone causes much danger to the ecosystem. In this chapter, biofilter which is one of the economic and efficient methods of combating VOC pollution is discussed.

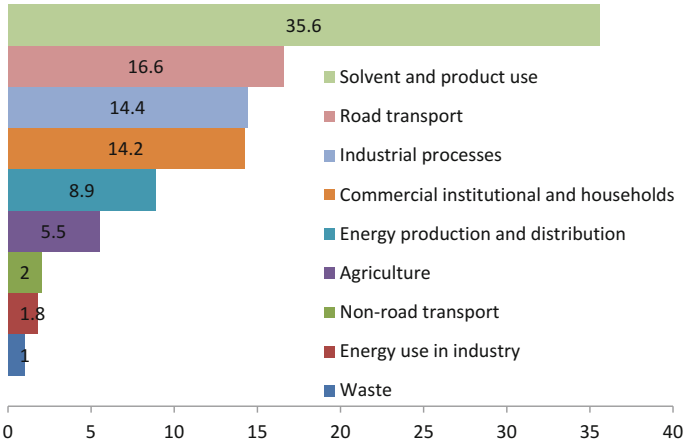


Fig. 15.1 Sources and emission percentage of VOCs

15.1.2 Effect of Volatile Organic Compounds

VOCs include esters, paraffins, ketones, aromatics, and olefins present in the exhaust air from the chemical, refineries, paints, and ink manufacturing industries, and the consuming industries. The use of volatile organic compounds in chemical industries has increased because of their use in the separation of desired product from its mixture. A large volume of VOCs released into the atmosphere every year would impair the air quality and are a threat to public health (Chan and You 2010).

VOCs play an important role in communicating between the plants and atmosphere. The release of high concentrations of VOCs and NO_x leads to ozone formation as per reaction 1.1 and thereby influences the greenhouse effect which affects climatic conditions and leads to health hazards such as respirational problems, nervous damage, and genetically disorders in humans. The differentiable concentration of carcinogenic VOCs of maximum permissible vulnerability in the milieu is epitomized (Tables 15.2, 15.3 and 15.4). In India, there is no independent guideline to determine VOCs discharge into environment. For example, toluene, even at low concentrations, is known to damage human liver, kidney, and paralyze the central nervous system. According to WHO, 65,00,000 deaths in 2012 were due to air pollution (WHO 2016). In India, air pollution is believed to cause 12,00,000 deaths in a year which is slightly less than the death due to tobacco usage. It is essential to combat VOCs release into the atmosphere using an efficient and economic method (Chakrabarti 2004).

For instance, toluene is an aromatic hydrocarbon belonging to the BTEX group of hazardous VOCs, which are used as an industrial feedstock and as a solvent. It is a hydrophobic and carcinogenic compound (type-II carcinogenic agent) (Berenjian et al. 2011).

15.1.3 Environmental and Health Hazards

VOCs are transported into the air as vapor or gas transform into its numerous mixtures. VOCs emit two types of pollutants namely—hydrophilic (eg., methanol)

Table 15.2 Class I solvents used in pharmaceutical industries based on their toxicity limit

Solvent	Exposure level in atmosphere (mg/day)	Risks
Benzene	2	Carcinogen
Carbon tetrachloride	4	Toxic and environmental hazard
1,2-Dichloroethane	5	Toxic
1,1-Dichloroethene	8	Toxic
1,1,1-Trichloroethane	15	Environmental hazard, teratogen

Table 15.3 Class II solvents used in pharmaceutical industries based on their toxicity limit

Solvent	Exposure level in atmosphere (mg/day)	Risks
Acetonitrile	4.1	Toxic
Chlorobenzene	3.6	Toxic, probable carcinogen
Chloroform	0.6	Toxic, probable carcinogen
Cyclohexane	38.8	Carcinogen
1,2-Dichloroethene	18.7	Carcinogen
Dichloromethane	6.0	Toxic
1,2-Dimethoxyethane	1.0	Toxic
N, N-Dimethylacetamide	10.9	Carcinogen
N, N-Dimethylformamide	8.8	Toxic
1,4-Dioxane	3.8	Toxic
2-Ethoxyethanol	1.6	Toxic
Ethyleneglycol	6.2	Toxic
Formamide	2.2	Toxic
Hexane	2.9	Toxic
Methanol	30	Carcinogen
2-Methoxyethanol	0.5	Harmful
Methylbutyl ketone	0.5	Harmful
Methylcyclohexane	11.8	Carcinogen
N-Methylpyrrolidone1	5.3	Mutagen
Nitromethane	0.5	Harmful
Pyridine	2	Mutagen
Sulfolane	1.6	Mutagen
Tetrahydrofuran	7.2	Toxic
Tetralin	1	Mutagen
Toluene	8.9	Toxic
1,1,2-Trichloroethene	0.8	Harmful
Xylene	21.7	Mutagen

and hydrophobic (eg., toluene). These VOCs result in the emission of harmful pollutants into the atmosphere, thereby increasing the risk of pollution problems and other health hazards viz., nausea, loss of appetite, tiredness, mental imbalance, memory loss, and color vision loss (Dees et al. 1996). VOCs by the activity of sunlight will be fractionated into its basic mixes like ozone, nitrogen oxides, and receptive VOCs and another form or mixes such as brown haze. VOCs play a major role in the formation of photochemical smog. Some VOCs have grave consequences on animals and plants. In liquid form and solutions, VOCs affect the water bodies and soil.

Table 15.4 Class III solvents used in pharmaceutical industries based on their toxicity limit

Solvent	Exposure level in atmosphere (mg/day)	Risks
Acetic acid	3.1	Probable toxic
Acetone	2.6	Carcinogen
Anisole	0.5	Carcinogen
1-Butanol	31.8	Toxic
2-Butanol	13.7	Carcinogen
Butyl acetate	4.0	Harmful
tert-Butyl methyl ether	0.7	Harmful
Cumene	7.9	Carcinogen
Dimethyl sulfoxide	6.8	Toxic
Ethanol	2.8	Toxic
Ethyl acetate	0.8	Toxic
Ethyl ether	4.2	Carcinogen
Ethyl formate	1.2	Mutagen
Formic acid	1.9	Harmful
Heptane	15	Mutagen
Isobutyl acetate	0.7	Harmful
Isopropyl acetate	0.9	Harmful
Methyl acetate	9.8	Carcinogen
3-Methyl-1-butanol	4.3	Carcinogen
Methyl ethyl ketone	0.3	Toxic
Methyl isobutyl ketone	1.5	Toxic
2-Methyl-1-propanol	1.1	Toxic
Pentane	5.2	Toxic
1-Pentanol	0.6	Toxic
1-Propanol	6.9	Toxic
2-Propanol	0.7	Mutagen
Propyl acetate	14.7	Harmful

For instance, day-by-day toluene, type-II carcinogenic pollutant releasing into air, water, land, or underground in the environment are increasing. Toluene exposure to people may occur during the use of gasoline and other products and enters into the body by skin contact. Its breakdown and removal in the body are difficult. Because of its short life expectancy in the atmosphere, toluene emitted is expected to be localized in a particular area. Toluene entering into the ground is not evaporated and reaches the ground and underground water sources. If the water contaminated with toluene is exposed to air, it is evaporated and remains in the atmosphere. Many soil bacteria are able to break down toluene. Severe and serious effects of toluene on birds or land animals are uncertain. Toluene is expected to bioaccumulate slowly. Some of the health issues due to VOCs are documented by World Health Organization (WHO) which is shown in Fig. 15.2.

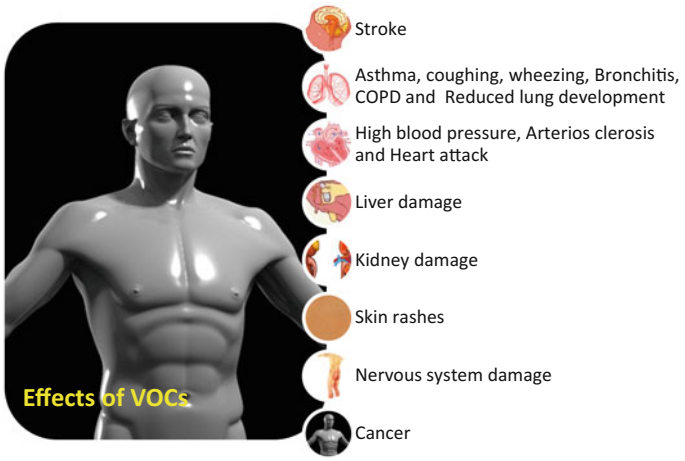


Fig. 15.2 Effects of VOCs on human body

The literatures on toluene bare in laborers and in creatures show that the toluene does not cause any tumor. The International Agency for Research on Cancer (IARC) and the Department of Health and Human Services (DHHSs) has not categorized toluene as a noxious substance. The EPA has discovered that the toluene is not responsible for the cancer in humans. The USEPA gauges permitting a ground-level ozone convergence of 65 sections for each billion (ppb) would deflect 1,700–5,100 unexpected losses across the nation in 2020 contrasted and the current 75-ppb widespread. A 2005 recent report by the European Commission figured that air contamination decreases the lifespan expectancy by a normal of nearly nine months over the European Union. The enterprise tasks that the severer standard could also save you an additional 26,000 instances of annoyed bronchial asthma and extra than a million cases of overlooked paintings or college (Tankersley 2010). Globally, 24,00,000 deaths are linked to air pollution than to automobile accidents as suggested by WHO, and University of Birmingham has shown a sturdy correlation between pneumonia-associated deaths and air pollution from motor vehicles.

The most noticeably, awful and regular citizen contamination emergency in India was the 1984 Bhopal disaster (Chakrabarti 2004). Released modern vapors from the Union Carbide processing plant, having a place with Union Carbide, the USA, executed more than 25,000 individuals outright and injured somewhere in the range of 1,50,000–6,00,000. The UK endured its most exceedingly terrible air contamination occasion when the December 4 Great Smog of 1952 framed over London. In 6 days more than 4,000 expired, and 8,000 more expired in the subsequent months. An incidental seepage of Bacillus anthracis spores from a biological warfare laboratory in the former USSR in 1979 close to Sverdlovsk is assumed to have been the reason for 100s of civilian demises. The most exceedingly terrible single episode of air contamination in the USA happened in Donora,

Pennsylvania in late October, 1948, when 20 public demised and more than 7,000 were bruised (Davis 2002).

15.1.4 VOCs Removal Techniques by Non-biological and Biological Methods

More rigorous requirements for the removal of VOCs from barometrical air in re-penny period require the development of inventive and financially savvy treatment choices. Several biological and non-biological methods (shown in Fig. 15.3) are accessible to expel smell and VOCs from squandering air stream. Numerous non-biological methods (shown in Fig. 15.4) such as incineration, absorption, wet scrubbing, ozone, adsorption, catalytic and thermal oxidation, condensation, membrane, activated carbon, photochemical oxidation had been developed for the remedy of VOCs. These physicochemical methods, which comprise dispersion, scrubbing, condensation, absorption, incineration, and UV oxidation, are used in the removal of VOCs. The most important choice and standards for VOC reduction technologies are cost, influent VOC concentration, vapor stream rate and the necessary regulator level. Moreover, pretreatment of the outlet vapor may also necessitate some regulator devices and could have an effect on undertaking value. Pretreatment alludes to the techniques and practices used to conditioning VOC flow before entering into reduction unit. Table 15.5 contains the average pretreatment contemplations for the choice of VOC reduction technology. Particulate elimination is a critical pretreatment method for catalytic and thermal combustion, adsorption, membrane, and biofiltration systems. Particulates can obstruct impetus beds or films bringing about diminishment of VOC elimination capacity of the device. The cooling of VOC vapor stream is an important pretreatment attention for adsorption and biofiltration frameworks. For adsorption framework, the precooler might be expected to soak the gas stream or to diminish the bay air temperature to adequate levels. It maintains a strategic distance from the dissolvable dissipation. High

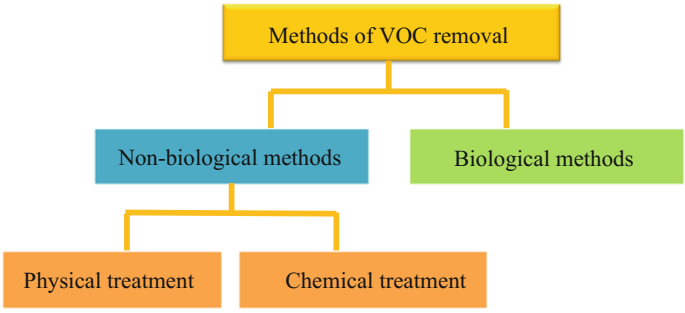


Fig. 15.3 Classification of VOC removal techniques

Fig. 15.4 Non-biological methods of VOC removal techniques

temperature in biofiltration beds may obliterate the microorganisms that change VOC into carbon dioxide, water, and mineral salts. Dehumidification is an essential pretreatment method for adsorption and buildup frameworks, while humidification is critical for biofiltration framework. Water vapor contends with VOC for adsorption destinations; henceforth diminishing the water vapor in the adsorption bay stream will increment the adsorption limit with respect to VOC. In condenser, water vapor can gather in the condenser tubes, subsequently decreasing the heat transfer ability of the framework. Then again, biofiltration framework expects dampness to keep the channel bed from drying and splitting, which would allow escape of unreacted VOC to the environment. Technologies, for example, scrubbing thermal incineration, and activated carbon adsorption are appropriate for various applications. They are frequently fetched, escalated when the off-gas contains moderately low VOC fixations (Lee et al. 2001). Amid burning, contaminations are combusted at temperatures in the vicinity of 700 and 1400 °C. In spite of the fact that the treatment execution is at the most part uniform with successful evacuation of most mixes, incinerators require expansive fuel input and may create unsafe auxiliary waste such as NO_x. Carbon adsorption allows high evacuation efficiencies for low toxin fixations; in any case,

Table 15.5 Typical pretreatment methods for the VOC abatement

Method	Waste gas flow rate (scfm)	VOC concentration (ppm)	Range of VOC ($g\ m^{-3}$)	References
Ozonation	No practical limit	No practical limit	–	Berenjian et al. (2011)
Absorption	No practical limit	No practical limit	8–50	Park et al. (2008)
Adsorption	No practical limit	100–5000	≤ 10	Tham et al. (2011)
Biofiltration	≥ 1000	≤ 1000	≤ 5	Ortiz et al. (2003)
Incineration	0–10000 thermal after burner 250–100000 recuperative 2000–500000 regenerative	60% lower explosive limit (thermal after burner) 25% lower explosive limit (recuperative) 10% lower explosive limit (regenerative)	2–90	Muñoz et al. (2007)
Catalytic oxidation	0–75000	25% of lower explosive limit	2–90	Khan et al. (2000)
Condensation	≤ 3000	≥ 1000	≥ 60	Muñoz et al. (2007)
Emerging methods membrane separation	≤ 500	≥ 5000	≥ 50	Parvatiyar et al. (1996)

scfm standard cubic feet per minute

this innovation has high capital and operational cost on the medium and its transfer or recovery. Furthermore, this procedure exchanges contaminants from the vapor to a solid stage that requires further remedy.

Adsorption frameworks work best at ideal fixation and ideal stream rate. VOC focus as low as 20 ppm is treatable with adsorption, yet fixation over 10,000 ppm may prompt extreme ascending of bed temperature. The stream rate must be sufficiently high to permit both dispersion and adsorption. To bring downstream rates, the required bed volume is vast and its cost ends up being noticeably restrictive. Stream rates between 1,000–50,000 scfm are perfect for adsorption framework. Condenser can process the waste gas stream of high VOC however at moderately low stream rates. The stream rates over 3,000 scfm may require altogether a huge warmth exchange zone. Layer partition frameworks are reasonable for low stream rate and high convergence of VOCs in the waste gas stream. These methods are favored at high (>1000 ppm) contamination fixations. A large number of these techniques are costly, particularly in vitality necessities.

The aeration of polluted water can be performed either by the means packed tower air circulation or mechanical surface air circulation with VOC emanations

capturing and treating the systems. The vapor stripping method and aqueous phase activated carbon adsorption bed were used traditionally for the removal of VOC contaminated liquids. For VOC polluted soil remediation, soil vapor extraction (SVE) is utilized. These strategies take into account the withdrawal of VOCs into the next stage. The aqueous phase activated carbon adsorption bed, and the treated aqueous phase is allowed to physically contact with the activated carbon, and therefore, the suspended matters or tainting substances are dragged on its surface. Finally, the activated carbon was filtered out, or it can be either recovered after treatment. Generally, packed bed and fluidized bed reactors are utilized for this process. When managing with halogenated VOCs and pesticides, this carbon technique has restricted viability. Inexpensive and logistics disputes would rise from positioning or disinfecting the consumed carbon, henceforth carbon adsorption is realistically more efficient for "enhancing" the post-treated fluid releases with low VOC fixations. Correspondingly, VOCs in air emanation could be treated with activated carbon by deflating it through activated carbon packed bed reactors. In any case, issues with the consumed carbon are identical as the aqueous-phase carbon process. More common and stable treatment for the separated VOC would be oxidation in the internal combustion engine (ICE), catalytic or UV, and thermal. Basically VOCs are fractionated into less detrimental mixtures like, carbon dioxide, hydrochloric gas, and water. Thermal oxidation parts are mostly single compartments refractory with porcelain blanket and coated with oxidisers, outfitted with propane or flammable gas burner, and a stack. In the compartment, burner average range limits between 0.4–2 (mil BTUs)/h with working temperatures from 760 to 870 °C and an extreme gas contact time is 1 s. The ICE works similarly; in any case, it is adjusted to high VOC fixations to enable the carbon-based mixes to be utilized as fuel. Secondary fuel is just added to enrich the oxidation process. To shrink the prerequisite for secondary fuel, air to air heat exchangers could be utilized to exchange heat from the fume gas to the entering feed. For treating halogenated VOCs, the fumes stream would require a gas scrubber to regulate the corrosive haze. Oxidation is considered ineffective for low VOCs at about 0.1–10 g m^{-3} as continuous oxidation is challenging to keep upto, unless with more supporting fuel. Catalytic oxidation includes the expansion of a catalyst to a thermal oxidiser to fasten the oxidation by means of adsorption and reacting oxygen and carbon-based mixes on the catalyst surface. This will bring down the response temperature around 320–540 °C in contrast with that required in a traditional thermal oxidiser. The reaction temperature is obtained due to direct pre-warming of feed; hence, it initiates catalytic oxidation when gone through a solid catalytic bed. The catalytic bed contains different metal oxides like nickel oxide or chromium oxide and may even contain noble metals, e.g., palladium. Thermal oxidisers could be utilized as pretreatment to catalytic units for high VOC (Berenjian et al. 2011).

UV oxidation strategy has been utilized to oxidize natural and hazardous mixes in squander water. Strong chemical oxidisers specifically respond to the contaminants with UV photolysis accomplished through synergistic UV light consolidated with ozone and hydrogen peroxide. For the most part, low strain (65 W) lights are utilized for ozone treatment frameworks and 15–60 W lights are utilized as a part of

hydrogen peroxide treatment. End results of UV oxidation are CO_2, H_2O, and salts. However, in the treatment of an extensive variety of VOCs, some pollutants might be vaporized instead of eliminated or obliterated, e.g., TCA. In such a case, post-treatment by activated carbon adsorption might be required. Bio-treatment of VOCs is a moderately less settled VOC expulsion technique, but it gives conceivable points of interest over more customary procedures with bringing down working and capital expenses and a little of carbon impression. This is because of lower vitality necessities as microorganisms are utilized in the process natural mixes at surrounding temperature rather than being subjected to warmth or radiation. Since 1923, biofiltration has been connected to deal with different VOC to debilitate contaminants.

The natural strategies, for example, biofiltration, bioremediation, and biodegradation, have been utilized as the more financially and reasonable option for the VOCs expulsion. The expenses of substance, vitality utilization and further treatment or disposal of optional waste are the significant issues in the regular treatment techniques. The organic techniques incorporating biofilters, biotrickling channels, bioscrubber, suspended development reactors, and film bioreactors (Doble and Kumar 2005) have been chosen in view of their key parameters crosswise, over bio-frameworks, which are recognized as dampness content in the medium, temperature, pH, and accessibility of fundamental and non-carbon supplements. As an extensive variety of VOCs is biodegradable, they can be dealt naturally with living beings coordinated to the kind of VOC to be pulverized. Some effortlessly biodegradable VOCs are esters, benzene, toluene, and phenols. One of the benefits of utilizing natural air contamination control strategies is that the poisons are really obliterated by organic oxidation. A drawback is that some pretreatment of air and contamination might be required, for instance, prefiltration for particulates, temperature modifications, and humidification of the gas stream. Biofiltration is taken a toll focused for on-stream rates over 1,000 scfm and VOC focus beneath 300–500 ppm. In the most recent decade, organic decrease innovations have pulled in an expanding notoriety due to low costs, operational effortlessness, and on the grounds that they are characteristically perfect technologies.

15.2 Degradation of VOCs Using Microorganisms

Microbes including bacteria and fungi are utilized as a part of the evacuation of VOCs. Experts have shown that heterotrophic microorganisms are the fundamental microorganisms utilized as a part of the disposal of VOCs (Delhomenie and Heitz 2005). Nevertheless, the fundamental favorable position of expanding contagious instead of bacterial for the biofiltration of hydrophobic toxins will destroy the substrates under outrageous natural conditions, for example, pH, low water substance, and constrained supplement concentrations. For the most part, heterotrophic microbial strains utilized as a part of VOC bioreactors can catalyze VOCs by two pathways: (i) devouring natural mixes over the span of catabolic pathway for vitality or (ii) utilizing VOCs as a carbon hotspot for anabolic procedures. Essential

species, for example, Pseudomonas, Candida, Mycobacterium, Alcaligenes, Exophiala, Acetinobacter, Fusarium, Cladosporium, Rhodococcus, Aspergillus, and Mucor are a portion of the living species which are distinguished and utilized for the debasement of VOCs by biofiltration framework. The channel bed inoculation relies upon both the idea of sifting material and the level of VOC biodegradability. For the most part, a biofilter contains 106 and 1010 cfu (colony forming units) of microscopic organisms and actinomycetes per gram of bed, individually. It can likewise contain 103–106 spores of growth for every gram of bed (Delhomenie and Heitz 2005). Asadi et al. (2009) analyzed biofiltration in which vigorous microbial species de-review contaminants in two stages. The initial step is oxidation of ketone functional group by molecular oxygen, and second step is breakdown of stimulated particle to CO_2 and water.

15.2.1 Biodegradation of VOCs Using Pure Culture

The microbial degradation of toluene using pure culture from various sources was studied extensively such as bioactive foam with *Alcaligenes xylosoxidas* (*Achromobacter*) isolated from oil and petrol storage tanks, *Cladosporium sphaerospermum*, *conidiophoresoh cladophialophora* sp., conidial chain of *cladophialophora* sp., sporothrix-like anamorph of *pseudeurotium zonatum*, conidiophore of *leptodontium* sp., yeast-like growth of *exophiala sp* were isolated from soil or groundwater samples, *Bacillus cereus* S1 isolated from wastewater treatment of Guangzhou petrochemical corporation, *Rhodococcus* EH831 isolated from a petroleum-contaminated soil and some pure culture was brought from microbial type culture collection center such as *Acinetobactor* sp. NCIMB 9689 and *Rhodococcus rhodochrous* AL NCIMB 13259, *Alcaligenes xylosoxidans*, *Stenotrophomonas maltophilia* T3-c, *Fusarium* and *Cladosporium* spp., *P.putida* MTCC 102, *Paecilomyces lilacinus* CBS 284.36, *Paecilomyces variotii* CBS 115145 reclassification of *Scedosporium apiospermum* TB1, *Phanerochaete chrysosporium* ATCC24725 92%, *Pseudomonas aeruginosa* ATCC 25619, *Pseudomonas* sp. CDBB-B 1230, *Pseudomonas fluorescens* from NCIMB 11671, *Pseudomonas putida* and *Pseudomonas fluorescens* as co-culture, *Pseudomonas putida* ATCC 17484, *Pseudomonas aeruginosa* ATCC 15692, *Pseudomonas arvilla* ATCC 23973, *Rhodococcus erythropolis*, *Rhodococcus* sp. B5 CCRC 17223 and *Rhodococcus fascians* AC6CCRC 17224, *Trametes versicolor* ATCC 42530. Among these pure cultures, based on the genus and morphology of species at various nativities levels, they are further classified into bacteria and fungi as follows.

15.2.1.1 Biodegradation of VOCs Using Bacteria

Bacteria are the oldest form of cellular life. They are also the most widely dispersed and conceivable microclimate on the planet. They vary in shape, size, and in

arrangements. General shapes include *Cocci, Bacilli, Spirilla,* and *Spirochetes*. They are identified by means of microscopic, macroscopic as well as by biochemical, serological, molecular, and genetic characteristics. Microscopic organisms are the most established type of cell life. They are most generally dispersed and possible microclimate on the planet. They change to fit as a fiddle, measure and in courses of action. General shapes incorporate *Cocci, Bacilli, Spirilla,* and *Spirochetes*. They are distinguished by methods of infinitesimal, perceptible, and additionally by biochemical, serological, molecular, and hereditary qualities. Non-putrid sulfide sets are produced by the microscopic organisms by methods of oxidizing ionic sulfide sets in the biofiltration along with O_2. Bacterial microorganisms, for example, *Pseudomonas putida, P. flurorescence, Rhodocous faccians, Alcaligens xyloxidans, Burkholderia cepacia, Hypomicrobium, Xanthobactor, Acinetobactor,* are used for the debasement of VOCs. Luis et al. (2006) detailed that the executive productivity of a biofilter achieves very nearly 100% when the inlet pollutant is 0.5 g m^{-3} or less while utilizing bacterial populace. Especially, *Pseudomonas putida* has delivered the most extreme VOC evacuation effectiveness (83%) with an inlet contamination of 1 g L^{-1}. A pilot scale consolidated bioreactor comprising of two regions, i.e., one region containing a bacterial suspension and the other region with a packed material for the development of fungus was utilized for the evacuation of xylene under stable state. Xylene alongside its three isomers was removed with a limit of 62 g m^{-3} h^{-1}. The aggregate xylene expulsion productivity of over 90% was accomplished with 24% in the main region and 67.6% in the second region (Li et al. 2003). Likewise, the impact of fluid gas stream proportions on the expulsion proficiency of the isomers was studied. At the point when the stream proportion of fluid and gas were more than 0.15, the evacuation proficiency was expanded in the primary region while diminishing in the second region.

15.2.1.2 Biodegradation of VOCs Using Fungi

Ziaie et al. (2009) reported the use of fungi microbial populace, for example, *Paecilomyces variotii, Pacilomyces, Phanerochaete chrysoporium, Fusarium solani and Cladosporium sphaerrospermum* for the debasement of VOCs. Because of the enzymatic oxidation of VOCs by lactase and peroxidase, fungi have a tendency to debase more perplexing particles. They can colonize and debase an incredible individual from substrates by utilizing them as a sole wellspring of carbon and vitality; thus, they are to a great extent, utilized in the biotechnological forms. Continuous operation with high concentration of VOC load prompts to supplement exhaustion, drought, and acidification which diminishe the microbial action and VOC's expulsion proficiency. Fungus was exceptionally favored than bacteria in view of their tolerant limit in development amid ecological variation like temperature, pH, and moisture content. Bacteria require more than 0.9 water content for its condition, though fungi can withstand its development even in the reduction of dampness content up to 0.6. Estimated airborne mycelia of fungus in coordinate contact with the gas stage can offer quicker contaminant mass exchange rate than

Table 15.6 Type of packing used in the removal of toluene by different microorganisms with removal efficiency

Microorganism	Type of packing material	Toluene removal efficiency (%)	References
Rhodococcus erythropolis	Peat biofilter	99	Malhautier et al. (2008)
Indigenous to filter media	Palm shells and activated sludge biofilter	100	Chetpattananondh et al. (2005)
Rhodococcus fascians	Peat moss and sugarcane stem (bagasse) biofilter	100	Tsai and Chi-Mei (2011)
Paecilomyces lilacinus	Perlite biofilter	53	Vigueras et al. (2008)
Acinetobacter sp. *Rhodococcus rhodochrous*	Peat and glass beads biofilter	100	Zilli et al. (2001)
Consortium	Vermiculate biofilter	90	Pineda et al. (2000)
Mixed culture	Polyurethane foam biofilter	100	Singh et al. (2010)
Consortium	Peat, vermiculite, mixture of vermiculite and activated carbon, tree bark, and porous glass rashig rings biofilter	65–95	Ortiz et al. (2003)
Paecilomyces variotii	Ceramic rings biofilter	70	Aitor et al. (2005)
Sphingobacterium multivorum Comamonas testosteroni Pseudomonas putida Pseudomonas fluorescens Chryseobacterium indologenes	Compost and perlite biofilter	70	Klapkova et al. (2006)
Penicillium sp. *Aspergillus niger Trichoderma viride*	–	75–92	Bhuvaneshwari et al. (2012)
Pseudomonas putida	Inorganic/polymeric composite chip biofilter	80	Park et al. (2008)
Cladosporium sphaerospermum	Biofilter	–	Weber et al. (1995)
Alcaligenes xylosoxidans	Biofilter	98	Daugulis et al. (2003)
Phanerochaete chrysosporium	Powdered compost biofilter	92	Zamir et al. (2011)
Mixed culture	Membrane biofilter	84	Parvatiyar et al. (1996)

(continued)

Table 15.6 (continued)

Microorganism	Type of packing material	Toluene removal efficiency (%)	References
Alcaligenes (Achromobacter) xylosoxidas	Ceramic enters to activated carbon bed bioactive foam emulsion bioreactor	89.59	Ghorbani et al. (2010)
Mixed culture	Coal biotrickling filter	100	Mathur et al. (2008)

the level of fluid in bacterial biofilter surface. A portion of the debasing fungi is given in Table 15.6 with the disposal limit and the bioreactors utilized. Weber et al. (1995) described that the development of eukaryotic microbes being better with toluene as sole carbon and vitality source. They used the fungi *Cladosporium sphaerospermum* for the expulsion of toluene from squander gasses. From the investigation of oxygen utilization and enzyme activities in the expulsion of toluene by *Cladosporium, sphaerospermum* showed that the toluene evacuation is by the underlying assault on the methyl composites. Further, they inferred that the fungus associated with biofiltration framework came about that the entire biodegradation of toluene is conceivable. Esterez et al. (2005) were accounted for the biodegradation of toluene by a secluded contagious strain, *Paecilomyces variotii*. This fungus has demonstrated the expulsion capacity at its most extreme, over the pH scope of 3.9–6.9 and the temperature between 23 and 40 °C. The action of strains diminished step by step at pH<4 and was kept up promoting the expansion of nitrate. This work presumed that the nitrate was a realistic nitrogen source for the growth of fungi *variotii*.

Vigueras et al. (2008) described the first report using *Paecilomyces lilacinus*, which utilized toluene as the sole carbon source. They utilized a gas-phase biofilters with perlite as supporting material and delivered the typical amputation ability of 50 g m^{-3} h^{-1} and evacuation proficiency of 53% with a total biomass populace of 31.6 mg for each gram of dry support. Enhanced execution of a compost biofilters by discontinuous stacking for the treatment of toluene vapor was explored. The framework was inoculated with white rot fungi, *Phanerochaete chrysosporium,* and was stacked 10 h for every day with various gas flow rates (0.096, 0.024, 0.06 m^3 h^{-1}) and toxin fixations (173.1 and 52.6 mg m^{-3}) and with no air circulation for the rest of the hours. The most extreme toluene expulsion proficiency of 92% and the amputation ability of 1913.7 mg m^{-3} h^{-1} were accomplished. The fungi *Paecilomyces variotii* CBS 115145 was tried with two unbending support materials to survey the development attributes of mycelia. The bioreactor containing 4.25 L and zone filled with ceramic raschig ring furnished with water maintenance limit and inner porosity had come about the most extreme amputation ability of 290 g m^{-3} h^{-1} under the lower water content. Prenafeta-Boldu et al. (2001) have utilized the microbial consortia isolated from soil close oil industry for the evacuation of toluene and recognized as *deuteromycete* and *ascomycete*. They revealed that the removal was hindered by 50% at the toluene fixation going in the vicinity of 2.4 and

4.7 mM. They inferred that the organism developed at low pH, and low water movement was powerful for the filtration of gas stream.

Qi and Moe (2006) reported that the removal of five segmental blends (acetone, methyl ethyl ketone, ethylbenzene, toluene, and *p*-Xylene) by means of two distinctive biofiltration frameworks. The stacking rate of VOC around 80.3 g m^{-3} h^{-1} was optimized and maintained for the action of air discharges. One biofilter was continuously operated under loading conditions and another was provided with intermittent filling of 8 h day^{-1}. After start-up of 3 weeks, the biofilter persistent stacking gave a higher evacuation productivity, while the discontinuous stacking demonstrated the proficiency after 6 weeks. The execution of biofilters with consistent stacking is around 97–99%. The outcomes demonstrated that the debasement of ketone in the two segments was gradually more, and sweet-smelling hydrocarbons were flimsy even after the constant operation of 2 months.

15.2.2 Biodegradation of VOCs Using Mixed Culture

Mixed culture produces high removal efficiency of VOCs than pure culture because it can release several toxic intermediates during biodegradation (Muñoz et al. 2007). In general, biofilm contains between 10^6 and 10^{10} CFU of bacteria and actinomycetes per gram of bed changes of the number of strains in the biofilm which relates to the removal efficiency of VOCs. Hence, the development of mixed culture as the inoculum for various studies taken from different sources are as follows: activated sludge in a wastewater treatment plant, (γ-*Proteobacteria*, few of the members of *Firmicutes*), aerobic bacterial sludge collected at Valladolid wastewater treatment plant (*Proteobacteria*, *Actinobacteria*, *Nitrospirae*, *Chloroflexi*, *Bacteroidetes*, *Planctomycetes*), microbial culture isolated from wastewater treatment plant of refinery solvent, and the mixed bacterial culture (*Sphingobacterium multivorum*, *Comamonas testosterone*, *Pseudomonas putida*, *Pseudomonas fluorescens,* and *chryseobacterium indologenes*) obtained from the aeration unit of local wastewater plant.

15.3 Biofilters

Biological decontamination of polluted vapor takes place by microbes, to convert unexpected constituents into harmless items, for example, CO_2, H_2O, and cellmass (Brauer 1986). Microbial off-gas remedy depends on the assimilation of VOC constituents in a fluid stage or biofilm by means of microbial oxidation. It is suitable for treating huge gaseous stream rate with low convergences of biodegradable contaminations. The significant sorts of bioreactors for treatment of air-phase contaminations are biofilters, biotrickling filters, and bioscrubbers. The fundamental expulsion method is comparatively similar for all types; nonetheless, contrasts exist

in the period of the organisms, which might be suspended or involved, and the condition of the fluid, which might be streaming or stationary. A few distinctive pressing materials might be utilized, including peat, manure, soil, and polyurethane foam. These supporting materials can be used along with microorganisms fit for the degradation specific to contaminants, or, on account of "selected" supporting media, for example, compost, the suitable microorganisms might be indigenous. Notwithstanding the supported media chose, the gaseous contaminants are exchanged from the gas stage into a biofilm where they are biodegraded into H_2O, CO_2, and cellmass. When all is said is done, the gas stream is humidified before entering the biofilters. Supplemental dampness might be given by water sprinkler system or utilization of soaker hoses installed in the supported medium (Devinny et al. 1999). Biofiltration is not filtration units as entirely characterized. Rather, they are a framework that utilizes a blend of fundamental procedures: assimilation, adsorption, desorption, and debasement of gaseous phase pollutants. Microorganisms develop a biofilm which is connected to the surface of a solid-packed medium. The filter bed medium usually comprises natural material (e.g., compost) or moderately inactive substances (e.g., polyurethane foam cubes), together which are mostly large surface area in the zone and some supplement supply. Biofilters are normally included in some type of water supply to control dampness and include supplements. Described to be in 1953, the ordinary sort of biofilters was utilized for treating malodorous sewer gasses in Long Beach, California, USA. At the present day with its applications comprehensively executed, biofiltration is an entrenched air pollution control innovation in numerous European countries, particularly the Netherlands and Germany. Almost, 40% of animal rendering plants in New Zealand utilize biofilters. In the USA and Canada, biofilters have prepared discoverable mass acknowledgment in many pig creation houses, aquaculture lakes, and other comparable places. In Asian nations like India and China, extraordinary research on biofilters is prompting their substantial scale usage (Saravanan et al. 2010).

The main reports on the utilization of a microbial technique to expel malodorous from a gaseous stream in a wastewater treatment plant were found in 1923 in Germany. Until the 1970s, microbial remedy of contaminations was for most time utilized as a part of soil and wastewater treatment. From the mid 70s, the strategy of expelling contaminants from squander gaseous stream by microbial debasement turned out to be more typical and viable. A driving power behind these improvements was more stringent controls of malodorous and waste disposal. An explanation behind these new controls, particularly in Europe was the diminished partition of industrialized and domestic areas. The directions and monetary help for biofiltration ventures expanded because of the utilization of biofiltration in the late 70s to the mid 80s. From that point on, more research was done and new applications were found. The toxins are oxidized by the wide assortment of microbes; usually they are existing in the supporting material in the biofilter. In the event of a latent supporting material being utilized, expert strains can be included. A toxin that is removed is different from gaseous odors (H_2S, NH_3) to profoundly dangerous mixes and solvents. In spite of the fact that biofiltration is a straightforward method, the debasement of vaporous emanation is an intricate wonder. It includes three

stages (supporting material, H_2O, and gas) and microbial kinetics (Morgado et al. 2004). The emanations treatment can be gaseous malodorous, VOCs or a mix of the two. In biotrickling system, microbes had grown on unbending supporting material or suspended in a liquid phase. As opposed to biofilters, there is a free streaming fluid stage that streams over the supporting material. As vapor goes through the reactor, pollutants are transmitted from the gas stage to a fluid stage, where they are accordingly degraded. Then again, in bioscrubbers, pollutant assimilation and pollutant debasement take place in different reactors. Absorption might be accomplished in packed column, spray towers, or bubble column. After introductory contaminant ingestion happens, the fluid stage is spread to a vessel where a packed section or suspended consortium of microorganisms performs pollutant degradation. Biotrickling and bioscrubber frameworks have the drawback of requiring more difficult structure and process; nevertheless, they offer the benefit of expanded operation control over pH, supplement content, and more parameters (Devinny et al. 1999). Leson and Winer (1991) presumed that biofiltration innovation could give noteworthy financial preferences over different techniques when connected to off-gases that contain promptly degradable toxins in low fixation. Ottengraf and Vandenoever (1983) set up that biofiltration can be a solid and economical strategy suitable for the counteractive action of air pollution.

Biofiltration method is generally utilized for expelling gaseous pollutants from an industrialized waste air or water stream. A large number of the contaminants evacuated by industrialized microbial filters are like those in indoor air; however, till date biofilters have not been utilized indoors. In spite of the fact that the pollutants come across indoors may substantially affect inhabitant well-being, their fixations in indoor air are low on contrasted with those experienced under-industrialized conditions. Industrialized microbial filters have a tendency to work under the VOCs convergence of 100 parts per million (ppm) to parts per thousands, while in indoor air, the concentrations seldom surpass 1 ppm and have a tendency to be under 100 parts per billion (ppb). Firstly, it was for the most part accepted that biofilters would not work at fixations under 1 ppm. Be that as it may, study from the University of Guelph has prompt the advancement of a biofilter particularly intended for indoor uses. Different studies have shown that the incorporation of green plants will encourage the recovery of defiled soils through the procedure of phytoremediation. The plants don't really debase the toxins specifically yet make an environment which encourages the action of valuable microorganisms. The plants assume a comparative part in these indoor biofilters. Microorganisms are settled to the packings and suspended in the fluid stage degrading the pollutants transmitted from the gas stage to the fluid stage as gas goes through the reactor.

There are various regular issues experienced in customary biofilter frameworks. Blocking is a standout among the most widely recognized issues confronted in full-scale usage. Blocking typically happens in the biofilter's estuary because of cellmass amassing in the territory of most prominent contaminant stacking. This can prompt extreme bioreactor working issues along with high pressure drops and low pollutant expulsion proficiency. The rate at which blocking can happen relies upon the idea of the supporting material, the organic stacking rate, the supply of

supplements, and different elements that impact the net yield of biomass. Keeping up legitimate dampness and supplement content in the supporting material are additionally an issue. The start-up is frequently challenging, prompting an unreasonable time of pollutant outflow from the biofilter. Pollutant outflows amid here, and now temperamental state stacking conditions have been accounted for various biofilter applications treating a wide range of mixes (Chang and Yoon 1995). A noteworthy confinement of utilizing membrane biofilters framework is from the necessity that the pollutant needs to disintegrate in the liquid phase and diffuse into the biofilm.

Evacuation of toluene by different device setups has been found in broad use throughout the previous couple of decades. Contingent upon the plan of bioreactors the disposal limit of the particular unpredictable particles, for example, toluene will differ. Predominantly utilized bioreactors are biofilters, biotrickling, bioscrubber, hollow fiber bioreactor, hybrid bioreactor, fibrous bed bioreactor, capillary bioreactor, biological activated carbon biotrickling filter, tubular bioreactor, flat sheet bioreactor, two face partitioning bioreactor, composite bead bioreactor, fixed film bioreactor, gas-fluid bed bioreactor, and bioactive foam emulsion reactor. Hollow fiber bioreactors have been utilized widely in immobilizing enzymes and microbial cells since they have huge surface region per unit volume.

Biofiltration is a novel microbial method used to treat polluted air that exploits the capacity of microorganisms to convert organic and inorganic toxins into less dangerous mixes than other ordinary methods. The multiplication of growth in biofilters was recurrent because of their capacity to endure, acid and low dampness surroundings. Fungi biofilters have demonstrated more noteworthy disposal capacities with respect to some hydrophobic mixes when contrasted with their bacterial systems. The aerobic degradation of toluene into CO_2, H_2O, and cellmass by bacterial species has been broadly contemplated. Biofiltration has turned out to be a relevant strategy for gas treatment since it is prudent contrasted with other 8 strategies. The contaminant evacuation productivity was high, and furthermore, least CO_2 was delivered by this innovation.

Biofiltration, at first connected to control scent discharges in soil beds, has turned into a setup innovation for VOC emanation control. Numerous studies and uses of biofiltration innovation on VOC expulsion were accounted for. All the more as of late, a few novel biofilters fit for achieving higher evacuation efficiencies were established. Studies were led utilizing polyurethane foam plugs and destroyed polyurethane foam blended peat while picking perlite and vermiculite as supporting media, presuming that the polyurethane foam did not have properties that were as ideal for biofiltration as different materials studied.

Biofiltration is generally another contamination control innovation. This eliminates the rank gas outflows (unstable natural mixes) of low concentrations all the more proficiently. It is a most modus working and productive in treating huge volumes of air streams soaked with low centralization of VOCs. It can treat more polluted wind streams from 60 to 150000 m^3/h with low VOC focusing viably and monetarily than most other accessible technologies. There have been more than 50 professional biofilters utilizing manure sort material introduced in Europe and the USA in the course of recent years.

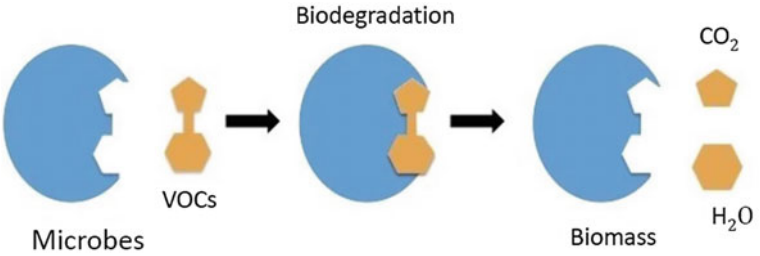


Fig. 15.5 Mechanism involved in the VOCs biodegradation process using microorganisms

This favorable spot allows the biofilter framework to be connected to littler scale ventures, for example, potentially the retrofitting building ventilations with a compressed VOC remedy framework. Existing bio-treatment of VOCs utilized as a part of gas treatment spins around the idea of biofiltration. There are different other biofiltration forms accessible, for example, biotrickling filters (BTFs), bioscrubbers, and the fresher innovation like membrane bioreactors (Doble and Kumar 2005). Biofiltration commonly works in view of two principles: primarily, the exchanges of toxins from the gaseous nourish to the packed medium and also the pollutants are biodegraded to cellmass, CO_2, H_2O, and other side products (shown in Fig. 15.5). The customary biofiltration framework is basically a packed bed bioreactor where the biocatalyst or microbes are immobilized in an inactive-supported medium to prepare biofilm. A perfect support material ought to have the accompanying properties: high void division, lightweight, low pressure drop, hydrophilic, and low bulk density. A suitably kept up regular natural supporting material like peat, vegetable mulch, bark, or wood chips may keep going for quite a while, however, designed (combined natural and engineered component) supporting materials will last any longer, up to 10 years. Many organizations offer these sorts of restrictive supporting materials with long-term assurance, not for the most part that gives the regular manure or wood chip bed biofilters. Infrequent water system of supplement arrangement onto the supporting bed and controlled moisture content keeps up the biofilm adequately. Contaminated air is nourished from the base through the microbial dynamic media, where contaminations diffuse into the fluid stage or they retained straightforwardly by the biofilm with treated gas released from the top. Shockingly, VOCs containing sulfates, chlorides, and nitrates have acidic side products that can antagonistically influence the biological community and diminish the viability of the bioreactor.

15.3.1 Type of Bioreactors Employed for Toluene Removal

Many types of bioreactors are employed in the degradation/removal of VOCs by many researchers. For successful application of any reactor types, it is important to

analyze the performance characteristics of the reactors and studying many parameters like the type of packing material, flow dynamics, loading rate, and microbial characteristics.

15.3.1.1 Biotrickling Bioreactor (BTBR)

In biotrickling filter (shown in Fig. 15.6), the supplement arrangement is ceaselessly flooded from the top and gathered at the base and reused. Parallel (co-current) or countercurrent stream between the vaporous and liquid stages was found to have no effect on the debasement execution. The biotrickling framework takes into account more effective expulsion of dissolvable VOCs from the air stream as well as allowing response control for supplement levels and pH. As there is a steady-state feed of supplements to the framework, abundance biomass develops rapidly and can prompt execution damage and pressure drops from uneven biomass circulation. In biotrickling filter, proper selection of packing material would disturb the efficiency of device in toluene removal and higher pressure drop through the biofilm also affects the toluene degradation. In packed flow reactor, undesired thermal gradients may exist, poor temperature control, channeling may occur, and unit may be difficult to service and control. In fluidized bed reactor, the mechanism is not well understood, severe agitation can result in catalyst destruction and dust formation and results in uncertain scale up. Fibrous bed bioreactor is associated with some problems such as cost, membrane fouling, and blockage. Packed bed bioreactor has its own favorable circumstances and in contrasted with different

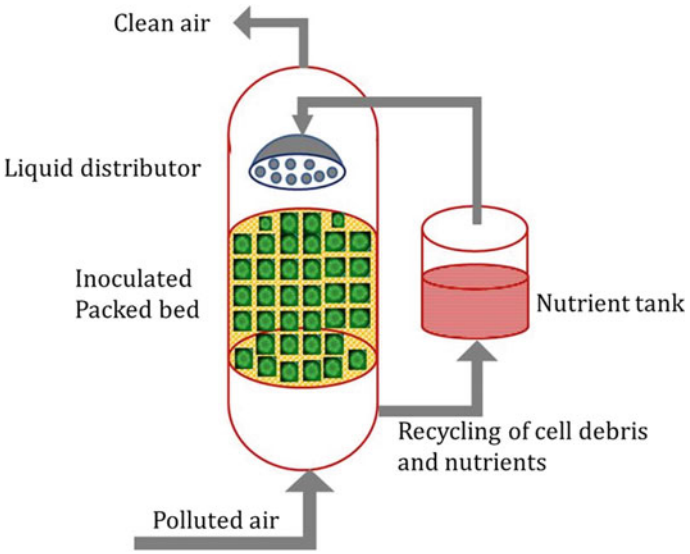


Fig. 15.6 Schematic representation of biotrickling filter for VOC removal

bioreactors, for example, straightforward operation, minimum cost, high response rate, enzymes, or cell immobilized with suitable transporter, which are stuffed in reactors bringing about high solid–fluid actual interfacial contact zone, and the speed of fluid crawling over the static solid particles considerably eases the film imperviousness to mass exchange and furthermore high debasing effectiveness of toluene utilizes distinctive microbes.

15.3.1.2 Bioscrubber Bioreactor (BSBR)

Bioscrubber (shown in Fig. 15.7) consists of an immersion tower and a bioreactor. In immersion tower, the gaseous stage pollutants are dispersed into a fluid by methods for countercurrent gas-fluid stream through idle supports. The outlet gas is released from the top, and the polluted fluid is pumped toward an aerator bioreactor. The microbes or activated sludge in the reactor is dispersed in a supplement-rich media, and the contact time for remedy is in accordance's to the method and concentration of VOCs in the inlet feed. After the biodegradation of pollutants, the medium is separated and the biomass is left to silt with partitions being reused by the bioscrubbing procedure once more. A liquid-phase framework with no high pressure drop takes into account all the more equitably appropriated temperature, supplement, and pH controls. Framework impediments are because of the restricted band of VOCs treatable. This range is constrained by water solubility of pollutants, in this way just applying to organics with low Henry's coefficient (<0.01) at low fixations (<5 g/m^3). Contrasted with different bioreactors considered, the

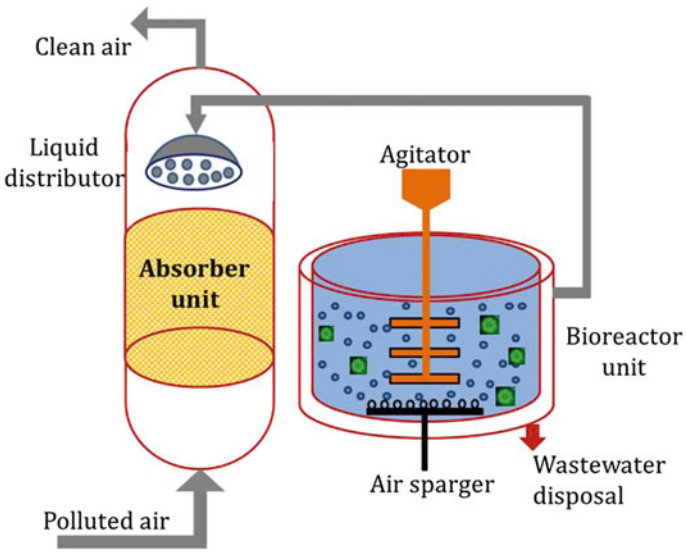


Fig. 15.7 Schematic representation of bioscrubber for VOC removal

bioscrubber requiring two sub-units could be a space constraint; particularly when numerous frameworks in arrangement or parallel are required to process higher VOC volumes. Bioscrubbers, in any case, are not also used within the biotechnology, contrasted with biofilters and biotricklers. A bioscrubber reactor is significantly more costly to introduce than different bioreactors. It has a synthetic scrubber at the heart of the process and takes after substance handling gear more than different bioreactors. Over sustaining can cause extreme biomass development, which can plug the bioscrubber. Working expense can be higher than other bioreactor procedures, and it needs costly and complex bolstering and counteracting frameworks. To control biomass development, lethal and hazardous mixes must be presented. Bioscrubber was not appropriate for pollutants with low fluid solubility (Deshesses and Huub 2003).

15.3.1.3 Two-Phase Partitioning Bioreactor (TPPB)

Two-stage partitioning bioreactors have been utilized as a part of combination with an immersion section containing organic compound to clean VOCs from air stream. The advantage of TPPB framework was that the VOCs can be evacuated in a single stage framework with high volatile organic compound loadings and high expulsion efficiencies (Daugulis and Boudreau 2003).

15.3.1.4 Fluidized Bed Bioreactor (FBR)

FBR utilizes fluidized particles as a biofilm transporter for the aerobic evacuation of organic mixes (Koch et al. 1991). Sand or activated carbon has been the most habitually utilized biofilm transporters. The deterioration of different VOCs incorporating halogenated aliphatics in groundwater was contemplated utilizing a pilot scale pure oxygen circulated air through FBR. Monitoring of bioreactor was easy in this type of reactor, and good mixing was achieved. It achieved 99% degradation of volatile organic compounds. Aerobic fluidized bed and GAC bioreactor treating typical pollutant fixations in groundwater were accounted for. Within a short hydraulic residence time (HRT 5 min), the contaminants of benzene, toluene, ethylbenzene, and xylene (BTEX) were removed from 5,420 to 64 parts per billion (98.9% expulsion). Especially, the benzene evacuation was surpassed 99.9% (under 1 ppb lingering benzene). By utilizing an anaerobic fluidized bed GAC bioreactor for the treatment of moderate-to high-strength leachate, an exceptionally proficient expulsion (98–99% of chlorinated aliphatic VOCs, 85–97% aromatic and ketone VOCs, and 97–99% semi-volatile organic compounds) was accomplished at HRTs of 3–12 h. The costs of these fixed film systems differ contingent upon the application qualities. Capital expenses are by and large focused with elective advances, for example, actuated carbon promotion sorption; however, the working costs, particularly long haul, are considerably lower than those of alternative technologies.

15.3.1.5 Fixed Film Bioreactor (FFBR)

Fixed film bioreactors have turned out to be conservative expertise for treat decomposable pollutants in vapor and liquid (Hickey et al. 1990). The fixed bed bioreactors utilize either fixed extended or fluidized beds of inactive or adsorptive media to withstand the biofilm for debasement of pollutants. Experimentally, inactive media are comprised of ceramics, plastics, stones, sands, and woodchips. Pollutants expulsion from the gaseous or liquid is accomplished through biofilm sorption. Adsorptive media, normally peat or granular-activated carbon (GAC) expels pollutants from the gaseous or liquid through both biosorption and physisorption. Even, the exceptionally adsorptive media, for example, GSC are expensive; it provides the improved production by constraining microbial restraint from hazardous pollutants and yielded increased expulsion proficiency, particularly amid the start-up of the reactor. The GSC media also improve the biosystem response to widely varying contaminant concentrations.

15.3.1.6 Upflow Packed Bed Reactor (UFPBR)

This technology guaranteed realistic reactor volume and low hydraulic residence time escorted with great energetic cellmass fixation in the reactor. The linkage of microbes over the packing with huge specific surface region prompts to maximum cellmass yield and high response rates since the bioreactor volume should be limited. Toluene removal was studied in upflow packed bed reactor using pretreated walnut shell as packing material (void volume of 66%). The highest toluene removal efficiency of 62% and the COD removal efficiency of 80% was achieved (Saghafi et al. 2010).

15.3.1.7 Foam Emulsion Bioreactor (FEBR)

It contains a suspension of exceedingly dynamic contamination removing microorganism and a liquid immiscible organic stage which was made into foam with the vapor being treated (Phipps 1998). The FEBR was like TPPB; however, the measure of organic stage was low and it utilizes a biocompatible surfactant for foam formation. To achieve huge amount of contamination evacuation rates, the FEBR depends on a very high microbial concentration of effectively developing microbes. In the meantime, bed blockage and related pressure drop issues are overcome by strategic utilizing moving foam rather than an immobilized culture developing on a bed. The mass exchange of BTX (benzene, toluene, and xylene) from the gaseous to the fluid stage was quick because of the expanded interfacial surface region of the surfactant foam. What's more, the toxins dissolvability was expanded by expanding the organic stage concentration; the ideal response time (15 s) of the bioreactor was not as much as biotrickling reactor. Low residence time expanded the disposal capacity of bioreactor. The nominal and maximum

elimination capacities of this bioreactor are higher than those of the biotrickling reactor, while the bed clogging and pressure drop were not encountered.

15.3.1.8 Membrane Bioreactor (MBR)

In MBR consists of membranes splitting the gas and liquid phases. The supplement and cellmass development in the fluid side while the vaporous contaminants are pressurized to actuate dissemination over the segment into the watery arrangement. The films can be additionally treated to have different worthwhile properties, e.g., hydrophobic and microporous. MBR for VOC treatment still requires more research to limit fouling and high cost (Artiga et al. 2005). Membrane bioreactor is associated with some disadvantages such as high cost, high cleaning process requirement, and membrane fouling. Major limitation of using membrane biofilter systems is from the necessity that the contaminant desires to liquefy in the watery stage and diffuses into biofilm on the grounds that contaminants with low water solvency will follow low degradation rates where contaminants transport into the biofilm will constrain the productivity. Foam bioreactor is associated with high cost and lower surface area for deposition of catalytic material and also this bioreactor is present in the initial stage of development.

15.3.2 Packing Materials

As the packed bed substantially supported by the biosystem, some of the standards required to obtain superior situations are high-unique superficial region which is excellent for microbial action, acceptable retaining water capacity and adequate water permeability. Simple carbon-based natural materials are used for packing such as composts, peats, and soils, because of their constancy, efficacy, and low cost (Doble and Kumar 2005). Recent strategies are enabling the column to have thoroughly spread constructions preventing bed compaction. Moreover, biocatalysts can be immobilised within a porous shape in structure (polymer beads) to allow for simpler protection and care of specific microorganisms. Pressure drop of the filter bed is an essential parameter for biofiltration process which significantly influences the operating cost. To meet the successful biofiltration process, the packing material must satisfy the following requirements. Definite superficial zone is denoted as “packed density” which directly relates to microbial population based on the wettability on the large free (or clear) passage diameter with minimum resistance to plugging or clogging parameter. Laboring or obstructing of a biofilter can manifest through mechanical catching of debris, similarly other particulate matters in biofilter. Laboring also can result from the increase cellmass concentration and crossing over the inside galaxy of packing. Struggling propensity for different packed zone can be anticipated or compared by looking at the void fraction and entry-free breadth.

Type of packing and its characteristics plays a major role in biofilter system for efficient performance. Many researchers were tried with different types of packing material in biofilter system for efficient removal of gaseous pollutants from air stream. Basically, there are two types of packing materials (i) organic supports/biodegradable matters—these backings have the benefit of containing a high-beginning microbial load and a few supplements. For the most part, they are anything but difficult to discover and cheap. The drawbacks are they have a tendency to be in the end biodegradable coming about lost movement and structure and require substitution (ii) dormant backings/non-biodegradable issues—these require the expansion of mineral supplements; however, they have the benefit of having a uniform structure and size, which diminishes compaction and permits better wind stream dispersion. Besides, the greater part of them opposes water expansion and they can be cleaned and recovered.

15.3.3 Suggestions and Future Scope of Work

- The present biofiltration system could be extended to mixed pollutants
- Hydrodynamic parameters in the column could be studied to understand the mass transfer aspects.
- Properties and conditions of packing could be studied elaborately to improve the performance and stability further.
- Some more parameters (e.g., microbial population, bed moisture content, porosity) could be included in the model for kinetic analysis.

15.4 Summary

With the quality of air peaking down so rapidly, along with its consequences, transition to a varied approach has become mandatory. Bioremediation of volatile organic compounds may prove as an effective key in the development of future technologies whose successful implementation will bring up a bloom a new world with new perspectives. Future projection in the field of technology is hard to visualize but possible to achieve. Thus, one major influence can change the way everything sustains.

References

Aitor A, Bertrand D, Pierre C, Richard A, Ines G-P, Sergio R (2005) Fungal biofilteration of toluene on ceramic rings. J Environ Eng 396–402

Artiga P, Oyanedel V, Garrido JM, Mendez R (2005) An innovative biofilm-suspended biomass hybrid membrane bioreactor for wastewater treatment. Desalination 179:171–179

Asadi A, Huat BBK, Hanafi MM, Mohamed TA, Shariatmadari N (2009) Role of organic matter on electroosmotic properties and ionic modification of organic soils. Geosci J 13(2):175–181

Berenjian A, Fathi A, Feghenabi S, Khodiev A (2011) Complete removal of toluene from air: a response surface methodology. Aust J Basic Appl Sci 5(6):286–288

Bhuvaneshwari S, Deepa Priya AG, Dhayananda Shakar AR, Sharath Kumar CS, Gopinath M, Dhanasekar R (2012) A comparative study on removal of toluene by batch reactor using different fungal culture isolated from municipal sewage water. Bonfring Inter J Indus Eng Manag Sci 2(1):25–29

Brauer H (1986) Biological purification of waste gases. Int Chem Eng 26(3):387–395

Chakrabarti S (2004) 20th anniversary of world's worst industrial disaster. Australian Broadcasting Corporation

Chan WC, You HY (2010) The influence of nonionic surfactant Brij 30 on biodegradation of toluene in a biofilter. Afr J Biotechnol 9(36):5914–5921

Chang A, Yoon H (1995) Biofiltration of gasoline vapors. In: Hodge DS, Reynolds FE (eds) Proceedings of the Conference on Biofiltration, University of Southern California, pp 123–130

Chetpattananondh P, Yada N, Charun B (2005) Biofilteration of air contaminated with methanol and toluene. Songklanakarin J Sci Technol 27(3):761–773

Daugulis AJ, Boudreau NG (2003) Removal and destruction of high concentrations of gaseous toluene in a two phase partitioning bioreactor by Alcaligenes xylosoxidans. Biotechnol Lett 25 (17):1421–1424

Davis D (2002) When smoke ran like water: tales of environmental deception and the battle against pollution. Basic Books. ISBN 0-465-01521-2

Dees C, Askari M, Henley D (1996) Carcinogenic potential of benzene and toluene when evaluated using cyclin-dependent kinase activation and p53-DNA binding. Environ Health Perspect 104(6):1289–1292

Delhomenie MC, Heitz M (2005) Biofiltration of air: A review. Crit Rev Biotechnol 25:53–72

Deshesses MA, Huub HJ (2003) Biotrickling filter air pollution control. Encyc Environ Microbiol. https://doi.org/10.1002/0471263397.env105

Devinny JS, Deshusses MA, Webster TS (1999) Biofiltration for air pollution control. CRC Lewis Publishers, Boca Raton, Florida, USA

Doble M, Kumar A (2005) Biotreatment of industrial effluents, 1st ed. Butterworth-Heinemann, p 336. ISBN-10: 0750678380

Estévez E, Veiga MC, Kennes C (2005) Biofiltration of waste gases with the fungi Exophiala oligosperma and Paecilomyces variotii. Appl Microbiol Biotechnol 32(1):33–37

Forbes P (2015) Monitoring of air pollutants: sampling, sample preparation and analytical techniques, vol 70. Elsevier

Ghorbani SF, Golbabaei F, Hamedi J, Mahjub H, Darabi HR, Shahtaheri SJ (2010) Treatment of benzene, toluene and xylene contaminated air in a bioactive foam emulsion reactor. Biotechnol Bioeng (Chin J Chem Eng) 18(1):113–121

Hickey RF et al (1990) Combined biological fluid bed-carbon adsorption system for BTEX contaminated ground-water remediation. Paper presented at the fourth national outdoor action conference on aquifer restoration, groundwater monitoring and geophysical methods, Las Vegas, NV

Khan IF, Aloke G (2000) Removal of volatile organic compounds from polluted air. J Loss Preven Proc Ind 13:527–545

Klapkova E, Martin H, Mark F, Carlos Riccardo S, Jan P (2006) Impact of biocatalyst and moisture content on toluene/xylene mixture biofilteration. Brazilian Arch Biol Technol 49(6):1001–1006

Koch B, Ostermann M, Hoke H, Hempel DC (1991) Sand and activated carbon as biofilm carriers for microbial degradation of phenols and nitrogen-containing aromatic compounds. Water Res 25:1–8

Lee DH, Lau AK, Pinder KL (2001) Development and performance of an alternative biofilter system. J Air Waste Manag Assoc 51:78–85

Leson G, Winer AM (1991) Biofiltration: an innovative air pollution control technology for VOC emissions. J Air Waste Manag Assoc 41(8):1045–1054

Li C, Moe WM (2003) Sequencing batch biofilter treatment of methyl ethyl ketone contaminated air. Environ Technol 24(5):531–544

Luis B, Jim H, Stuart N, David S, Andy PS, Christopher MT, Janet W, Nichola HW, Gareth RW, Alan RF, Andrew DG (2006) Analogues with fluorescent leaving groups for screening and selection of enzymes that efficiently hydrolyze organophosphorus nerve agents. J Med Chem 49:246–255

Malhautier L, Avezac M, Rocher J, Roux JC, Fanlo JL (2008) Toluene degradation capabilities of strains isolated from a peat biofilter used for the treatment of a complex mixture of VOCs. Chem Eng 15:337–344

Mathur Anil K, Majumder CB (2008) Biofiltration and Kinetic aspects of a biotrickling filter for the removal of paint solvent mixture laden air stream. J Hazar Mater 152:1027–1036

Moe WM, Irvine RL (2001) Polyurethane foam based biofilter media for toluene removal. Water Sci Technol 43:35–42

Morgado J, Merlin G, Gonthier Y, Eyraud A (2004) A mechanistic model for m-xylene treatment with a peat-bed biofilter. Environ Technol 25:123–132

Muñoz R, Villaverde S, Guieysse B, Revah S (2007) Two-phase partitioning bioreactors for treatment of volatile organic compounds. Biotechnol Adv 25:410–422

Ortiz I, Revah S, Auria R (2003) Effects of packing material on the biofilteration of benzene, toluene, and xylene vapors, Environ Technol 24:265–275

Ottengraf SPP, Vandenoever AHC (1983) Kinetics of organic-compound removal from waste gases with a biological filter. Biotechnol Bioeng 25:3089–3102

Park B-G, Won Sik S, Jong Shik C (2008) Simultaneous biofilteration of H2s, NH3 and toluene using an inorganic/polymeric composite carrier. Environ Eng Res 13(1):19–27

Parvatiyar Madan G, Rakesh Govind, Dolloff F Bishop (1996) Biodegradation of toluene in a membrane biofilter. J membrane sci 119:17–24

Phipps DW (1998) Biodegeneration of volatile organic contaminants from air using biologically activated foam. US Pat 57:143–179

Pineda J, Auria R, Perez-Guevara F, Revah S (2000) Biofilteration of toluene vapours using a model support. Bioproc Eng 23:479–486

Prenafeta-Boldu FX, Kuhn A, Luykx DMAM, Anke H, Van Groenestijn JW, De Bont JAM, Mycol Res 105:477–484 (2001)

Qi B, Moe WM (2006) Performance of low pH biofilters treating a paint solvent mixture: continuous and intermittent loading. J Hazard Mater 135(1–3):303–310

Saghafi S, Bakhshi Z, Ghasem D, Najafpour EK, Rad HA (2010) Biodegradation of toluene and xylene in an UAPB bioreactor with fixed film of pseudomonas putida. Am-Eurasian J Agric Environ Sci 9(1):01–07

Saravanan V, Rajasimman M, Rajamohan N (2010) Biofiltration kinetics of ethyl acetate and xylene using sugarcane bagasse based biofilter. Chem Eng Res Bull 14:51–57

Sharma RK, Agrawal M, Marshall FM (2009) Heavy metals in vegetables collected from production and market sites of a tropical urban area of India. Food Chem Toxicol 47:583–591

Singh RS, Rai BN, Upadhyay SN (2010) Removal of toluene vapour from air stream using a biofilter packed with polyurethane foam. Proc Safety Environ Protect 88:366–371

Tankersley J (2010) August 14, 2012. EPA proposes nation's strictest smog limits ever. Los Angeles Times

Tham YJ, Abdul LP, Abdullah AM, Devi AS, Yap TYH (2011) Performances of toluene removal by activated carbon derived from durian shell. Bioresour Technol 102(2):724–728

Tsai Y-C, Chi-Mei L (2011) Addition of rhodococcus fascians Ac6 to prevent inhibition of the toluene degradation from ethyl acetate in biofiltration of Vocs—contaminated air stream. Ipcbee 8:276–280

Vigueras G, Shirai K, Martins D, Franco TT, Fleuri LF, Revah S (2008) Toluene gas phase biofilteration by Paecilomyces lilacinus and isolation and identification of a hydrophobin protein produced thereof. Appl Microbial Biotechnol 80:147–154

Weber FJ, Hage KC, De Bont JAM (1995) Growth of the fungus Cladosporium sphaeropermum with toluene as the sole carbon and energy source. Appl Environ Microbiol 61(10):3562–3566

World Health Organization (2016) (press release). http://www.who.int/mediacentre/news/releases/2017/pollution-child-death/en/. Accessed 01 July 2017

Yoon IK, Park CH (2002) Effects of gas flow rate, inlet concentration and temperature on biofiltration of volatile organic compounds in a peat-packed biofilter. J Biosci Bioeng 93: 165–169

Zamir SM, Rouein H, Bahram N (2011) Removal of toluene vapors using a fungal biofilter under intermittent loading. Proc safety Environ prot 89:8–14

Ziaie J, Behnamnia M, Kalantari KM (2009) The effects of brassinosteroid on the induction of biochemical changes in Lycopersicon esculentum under drought stress. Turk J Bot 33:417–428

Zilli M, Emilio P, Luciane S, Attilio C, Marco DB (2001) Toluene and styrene removal from air in biofilters. Proc Biochem 37:423–429

Chapter 16
Bioremediation of Industrial and Municipal Wastewater Using Microalgae

Baskar Gurunathan, I. Aberna Ebenezer Selvakumari, R. Aiswarya and S. Renganthan

Abstract The present scenario of increased population and industrial development leads to deterioration of freshwater and decreases the quality of water all over the world. This causes freshwater shortage in most of the area. Moreover, organic and inorganic substances released from various sources into the existing natural water bodies or environment lead to pollution. The primary and secondary treatment of wastewater had been introduced in many numbers of places to extinguish the easily settled material and to oxidize the organic matter in wastewater. But these methods were not found to be efficient because the effluent from secondary treatment is loaded with increased amount of inorganic nitrogen and phosphorus, and so, it leads to eutrophication and much more long-standing problems because of discharged heavy metals and refractory organics. On the other side, wastewater contains numerous ingredients, and interestingly, some of the compounds in the wastewater, like nitrogen and phosphorus, are identified as beneficial ingredients for microalgae cultures. Therefore, algal bioremediation can be considered as a feasible alternate technology for treating the wastewater in a cost-effective and assertable way compared to conventional water treatment process. These microalgal cultures are autotrophs, and they play a notable role in remediation of wastewater by their photosynthetic ability. A win–win situation of using microalgae in the bioremediation of industrial or municipal wastewater provides tertiary biotreatment of wastewater coupled with the production of potentially valuable biomass as bioresource for biofuel or high-value by-products. There is a mutual advantage in this method in which using wastewater for microalgal culture will minimize the use of freshwater, reduce the cost of nutrient addition, and also removal of nitrogen and phosphorous, and reduce CO_2 emission. This chapter covered the overview of the role of microalgae in treatment of industrial as well as municipal wastewater.

B. Gurunathan (✉) · I. A. E. Selvakumari · R. Aiswarya
Department of Biotechnology, St. Joseph's College of Engineering, Chennai 600119, India
e-mail: basg2004@gmail.com

S. Renganthan
Department of Biotechnology, A.C.Tech., Anna University, Chennai 600025, India

S. J. Varjani et al. (eds.), *Bioremediation: Applications for Environmental Protection and Management*, Energy, Environment, and Sustainability,
https://doi.org/10.1007/978-981-10-7485-1_16

Keywords Industrial wastewater · Tertiary treatment · Bioremediation
Microalgae · Autotrophs

16.1 Introduction

Water connects every aspect of life. Even though the world is covered with two-third of water, majority of it is not potable. The rough approximation says that only 2.5% of water in our planet is freshwater and the rest belongs to saltwater; 69% of the freshwater is present as frozen ice. As life on earth is ultimately depends on freshwater, it is a typical important resource in this planet. As the population continues to grow, earth water resources are subjected to ever-increasing pressure. Due to increased population growth, the withdrawals of water for domestic, agricultural, and other own uses are constantly increased. The rapid industrialization also imposes several dangerous risks to both availability and quality of water resources all over the world. In recent days, the freshwater and its importance are recognized universally than earlier days and arguments concerning satisfactory water management can be turned up almost in every scientific, social, and political agenda all over the world; water resources seem to encounter severe quantitative and qualitative risks because of various reasons. It is truism nowadays that the society started to recognize the pollution-associated problems as a major concern for society. Environmental laws are addressed for all general applicability, and the enforcement of these laws has been strictly evaluated. Thus, this present scenario evidently proves that the fight against pollution has started worldwide regarding health, environment, and economy.

The effects of water pollution not only affect human life but also devastate the aquatic organisms and terrestrial animals and birds. More seriously, contaminated water destroys the whole aquatic life and reduces their reproductive ability. Ultimately, the water becomes unfit for human consumption or domestic usage.

Due to the pollution and exhaustion of water resources, today's generation is facing a major water crisis. Polluted water is in any way the type of water which has been in some manner negatively affected in quality by anthropogenic influence. According to U.S. geological survey, it is proposed that by the year 2025 international water thirst will go beyond the supply by 56%. To resolve this water crisis and to cope with the world's growing population's demand for freshwater, various wastewater treatment techniques concentrated at remediating polluted wastewater. There are chiefly two groups of wastewater, i.e., municipal wastewater and industrial wastewater. Municipal wastewater resides in domestic wastewater hailed from family units, sewer overflows, and storm drains, whereas a wide range of industrial wastewater may contaminate with feedstock materials, by-products, washing and cleaning agents, solvents, and product materials in soluble or particulate form. The indiscriminate disposal of domestic and industrial wastewater gives rise to heavy water pollution that puts at risk all set of inhabiting organisms (Aziz and Ng 1992). Therefore, the remediation necessarily centralized on the treatment

techniques and actions to moderately or entirely remove contaminants (nitrates, phosphates, and heavy metals) from wastewater before releasing them into waterways.

Almost all wastewater treatment plants implement conventional wastewater treatment methods to cut down the contaminant concentrations and to promote the quality of wastewater sewage before it discharges to groundwater or moves back into various water bodies. Even though there may be many differences in wastewater treatment plants, most will have some common steps including preliminary treatment, primary treatment, secondary treatment, tertiary treatment, disinfection, and solids handling. These methods are engaged only for treating of municipal wastewater and are not constructed and equipped for handling noxious industrial waste. These toxic and hazardous wastes are pretreated at the source of generation (industries) for cutting down their concentration level and are transmitted to wastewater treatment plant as industrial wastewater (Camargo and Alonso 2006).

In short, municipal and industrial wastewater is initially collected and subjected to following treatment processes: primary and secondary treatment. During primary treatment, the bar screens are used to remove large solids from the wastewater. The pumping of water to pre-aeration tanks is followed where grit (sand and other solids) is removed. Then, the wastewater is transferred into a primary clarifier, and the suspended solids are allowed to settle down known as primary settlement. After the primary clarifier, the wastewater may still contain increased amount of organic content, and so, it undergoes secondary treatment. In this treatment, the organic matters in the wastewater are degraded by microorganisms within the aeration tanks, and the waste sludge formed is allowed to settle in the secondary clarifier (Han et al. 2000). Next, sludge that settled down on the bottom of both the primary and secondary clarifiers is pumped into a digester to produce methane and carbon dioxide. Still, the water leaving the secondary clarifier may contain estimable amounts of nitrogen and phosphorus and release of this water may act as a reason for eutrophication in rivers and other water bodies. As these treatment processes posses no special methods to remove any heavy metals from wastewater, these contaminants have a direct impact on environment by two aspects. First, these contaminants will persist in natural ecosystem for an extended period, and second, they would accumulate in biological food chains and might gain the tendency to cause chronic and acute disorders in living beings.

16.2 Bioremediation

The widely used treatments that emphasize on sustainable wastewater treatment worldwide are sewage treatment plants (STP) and effluent treatment plants (ETP). These two treatments exclusively depend on costly chemicals and heavy inputs of energy. But the industries are very much interested in initiating cheaper methods that could provide a long-term sustainable solution for treatment of wastewater.

Moreover, it is very important to remove all the nitrogen and phosphate contents from wastewater, and this can be done by tertiary treatment known as bioremediation. It is defined as the elimination, attenuation or transformation of polluting substances by the application of biological processes (Validi 2001). In this system, rapid sand filters, microstraining, and fluidized bed systems are commonly used. Therefore, the microorganism involved plays a crucial role in neutralizing the pollutants from wastewater. This process may occur on its own but can effectively occur only by the addition of nutrients, oxygen, etc., that enhance the pollution, eating microbial growth within the medium. Thus, many associations and industries across the world are intensively looking into bioremediation as a viable source to clean wastewater. This is because bioremediation can also prove less expensive than other technologies due to significant time and planning needed for successful treatment.

Bioremediation can work on wide range of organic and inorganic compounds. Many microbes are involved in this process. Aerobic bacteria including *Pseudomonas, Alcaligenes, Sphingomonas, Rhodococcus,* and *Mycobacterium* have been reported to degrade pesticides and hydrocarbons, both alkanes and poly-aromatic compounds (Muthukumaran et al. 2005). Anaerobic bacteria are not conventionally used as aerobic bacteria. There is a booming interest in anaerobic bacteria used for bioremediation of polychlorinated biphenyls and dechlorination of trichloroethylene (TCE) and chloroform. Lignolytic fungi such as *Phanaerochaete chrysosporium* are also widely used to attenuate diverse range of toxic environmental pollutants. In recent days, microalgae gain a keen focus in bioremediation of wastewater.

16.3 Phycoremediation

It can be represented in a broader sense as the application of macroalgae or microalgae in bioremediation process for the elimination or bioconversion of contaminants including nutrients and xenobiotics from wastewater. Phycoremediation is particularly drawing attention because it can handle more than one problem on-site, a solution not capable of conventional chemical process. Thus, the microalgae usage in nutrient cutoff from various effluents has been described by many authors.

(a) **Features of Phycoremediation**

i. Compatible with existing operations (Pittman et al. 2011)
ii. Profits from both algal biomass and additional biochemicals that are extracted from algal source (Olguin 2003)
iii. This can be performed in batch-wise, semicontinuous, or continuous manner (Rao et al. 2011)
iv. CO_2 sequestration can act as a resolution for the endangerment of global warming (Redalje et al. 1989)

v. Compatible in handling fluctuations in terms of both quality and quantity of effluent feed (Rawat et al. 2011)
vi. Environment oxygenation (Rawat et al. 2011)
vii. Biofuel and biofertilizers are also probably obtained as by-products (Park et al. 2011)
viii. Efficient in minimizing automation, maintenance, and requirement of skilled operators (Ward et al. 2014)
ix. Selectively, the contaminant (metals) alone is removed under considerations
x. Sustainable and eco-friendly from an ecological aspect (Rawat et al. 2011).

(b) **Characteristics of Microalgae**

As the microalgae belong to unicellular organism, they are microscopic and are phototropic usually found as individuals or in groups in marine or freshwater environment, and it consumes carbon dioxide, nitrogen, and phosphorus and releases oxygen. Their photosynthetic mechanism is analogous to land-based plants, but as they possess simple cellular structure and are immersed in an aqueous environment, they could efficiently access water, CO_2, and other nutrients, and thus, they are highly skilled in converting solar energy into biomass (Barsanti and Gualtieri 2006). Most microalgae species can concentrate extracellular polysaccharides like gelatinous mass which surrounds their cells. The membrane components of microalgae are lipids and fatty acids, and they act as storage products, metabolites, and energy source. In terms of biomass, microalgae are the largest primary producers worldwide. These are the universal primary producers having high affinity toward polyvalent metals and also effectively clear the heavy metals, nitrates, and phosphates present in wastewater (Laliberte et al. 1997). Therefore, the application of algae in the bioremediation of wastewater offers probable benefits over other techniques in use.

(c) **Promising Attributes of Microalgae**

(1) Having higher photosynthetic capabilities than compared to higher plants (Bhatnagar et al. 2011)
(2) Able to transform solar energy and CO_2 emissions from power plants and reduce the energy requirements (Rosenberg et al. 2011)
(3) Capable of making the disposal easy by incorporating the leftover nutrients such as nitrogen and phosphorus from effluent for their own growth purpose (Bhatnagar et al. 2011)
(4) Manage extreme adverse conditions (Bhatnagar et al. 2010)
(5) Capable of reducing greenhouse gas emissions (Rao et al. 2011)
(6) Have extensive applications of harvested biomass (Bhatnagar et al. 2011).

The utilization of microalgae in wastewater treatment is further strengthened by some of the useful features of microalgae (Table 16.1). Therefore, cultivation of microalgae in wastewater offers the multiple advantages in wastewater treatment, mitigation of greenhouse gases, and production of algal biomass simultaneously.

Table 16.1 Comparison of wastewater treatment potential of microalgae and higher aquatic macrophytes (Renuka 2014)

Characteristics	Microalgae	Higher aquatic macrophytes
Doubling time	1–2 days	Prolong time is required for doubling of biomass
CO_2 sequestration potential	Higher photosynthetic efficiency and thus offers dual advantage in reduction of greenhouse effect and the resultant global warming	Capable of lower photosynthetic efficiency hence, CO_2 mitigation potential is also minimal
Space requirement	Smaller dimensions	More space for their maintenance and growth
Processing	Scale-up can be done efficiently because they can be harvested easily (filamentous nature or flocculation ability)	Difficulties in scaling up at commercial levels because of rooted nature of macrophytes

(d) **Goals of Phycoremediation**

(a) Tertiary treatment of wastewater
(b) Nutrient removal from municipal and industrial wastewater with high organic matter
(c) Treatment of acidic and metal wastewater effluents
(d) CO_2 sequestration

(e) **Advantages of using Algal Technology**:

i. Cost-effective
ii. Eco-friendly
iii. Safe process
iv. High nutrient value algal biomass is good enough to use as a live feed for aquaculture
v. Removal of waste CO_2 due to photosynthetic fixation.

16.4 Microalgae in Wastewater Treatment

The biodiversity of microalgae is very large and estimated to be about 200,000–800,000 species, out of which about 50,000 species are only described. These diverse microalgae species are capable of propensity to adapt themselves to extreme conditions, and so the inhospitable habitats of microalgae attract the scientific community to examine, identify the promising strains/species/genera, and take steps to accomplish potential microalgae-based technologies for wastewater treatment. Therefore, microalgae are believed to be a promising agent in solving energy and environmental challenges all over the world. Phycoremediation is a perfect environmental approach to reduce nitrogen and phosphorus and removal of various

heavy metals from wastewater effluent (Mehta and Gaur 2005). Microalgae can consume considerable amount of these nutrients because they require increased amount of nitrogen and phosphorus for their own protein synthesis (45–60% microalgae dry weight) and metals as micronutrients for their growth and survival (Martinez et al. 2000). Therefore, wastewater can be utilized as an inexpensive nutrient source for the microalgae cultivation (Gomez et al. 2015). These microalgae in wastewater reduce the need of chemical fertilizers and their associated hardship on life cycle to a larger extent. Through the application of discharged wastewater, the zero-waste concept is also implemented. In addition, the harvested biomass of the microalgae from treatment ponds is commonly used as nitrogen and phosphorus supplements for agricultural purpose, and additional energy can be obtained from methane when subjected to fermentation. Accumulation of highly toxic substances such as arsenic, zinc, and selenium can be done by these microalgae in their cells and thus can be used to eliminate toxic adverse agents from the aquatic environment (Xue et al. 1988). Another type of pollution includes radiation, an important type of pollution, and some wastewater discharge may contain natural radioactive materials or became radioactive through contamination. The microalgae play a crucial role in taking up and accumulating many radioactive minerals in their cells even from greater concentrations in the wastewater. Taking into account all these abilities of microalgae in remediation of polluted wastewater, it is significant to prioritize algal technology in wastewater treatment systems in future years.

16.5 Methodology

16.5.1 Microalgae Wastewater Treatment in Waste Stabilization Ponds (WSP)

The most popular and simplest method of microalgae wastewater treatment is waste stabilization ponds (WSPs). This method is now accepted as simple widely used major treatment process globally and a well-known alternative to other biological treatment systems. This method can be applicable in areas of the globe that have copious sunlight and readily available land and so regarded as cost-effective. Therefore, WSP could notably highly favor in Australia, Africa, India, Canada, and certain areas of the USA.

Role of Algae in WSPs Wastewater treatment

Waste stabilization ponds (WSPs) are ‘green treatment.’ They can be easily achieved by the mutual growth relation of microalgae and heterotrophic bacteria. As a by-product of photosynthesis, microalgae produce oxygen from wastewater. The oxygen formed is utilized by the bacteria, and the organic compounds in

wastewater are aerobically biooxidized. An end-product of this biooxidation is carbon dioxide, and during photosynthesis in algae, CO_2 is fixed into cell carbon.

Two types of WSPs are as follows:

i. Facultative ponds (FPs)
ii. High-rate algal ponds (HRAPs).

16.5.2 Facultative Ponds

These facultative ponds exhibit aerobic conditions in the top surface of the pond due to the production of oxygen during photosynthesis by microalgae and anaerobic conditions in the bottom layers. Therefore, they are characterized by an upper aerobic zone, region with available free oxygen in the pond, and a lower anaerobic zone, with empty free oxygen area in lower depth. Active wastewater treatment processes occur in both levels of FP. These ponds are most commonly used to treat domestic sewage and other wide range of organic industrial effluent discharge, and it involves a complex relationship between other aerobic and anaerobic microbes and microalgae.

According to Oswald (1991), the competency of the above concept is demonstrated by the following parameters:

i. Growth rate of microalgae
ii. Contaminant concentration in the medium
iii. Heavy metal concentration in the medium
iv. Vital percentage of removal of metals from the medium
v. Capital and operating costs.

The major function of FP is to maintain the microalgae population within the basin with high-level heterotrophic activity supplied with required dissolved oxygen. This aids the decomposition and stabilization of organic matter at an optimum rate. Due to periodic odor issue, FPs are typically not designed to establish closer than 0.5–1 km from residential areas. Ideal site locations for FPs are open zones because it facilitates mixing by allowing maximum surface wind sweep over the pond. As long as the wastewater influent mixes rapidly within the pond supernatant, it is not important to consider the shape of the FP. These ponds are in the range of depth from 2 to 5 feet (0.7–1.5 m) with a freeboard of 3–5 feet (1.0–1.5 m) above high water mark level. This strategy is followed to accommodate any wave action. Typical loading rates for FPs are based on general climate zones (Table 16.2).

Advantages

i. Effectively removes settling solids, BOD, pathogens, fecal coliform, and ammonia to some extent

Table 16.2 Loading rates of facultative ponds

Surface Loading (kg BOD $ha^{-1}d^{-1}$)	Population per ha	Detention (days)	Climate conditions
<10	<200	>200	Extremely cold with seasonal ice cover
10–50	200–1000	200–100	Cold seasonal climate, hot climate for short duration
50–150	1000–3000	100–33	Temperate to semitropical climate
150–350	3000–7000	33–17	Tropical, uniformly distributed, regular sunlight and temperature, no seasonal cloud cover

ii. Easy to operate

iii. Requires little energy

iv. Comparatively, the removal of contaminants will be relatively small than other secondary treatment processes.

Disadvantages

i. Settled sludge requires periodic removal
ii. Ammonia levels in effluent are difficult to control and predict
iii. Due to the reduction in the microbial activity, sludge accumulation will be higher in cold climates
iv. In case of uncontrolled emergent vegetation, mosquitoes and similar insect vectors cause severe problem
v. Requires relatively large land area
vi. During spring seasons, strong odor occurs and this also happens when the aerobic blanket disappears.

16.5.3 High-Rate Algal Ponds (HRAPs)

Nutrient removal by microalgae is very favorable than other conventional technologies (Muthukumaran et al. 2005; De La Noue et al. 1992). In contrast to facultative ponds, HRAPs are aimed to assist algal growth. The shallow HRAP oxidation ponds are specifically constructed to promote the suspended microalgae growth by using wastewater effluent rich in organic nutrient source. These systems are extensively employed all over the world in many places for wastewater treatment for past many years. These ponds are also constructed as circular, shallow, or channeled raceways with low energy input mixing. These shallow ponds (0.3–0.6 m) allow maximum light penetration, and depending on climatic conditions, they can also operate at short hydraulic retention time (HRT) in the range of 4–10 days in minimal surface area. Continuous mixing should be provided in order to keep the microalgae cells in

suspension, and this also favors the periodic exposure to light. Mixing also prevents the sludge formation that results in the formation of anaerobic conditions. Most of these ponds are operated only in the velocities between 10 and 30 cm/s due to energy cost dependence on velocity (Dodd 1986). HRAPs are the most cost-effective reactors and convenient reactors available for liquid waste management, and it is also applicable for efficient solar energy capture. In contrast, mechanical aerated ponds are high energy consuming and requires high amount of energy in the range of 0.80–6.41 kWh per kg of BOD removed.

Advantages

i. Simple operation

ii. Cost-effective

Limitations

i. Variable effluent quality
ii. Land requirement.

16.5.4 Cell Immobilization

In the above systems, microalgae harvesting is a major cost factor. In addition, maintenance of microalgae in the open ponds cannot be governed well which complicates the process of harvesting. It is therefore recommended to seek ways to bypass the removal of microalgae from suspensions, i.e., by cell immobilization. This technique improves the removal of pollutants. The commonly used material for microalgae immobilization is alginate. Chitosan- and carrageenan-immobilized microalgae (*Phormidium, S. bicellularis, S. quadricauda*) are also capable of removing nitrogen and phosphorus from secondary effluent wastewater discharge (Table 16.3). Chitosan and polyvinyl foams can also be used as cost-effective immobilizing polymers with a long-standing performance.

As reported, nitrogen and phosphorus can efficiently remove from effluents by immobilized alginate matrix (more than 90%) in species such as *Chlorella* and *Scenedesmus* (Martinez et al. 2000).

Advantages

i. Alginate warrant against the extreme physical and chemical changes during the process of immobilization
ii. Superior permeability
iii. Minimal toxicity
iv. High penetrability of immobilizing alginate matrix provides a convenient environment for microalgae growth and survival (Mallick 2002).

Table 16.3 Immobilized microalgae species

Pollutant	Immobilizing material	Microalga species	References
Nitrogen	Alginate Polyvinyl foam Polyurethane Polyvinyl foam filter paper	*Anabaena* Sp.; *Anabaena doliolum; Clorella vulgaris; C. sorokiniana; Chlamydomonas reinhardtii; Isochrysis galbana; Scenedesmus obliquus*	Chen (2003), de-Bashan et al. (2002), Tam et al. (1997), Hernandez et al. (2006)
Phosphorus	Alginate	*Chlorella vulgaris; C. sorokiniana; C. emersonii*	Hernandez et al. (2006), Robinson (1998), Doran and Boyle (1979)
Nitrogen + phosphorus	Alginate Carrageenan Chitosan Polyurethane Polyvinyl foam and polyvinyl acetate sulfate Polystyrene Cellulose fibers Micro- and macroporous fibrous tissue	*Anabaena doliolum; Chlamydomonas reinhardtii; Chlorella vulgaris* *Anabaena doliolum; Chlorella vulgaris; C. kessleri;* *Anabaena doliolum* *Chlorella pyrenoidosa; Phormidium laminosum* *Chlorella vulgaris; C. kessleri; Scenedesmus quadricauda* *Phormidium laminosum*	de-Bashan et al. (2002), Faafeng et al. (1994), JimenezPerez et al. (2004), Kaya and Picard (1996), Shi (2009), Mallick (2002), Garbisu et al. (2000)

Limitations

i. Hollow fibers are expensive
ii. Covalent immobilization may lead to loss of cell viability
iii. Mass transfer limitations occur in higher rate when cell entrapment methods are employed (Kushwaha et al. 2011).

16.5.5 Use of Strains with Special Attributes

Special attributes of algal strains enhance the efficiency of nutrient removal. The special attributes include a quick sedimentation behavior, tolerance to intense high

temperatures, chemical composition with superior high value-added products, and a capacity for growing mixotrophically. A *Phormidium* strain was identified to remove nutrients more efficiently than compared to green algae isolated from polar environments. Therefore, this was suggested as a suitable strain for wastewater treatment in cold regions during spring and autumn seasons. On the other hand, Hammouda et al. (2005) reported that *Phormidium bohneri* could be used as a good candidate for high-temperature wastewater treatment (around 30 °C). Additionally, these strains also possess rapid sedimentation behavior.

Spirulina (Arthrospira) is one of the most favored microalgae for wastewater treatment (Laliberte et al. 1997). The advantages of using *Spirulina* are highlighted as follows (Olguin et al. 2003): (1) can be able to flocculate more easily that facilitates harvesting easier and also cheaper when compared to other microalgae species; (2) the biomass contains large amount of protein content (60–70% dry weight); (3) can also be successfully used as a feed supplement for different mammals (Becker 1994); (4) have many value-added compounds such as polyunsaturated fatty acids that have promising therapeutic effects in humans; (5) biomass enriched with polysaccharides can be efficiently utilized as bioadsorbent for heavy metals (Hernandez et al. 2006); (6) can survive at extreme pH that minimizes the chances of contamination with other species (Zhang et al. 2012); (7) some strains can also survive in very high ammonia nitrogen concentration (130 mg 1–1); and (8) few strains can grow under heterotrophic and mixotrophic conditions (Bhatnagar et al. 2011).

16.6 Bioreactor Design

To enhance sufficient nutrient removal and successful microalgae biomass accumulation, a precise reactor design is required for huge algal cultivation. There are a wide variety of bioreactors used for wastewater treatment process such as open raceway ponds, photobioreactors, open maturation/oxidation ponds, vertical tank reactors and polybags. However, constructing an efficient microalgae growth chamber for organic nutrient removal from waste effluents and biomass production is an ongoing task.

16.6.1 Open Raceway Ponds

In the treatment of municipal and industrial wastewater treatment, there is a constant need for supply of oxygen for sound degradation of organic matter to smaller molecules. The process of supplying oxygen in large-scale treatment plant is expensive and tiresome and requires large number of skilled expertise and manpower. Therefore, these difficulties can be beaten down by microalgae cultivation in

the ponds and tanks where wastewater treatment is carried out (Chinnasamy et al. 2010). During photosynthesis, the algae release O_2 into the environment that ensures biodegradation process with continuous supply of oxygen.

The open raceway ponds are artificially made shallow ponds where the algae species were cultivated in outdoor. In order to maintain the growth and productivity of microalgae, closed loop oval-shaped channels with recirculation filled with low depth water in huge volume over high surface area are generally constructed with enough circulation and mixing. Mixing and circulation are usually equipped via paddlewheel systems (Chisti 2012). CO_2 is usually bubbled from bottom of the open ponds with diffuser systems. The requirement of nitrogen and phosphorus for the growth of microalgae is given by the treated wastewater instead of addition of commercial N and P source in the medium.

The HRAPs discussed above are a combination of augmented oxidation ponds and an algal reactor. HRAPs are shallow, paddle wheel mixed open raceway ponds and facilitate efficient wastewater treatment. Compared to other conventional oxidation ponds, this is primarily more efficient because the saturated oxygen released during photosynthesis acts upon the aerobic wastewater treatment process and consumption of wastewater nutrients into algal biomass.

Advantage

i. Low production cost with minimal nutrient requirement
ii. Low competition with agricultural land
iii. Low maintenance cost

Limitations

i. Control of environment in and around the pond is difficult
ii. Bad weather may affect algal growth
iii. Contamination with undesirable species may often results
iv. Disproportionate light intensity and dispersion within the pond.

16.6.2 Photobioreactor

Photobioreactor is a closed controlled system where microalgae can be cultivated in axenic condition, i.e., highly sterilized ascetic environment. These reactors have been designed to maximize the volumetric productivity of microalgae cultures. This method of algal cultivation is found to be more efficient because closed reactor systems enable stringent process control which leads to increased treatment process. This reactor can be used as a ‘bubble column’ or as an air-lift reactor by the use of an internal riser. Additionally, the reactor is equipped with an adjustable lighting system and gas supply to enhance the treatment process.

Advantages

i. Cultivation of microalgae in controlled condition is a promising approach for higher productivity
ii. Increases the surface-to-volume ratio and favors maximum utilization of light
iii. Gas transfer is strictly controlled
iv. Evaporation of growth medium is reduced
v. Uniform temperature can be retained
vi. Offers excellent protection from outside contamination
vii. Can accommodate in smaller area and is space saving. It can also mounted vertically, horizontally, or at angle indoors or outdoors
viii. Fouling problem is limited

Disadvantages

i. Expensive capital cost
ii. Production efficiency is not much competent than open pond culture
iii. Technical difficulties in sterilizing bioreactors.

16.6.3 Activated Sludge Process

This process includes the following steps:

(1) The chemical step including consumption of phosphorous precipitation chemicals such as ferric chloride or ferrous sulfate is very high, and cationic electrolytic polymers are widely used to concentrate the suspended biomass and increase removal of sludge from wastewater.
(2) Oxygen supplementation
(3) Handling of sludge: The sludge from the WWTPs contains excess water that needs to be conditioned ahead of the thickening process. The excess sludge production during the primary and biological steps requires a strict sludge management practice to avoid GHG emissions during storage (Ward et al. 2014).
(4) Settling of sludge is done by large sedimentation unit, and long time is essential to settle the final sludge. Cationic polymers are mandatory to improve dewatering of sludge.

16.7 Harvesting Strategy

As harvesting contributes its significant role in the cost of operation, it is a worth noticeable aspect to be examined in the algae-based wastewater treatment process. Cost-effective phycoremediation requires suitable algae harvesting methods. A wide range of properties that impact the separation of microalgae from wastewater treatment plant include size, shape, specific gravity, surface charge, motility, growth phase, presence of appendages, and extracellular organic matter (EOM) composition and concentration (Barsanti and Gualtieri 2006). The other factors include properties of the culture medium, cell concentration, pH, and ionic strength that may also have strong impact on the efficiency of harvesting algal species. As *Chlorella* sp., posses specialized properties such as single spherical cells with diameters of 3–5 μm and higher specific gravity than water allows them to grow well in wastewater (Bhatnagar et al. 2010). Bioflocculation is affected by the EOM concentration depending on the microalgal growth stage and concentration of substrates. There are some problems in wastewater treatment process using microalgae including their residual turbidity, clogging of filters, by-product formation, and the presence of taste- and odor-causing compounds.

Thus, microalgae harvesting basically concerns a two-step process. The first step involves coagulants application in order to destabilize the algal cells followed by sedimentation or floatation. Later in the second step, filtration or sedimentation is followed to dewater and wet the algal sludge. In these applications, destabilization of particles is achieved by using polyvalent metal salts. Chloride and sulfate salts of aluminum and ferric iron were the most efficient coagulants. Aluminum plays a crucial role in cell lysis, and ferric salts cause some cell discoloration. An alternative method of electrocoagulation has also been used successfully for the recovery of marine and freshwater microalgae, respectively. By optimizing voltage and run times, this method can achieve greater than 98% of algae recovery and enhance the harvesting. For efficient wastewater sludge management, dewatering polymers are most commonly used and were also examined for algae harvesting (Han et al. 2000). Other than these, there are many cationic, anionic, and nonionic polymers that could also be used for indigenous algal recovery (Table 16.4). No flocculation was observed with the anionic and nonionic polymers; however, the use of cationic polymer may result in low residual supernatant turbidity even though used at relatively low concentrations. Several natural polymers have the ability to generate desired microalgae product that is free from incompetent and harmful contaminants (Ahmad et al. 2011).

Table 16.4 Different microalgae harvesting techniques

Production approach	Harvesting approach	Scale	References
Open raceway ponds	Fractionation of foam, cavitation bubble disruption	Not disclosed	Garcia et al. (2000)
Two-stage process: CSTR to PFR	Flocculation followed by settling	Small pilot (6000 gal pond)	Papazi et al. (2010)
Raceway ponds	Autoflocculation, centrifugation or flocculation then settling	Pilot (1 acre site)	Chisti (2012)
Closed tubular reactors	Not specified	Bench	Sears (2007)
Raceway ponds for Spirulina	Filtration	Large full (90 acre site)	Craggs et al. (2003)

16.8 Advantage—Dual Role of Microalgae

The chief advantage of using microalgae in wastewater treatment process is their dual role in phycoremediation of domestic and industrial wastewater and production of biomass which is suitable for rational biofuel production.

16.9 Nonfuel Applications

(a) Sewage and wastewater treatment
(b) Animal feed supplementary
(c) Chemicals and fertilizers
(d) Biopolymers and bioplastics
(e) Paints and dyes
(f) Lubricants
(g) Pollution control (CO_2 sequestration).

16.10 Fuel-Based Applications

Various types of biofuel can be produced using algae. It includes the following:

(a) Biodiesel by transesterification of algal oil (Aravantinou et al. 2013)
(b) Bioethanol produced by fermentation and distillation of sugars (Arbib et al. 2014)
(c) Biobutanol generated from the leftover green waste (Li et al. 2008)
(d) SVO, algal oil directly used as a fuel

(e) Biogas (methane), the main focus of most of the works in microalgae for biofuel production
(f) Other hydrocarbon fuel variants such as gasoline.

16.11 Applications

16.11.1 Treating Municipal Wastewater

Municipal waste streams are enriched with carbon, nitrogen, and other minerals and can also be used as substrate for microalgae cultivation (Hammouda et al. 1995; Hoffmann 1998). Thus, the cost spent for nutrient and water supply will be greatly reduced. The wastewater used for algal cultivation is called centrate, a feasible alternative for algal media used for cultivation. This is generated by dewatering activated sludge by centrifugation (Woertz et al. 2009). The use of this substitute is highly beneficial because of the following reasons: First, higher concentrations of carbon, nitrogen, and phosphorus in the centrate can supply enough nutrients for microalgae growth. Second, various types of minerals found in centrate such as K, Ca, Mg, Fe, Cu, and Mn (Shi 2009), served as essential micronutrients for the microalgal growth and metabolism. Third, the situation seldom met with scanty of centrate throughout the year because the volume of the centrate produced daily is extremely large. This approach of microalgae cultivation using engineered systems in wastewater treatment and biomass recycling has broadened the microalgae application for algae-based biofuel production to its greatest potential. Thus, the centrate can be used for algae cultivation that could play the dual role of waste organic reduction in municipal wastewater stream and biomass or bioenergy production that could be used for various other applications. The prevalent microalgae species used for municipal wastewater treatment are from the family of Chlorella and the most commonly used microalgae such as *Chlorella pyrenoidosa* (Tam and Wong 1989) and *Chlorella Vulgaris*. According to the culture conditions subjected to these algal species, the cell composition may differ. The algal culture grown under different environmental factors such as light input (light intensity) and light–dark cycle, temperature, nutrients status, and salinity has direct impact on the cell composition of these single cell algal species, and it varies in many folds. These culture condition parameters influence the cellular metabolism as well as photosynthesis and productivity of cell biomass (Lee and Lee 2001; Martinez et al. 2000). However, different species and strains show different responses when there is any alteration in culture conditions.

In this method, the preparation of centrate follows removal of large solid particles by sedimentation and filtration with filter cloth (Wypall X70, Kimberly-Clark Professional). The centrate was autoclaved at 121 °C after filtration and allowed to

cool to room temperature. This eliminates the presence of any indigenous bacteria in the centrate used for algal cultivation. The autoclaved centrate can be stored at 4 °C for 5 days to settle down any visible solid particles if present. In this way, all the undesired solid particles are removed, and the transparent supernatant was used for microalgae cultivation. Algal growth was apparently increased in the centrate because it consists of higher levels of nitrogen, phosphorus, and COD and the microalgae growth leads to reduction in N, P, and COD level. The presence of metal ions especially Al, Ca, Fe, Mg, and Mn in centrate was also found to be abolished in an efficient manner. Therefore, it is concluded that microalgae species can be efficiently used for municipal wastewater treatment.

16.11.2 Treating Food Processing Industrial Wastewater

Food and milk processing industries consume large quantities of water. The food industrial effluents are in general characterized by high BOD and COD along with fats, oil–grease, and many other recoverable nutrients like nitrogen, phosphorous, and potassium. Treating these dairy effluents is highly urged for not only the environmental safety purpose, but also recycling of the already used water for other industrial usage (Mulbry et al. 2008). The physicochemical treatment of these effluents is not efficient because it is expensive and only minimal removal of soluble COD could be achieved. Moreover, the implementation of chemical treatments is also improvident because the various chemical additives used in treatment technique might induce secondary pollution and contaminate the treated water. Thus, dairy industry wastewater assures an entangled system which consists of different components, including pollutants from the processed raw materials, chemicals used for treatment process, and debris of technological additives used in each operation.

Dairy wastewater has increased amount of dissolved organic components like whey proteins, lactose, fat, and minerals (Shamsudin et al. 2011). These effluents are largely neutral or slightly alkaline and also can become quite acidic quickly, because of the ability of fermentation of milk sugar to lactic acid. The lower pH may lead to the precipitation of casein. Oil and grease content in wastewater is primarily composed of a fatty matter from animal and vegetable sources, hydrocarbons of petroleum origin, etc. The organic load present in these discharged effluents indicates the high-level chemical oxygen demand (COD), biochemical oxygen demand (BOD), oil and grease, nitrogen, and phosphorus. Wastewater treatment is an important aspect of resource management control and an essential part of dairy food plant operations, and microalgae cultivation in this wastewater favors sufficient reduction in the BOD and COD level and NP concentrations in the effluent water stream (Wang et al. 2010). Thus, microalgae species can be efficiently used for food and dairy industrial wastewater treatment.

16.11.3 Treating Paper Industrial Wastewater

Owing to large amount of chlorinated lignin derivatives, kraft mill bleaching effluent is plentiful and intensely colored (Tarlan et al. 2002) that are resistant to microbial degradation (Amy et al. 1988). These effluents are rich in compounds with carbonyl, quinoid, coniferaldehyde, and ethylenic groups. This leads to high chemical oxygen demand (COD) and absorbable organic xenobiotics (AOX) wastewater discharge. Various biological treatment methods such as activated sludge, lagoons, or anaerobic processes can be used for degradation of chlorinated organic contents in the effluent before they got discharged into receiving water bodies. But the intense color of the effluent obtained from the lignin-derived products remains unchanged and a complicated task to treat (Archibald and Roy-Arcand 1995). Interestingly, some of the fungi, especially white-rot fungi, are investigated in highly aerobic environment for the treatment of these effluents, and the most rapid and extensive degradation of lignin is achieved. But the major drawback of this fungal treatment of effluent is the requirement of high glucose for the survival of these microorganisms. In several recent studies, investigators reported that algae can replace fungi in removing color and AOX (Aziz and Ng 1993; Dilek et al. 1999). Within 50 days incubation of microalgae at room temperature and grown under natural or artificial lighting conditions, around 50–80% color reduction can be achieved by using a mixed culture of microalgae from a kraft mill effluent. The actions of these microalgae were reported to depict a natural pathway for the conversion of lignin to other materials within the carbon cycle. These observations encouraged our present examination of algal removal of color from pulp and paper effluents, and this treatment reported to be highly efficient.

16.11.4 Treating Agro-Industrial Wastes

The cultivation of microalgae requires large amount of nutrients, but this may have huge environmental and economic impacts that made them not applicable (Halleux et al. 2008). Therefore, wastewater, especially derived from agro-industrial facilities, can act as an alternative to the synthetic culture media. High nutrient content is observed in these effluents (Markou and Georgakakis 2011). Microalgae are capable of transforming the organic carbon sources in the effluent to lipid as intracellular products. Use of hydrolysates from agricultural residues as cultural medium for microalgal growth is one of the cost-effective methods, as many species of microalgae take up organic compounds from the medium. Organic carbon sources like starch hydrolysates from sweet sorghum, cassava, waste molasses, and rice straw were utilized to cultivate microalgae as cost-effective approach to displace glucose. Therefore, the microalgae growing in the wastewater will utilize the organic nutrients present in it, and the combined microalgae–bacterial systems can

aid agro-industrial wastewater treatment; so, this has been attaining a special consideration in recent years. The mutual relationship is found in these combined systems. The O_2 produced during algal photosynthesis is used by bacteria for its metabolism, whereas bacteria release CO_2 that assists the growth of microalgae. The use microalgae–bacterial system for wastewater treatment eliminates the oxygen supplementation done by external sources. Compared to other conventional processes, this treatment allows recovery of nutrient as biomass and reduces the emission of CO_2 into the atmosphere. This is achieved by microalgae which contribute to CO_2 mitigation. Therefore, the substrate reuse can improve the chances of microalgal biomass production for its further restraint, like anaerobic digestion (González-Fernández et al. 2011). This treatment process has dual advantages of wastewater treatment and also algal biomass recovery that may be used for various other applications.

16.12 Cost Analysis

This technique is highly cost efficient because it economizes power and chemicals used (Olguin 2003). This process is 70–90% cheaper than other technologies and basically need low capital investment (Table 16.5).

Table 16.5 Operational and financial comparison by VIAT

Cost parameter	Conventional effluent treatment	Phycoremediation	Annual cost benefit
Acidity—high levels of dissolved carbon dioxide	Neutralization with caustic soda	Algal treatment to absorb the acidic contents and neutralize the effluent	Rs. 50 lakhs spent for caustic soda annually is saved
			The total cost of the services (labor/electricity, etc.) used in the operation is saved
Sludge Formation	Evaporation of effluents deposits sludge. That needs to be buried in a landfill. About 290 tonnes of sludge produced annually	Algal remediation produces a nutrient-rich commercially valuable fertilizer that has high demand in the market	The sludge disposal used to cost an estimated Rs. 3 lakhs annually. This cost is saved
		There results in no residual sludge formation	Additionally revenues from the sale of algal biomass fertilizer
Structures and Space	11,000 m^2 of masonry tank for evaporating the effluent	3000 m^2 of tank for containing and evaporating the effluent.	About 75% of the effluent treatment facility space is saved.

16.13 Additional Features

(a) Phycoremediation is a naturally happening phenomena because microalgae are natural living organisms. These microalgae presciently exist in nature and are used for the depletion of undesirable materials from municipal and industrial effluents for treatment of wastewater. We come across these microalgae species on a daily basis, which have zero ill effects. Even after the completion of phycoremediation also, the environment closely restored its original pure condition.
(b) The ultimate feature about phycoremediation method is that it can specifically treat various effluents, for example, in the treatment effluents with large amount of heavy metals or dyeing effluents from various industries.
(c) Phycoremediation is highly case specific, and this process can be operated in batch-wise, semicontinuous, or continuous manner.
(d) Large fluctuations can handle in high flexible manner in terms of both quality and quantity of effluent feed from various industries.
(e) This technology has been affirmed to be persuasive in treating array of effluents from dye, food, chemical, pharmaceutical, and dairy industries.
(f) Phycoremediation technology took away a part of highly automation, maintenance, and requirement of skilled personnel operators and so considered as robust.
(g) Phycoremediation guarantees nil sludge generation, and so, there is no disruption of surroundings and pollution.
(h) The chemicals used in the nutrient media are of negligible cost, and the addition of these nutrients is only required in initial stage to enhance algal growth. Once the culture is established, there is no need for the addition of extra costlier nutrients.
(i) Due to rapid multiplication of microalgae, the cultures could replenish themselves with fresh feed of effluents. Hence, this process requires only one time addition of microalgae.
(j) After adaptation of microalgae into an environment, it removes the obnoxious odors present in the effluent and converts it into rich algal smell.
(k) Moreover, microalgae treatment transforms the colored effluent into colorless.

16.14 Challenges

Algae-based wastewater treatment technology requires optimal sunlight and warm temperature, and so, it is suitable for tropical countries. Environmental factors also play a major part in algae cultivation. Lighting and optimal temperature maintenance

in algae ponds are difficult. In the mass cultivation of microalgae using wastewater besides these environmental factors, there are a number of biological and operational problems.

The challenges in large-scale cultivation of microalgae for wastewater treatment includes supply of nutrients and recycling, gas transfer and exchange, photo synthetically active radiation (PAR) delivery, integrity of culture, environment control, land availability and harvesting. When the treatment plant suffers from insufficient gas exchange, the microalgae culture may become carbon limited, and the photosynthetic by-product acts as inhibitor for the growth of algal culture (Lee and Lee 2001). For maximum efficiency of microalgae culture, in terms of nutrient supply Redfield ratio of 16:1 nitrogen-to-phosphorus ratio is required (Rhee 1978). This ratio represents an average species-specific N to P ratios, and this may vary from 8 to 45. Therefore, it is not a universal biochemical optimum for algal cultivation. To prevent the limitations of this technique, the nutrient molar ratio in the wastewater effluent should reach the approximate ratio and match the stoichiometric ratio of the algal biomass. This signifies that nitrogen or phosphorus should be added by external means in order to reach the proper N to P ratio in the wastewater effluent. Moreover, some of this wastewater typically contains organic pollutants or heavy metals, and the microalgae-based treatment aids the removal of these constituents from effluent. Microalgae cells can adsorb heavy metals and can be removed from effluents by adsorption. These species can also degrade organic phenol and acetonitrile. The additional problem includes optimization of carbon dioxide delivery. An engineering challenge exists in the CO_2 supply through direct bubbling. This challenge is directly related to carbon dioxide supply and removal of excess oxygen. Saturated concentration of oxygen in the treatment plant may begin to inhibit photosynthesis and cause photooxidative damage. Open ponds are potentially suffered from carbon limitation due to various mass transfer limitations. Intense sunlight available for microalgae culture in open ponds may often be disastrous to algal species because they were in the situation to utilize all available photons that lead to absorb the excess energy by cells or lost in the form of fluorescence or heat. This prolonged exposure to high-intensity sunlight can circumvent the energy-dissipating machinery of the cells resulting in photoinhibition and cell damage (Borowitzka 1998). In contrast, the light will be limited for algae in deeper portions of a culture because algae found in top portions might absorb the majority of light. Thus, cultures often suffer from photoinhibition and photodeprivation simultaneously. Monocultures of high lipid-producing strains are fast growing species of microalgae (Tian et al. 2014), and therefore, careful maintenance of monocultures is required for wastewater treatment systems (Table 16.6). When diverse wastewater effluent resources are used, there occurs domination by naturally occurring mixed cultures of algae. Moreover, a major challenge remained was

Table 16.6 Summary of challenges in microalgae wastewater treatment plant

Challenges	Description
Space requirements	The mass microalgae system is designed as shallow raceway ponds for purpose of wastewater treatment, and requirement of more space is the major disadvantage
Temperature maintenance	As this system is developed in open environment, temperature control cannot be done efficiently
Climatic condition	Since the microalgae species rely on the process of photosynthesis, availability of sunlight is very critical to achieve efficient growth of microalgae. Therefore, this treatment plant can be efficiently designed only on tropical regions. Moreover, the system operates more efficiently only in warm climates where little chances of freezing exist
External nutrient supply	In mass cultivation, the microalgae grow efficiently only in the presence of appropriate N:P molar ratio, and therefore, it is necessary to supply this nutrients by external means if the required ratio is not found in wastewater effluent
Biological limitations	Gas transfer limitation may inhibit the growth of microalgae, and intense light supply may result in photoinhibition
Operational problems	Supply of CO_2 and removal of O_2 remain challenging for engineering operations
Addition of cost	The major drawback is often offset in less developed urban areas where large amount of land and operation cost are inexpensive

discovering ways to create proper controlled environment for efficient microalgae growth without adding unreasonable expenses. Finally, the ultimate challenge in the large-scale production of microalgae is requirement of large expense of land.

16.15 Summary

Recent estimates of Central Pollution Control Board (CPCB) indicate that 40 billion liters of wastewater was generated from urban areas of the country and only about 20–30% of the generated wastewater is treated. In most of the urbanized areas, the wastewater is inadequately treated and thence has become the reason for eutrophication and increased bacterial infection resulting in deteriorating health and hygiene because of the contamination of surface and groundwater resources. The average daily per capita water supply in India is about 88.73 l and sewage accounts to 137.82 l. One of the most notable advantages of microalgae is that they can habituate and grow in polluted environments. They naturally grow with minimal freshwater requirements and attain higher cell densities in nutrient-rich wastewater. Wastewater is a good source for algal growth and subsequent derivation of various

other algal-based by-products. Thus, these species can be possibly utilized for low-cost and environmentally friendly wastewater treatment compared to other commonly used conventional treatment processes in order to reduce BOD, remove N and P, and remove heavy metals and discoloration of polluted domestic and industrial effluents.

References

Ahmad AL, Yasin NH, Derek CJC, Lim JK (2011) Microalgae as a sustainable energy source for biodiesel production: a review. Renew Sustain Energy Rev 15:584–593

Amy GL, Bryant CW, Allerman BC, Barkley WA (1988) Biosorption of organic halide in a kraft mill generated lagoon. J Water Pollut Control Fed 60(8):1445–1453

Aravantinou AF, Theodorakopoulos MA, Manariotis ID (2013) Selection of microalgae for wastewater treatment and potential lipids production. Bioresour Technol 147:130–134

Arbib Z, Ruiz J, Alvarez-Diaz P, Garrido-Perez C, Perales JA (2014) Capability of different microalgae species for phytoremediation processes: wastewater tertiary treatment, CO2 bio-fixation and low cost biofuels production. Water Res 49:465–474

Archibald F, Roy-Arcand L (1995) Photodegradation of high molecular weight kraft bleachery effluent organochlorine and color. Water Res 29(2):661–669

Aziz MA, Ng WJ (1992) Feasibility of wastewater treatment using the activated-algae process. Bioresour Technology. 40:205–208

Aziz MA, Ng WG (1993) Industrial wastewater treatment using an activated algae reactor. Water Sci Technol 28:71–76

Barsanti L, Gualtieri P (2006) Algae: anatomy, biochemistry, and biotechnology. CRC Press, Boca Raton

Becker EW (1994) Microalgae: biotechnology and microbiology. Cambridge University Press

Bhatnagar A, Chinnasamy S, Singh M, Das KC (2011) Renewable biomass production by mixotrophic algae in the presence of various carbon sources and wastewaters. Appl Energy 88:3425–3431

Bhatnagar A, Bhatnagar M, Chinnasamy S, Das K (2010) Chlorella minutissima—a promising fuel alga for cultivation in municipal wastewaters. Appl Biochem Biotechnol 161:523–536

Borowitzka MA (1998) Limits to growth, in wastewater treatment with algae. In: Wong YS, Tam NFY (eds). Springer, pp 203–226

Camargo JA, Alonso A (2006) Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: a global assessment review article. Environ Int 32:831–849

Chen YC (2003) Immobilized *Isochrysis galbana* (Haptophyta) for long-term storage and applications for feed and water quality control in clam (*Meretrix lusoria*) cultures. J Appl Phycol 15(5):439–444

Chinnasamy S, Bhatnagar A, Hunt RW, Das KC (2010) Biomass and bioenergy production potential of microalgae consortium in open and closed bioreactors using untreated carpet industry effluent as growth medium. Bioresour Technol 101:3097–3105

Chisti Y (2012) Raceways-based production of algal crude oil. In: C. Posten & C. Walter (eds). Microalgal biotechnology: Potential and production, pp 113–146

Craggs RJ, Daves-Colley RJ, Tanner CC, Sukias JP (2003) Advanced pond system: performance with high rate ponds of different depths and areas. Water Sci Technol 48:259–267

de-Bashan LE, Moreno M, Hernandez JP, Bashan Y (2002) Removal of ammonium and phosphorus ions from synthetic wastewater by the microalgae *Chlorella vulgaris* coimmobilized in alginate beads with the microalgae growth-promoting bacterium Azospirillum brasilense. Water Res 36(12):2941–2948

De La Noue J, Laliberte G, Proulx D (1992) Algae and waste water. J Appl Phycol 4:247–254

Dilek FB, Taplamacyoolu H, Tarlan E (1999) Colour and AOX removal from pulping effluents by algae. Appl Microbiol Biotechnol 52:585–591

Dodd J (1986) Elements of pond design and construction. Microalgal mass culture. CRC, Boca Raton (FL), pp 265–283

Doran MD, Boyle WC (1979) Phosphorus removal by activated algae. Water Res 13:805–812

Faafeng BA, van Donk E, Kallqvist ST (1994) In situ measurement of algal growth potential in aquatic ecosystems by immobilized algae. J Appl Phycol 6(3):301–308

Garbisu C, Alkorta I, Llama MJ, Serra JL (2000) Immobilization of living micro algae and their use for inorganic nitrogen and phosphorous removal from water

Garcia J, Mujeriego R, Hernandez-Marine M (2000) High rate algal pond operating strategies for urban wastewater nitrogen removal. J Appl Phycol 12:331–339

Gomez-Serrano C, Morales-Amaral MM, Acien FG, Escudero R, Fernandez-Sevilla JM, Molina-Grima E (2015) Utilization of secondary-treated wastewater for the production of freshwater microalgae. Appl Microbiol Biotechnol 99:6931–6944

González-Fernández C, Molinuevo-Salces B, García-González, MC (2011) Evaluation of anaerobic codigestion of microalgal biomass and swine manure via response surface methodology. Appl Energ 88(10):3448–3453

Halleux H, Lassaux S, Renzoni R, Germain A (2008) Comparative life cycle assessment of two biofuels ethanol from sugar beet and rapessed methyl ester. Int J LCA 13(3):184–190

Hammouda O, Gaber A, Abdel-Raouf N (1995) Microalgae and wastewater treatment. Ecotoxicol Environ Saf 31(3):205–210

Han SQ, Zhang ZH, Yan SH (2000) Present situation and developmental trend of wastewater treatment and eutrophication waters purification with alga technology

Hernandez JP, de-Bashan LE, Bashan Y (2006) Starvation enhances phosphorus removal from wastewater by the microalga *Chlorella* spp. co-immobilized with *Azospirillum brasilense*. Enzyme Microb Technol 38(1):190–198

Hoffmann JP (1998) Wastewater treatment with suspended and non-suspended algae. J Phycol 34:757–763

Jimenez-Perez M, Sanchez-Castillo P, Romera O, Fernandez-Moreno D, Pérez-Martınez (2004) Growth and nutrient removal in free and immobilized planktonic green algae isolated from pig manure. Enzyme Microb Tech 34(5):392–398

Kaya VM, Picard G (1996) Stability of chitosan gel as entrapment matrix of viable *Scenedesmus bicellularis* cells immobilized on screens for tertiary treatment of wastewater. Bioresource Technol 56(2–3):147–155

Kushwaha JP, Srivastava VC, Mall ID (2011) An overview of various technologies for the treatment of dairy wastewaters. Crit Rev Food Sci Nutr 51:442–452

Laliberte G, Olguín EJ, de la Noue J (1997) Mass cultivation and wastewater treatment using Spirulina. In: Vonshak A (ed) Spirulina platensis-physiology, cell-biology and biotechnology. Taylor and Francis, London (UK), pp 159–173

Lee K, Lee CG (2001) Effect of light/dark cycles on wastewater treatment by microalgae. Biotechnol Bioprocess Eng 6:194–199

Li Y, Horsman M, Wu N, Lan CQ, Dubois Calero N (2008) Biofuels from microalgae. Biotechnol Prog 24:815–820

Mallick N (2002) Biotechnological potential of immobilized algae for wastewater N, P and metal removal: a review. Biometals 15:377–390

Martinez ME, Sanchez S, Jimenez JM, El Yousfi F, Munoz L (2000) Nitrogen and phosphorus removal from urban wastewater by the microalga *Scenedesmus obliquus*. Bioresour Technol 73:263–272

Markou G, Georgakakis D (2011) Cultivation of filamentous cyanobacteria (blue–green) in agro-industrial wastes and wastewater: a review. Appl Energ 88(10):3389–3401

Mehta SK, Gaur JP (2005) Use of algae for removing heavy metal ions from wastewater: progress and prospects. Crit Rev Biotechnol 25:113–152

Mulbry W, Kondrad S, Buyer J (2008) Treatment of dairy and swine manure effluents using freshwater algae: fatty acid content and composition of algal biomass at different manure loading rates. J Appl Phycol 20:1079–1085

Muthukumaran M, Raghavan BG, Subrahmanian V et al (2005) Bioremediation of industrial effluent using micro algae. Indian Hydrobiol 7:105–122

Olguin EJ (2003) Phycoremediation: key issues for cost-effective nutrient removal process. Biotechnol Adv 22:81–91

Oswald WJ (1991) Introduction to advanced integrated ponding systems. Water Sci Technol 24 (5):1–7

Papazi A, Makridis P, Divanach P (2010) Harvesting *Chlorella minutissima* using cell coagulants. J Appl Phycol 22:349–355

Park JBK, Craggs RJ, Shilton AN (2011) Wastewater treatment high rate algal ponds for biofuel production. Bioresour Technol 102:35–42

Pittman JK, Dean AP, Osundeko O (2011) The potential of sustainable algal biofuel production using wastewater resources. Bioresour Technol 102:17–25

Robinson PK (1998) Immobilized algal technology for wastewater treatment purposes. In: Wong YS, Tam FY (eds) Wastewater treatment with algae. Springer, Berlin, pp 1–16

Rao HP, Kumar RR, Raghavan BG, Subramanian VV, Sivasubramanian V (2011) Application of phycoremediation technology in the treatment of wastewater from a leather-processing chemical manufacturing facility. Water Soil Air 37:7–14

Rawat I, Ranjith Kumar R, Mutanda T, Bux F (2011) Dual role of microalgae: phycoremediation of domestic wastewater and biomass production for sustainable biofuels production. Appl Energy 88:3411–3424

Redalje DG, Duerr EO, de la Noue J, Mayzaud P, Nonomura AH, Cassin RC (1989) Algae as ideal waste removers: biochemical pathways. Biotreatment of agricultural wastewater. CRC Press, Boca, Raton, pp 91–110

Rhee GY (1978) Effects of N:P atomic ratios and nitrate limitation on algal growth, cell composition and nitrate uptake. Limnol Oceanogr 23:10–25

Rosenberg JN, Mathias A, Korth K, Betenbaugh MJ, Oyler GA (2011) Microalgal biomass production and carbon dioxide sequestration from an integrated ethanol biorefinery in Iowa: a technical appraisal and economic feasibility evaluation. Biomass Bioenergy 35:3865–3876

Sears JT (2007) Method, apparatus and system for biodiesel production from algae. PCT Patent Application WO2007/025145 (1 March 2007)

Shamsudin MN, Yodfiatfinda, Zainal AM, Yusop Z, Radam A (2011) Evaluation of market competitiveness of SMEs in the Malaysian food processing industry. J Agri Market 4:1-20

Shi J (2009) Removal of nitrogen and phosphorus from municipal wastewater using microalgae immobilized on twin-layer system. PhD dissertation, University of cologne

Tam NFY, Wong YS, Simpson CG (1997) Removal of copper by free and immobilized microalga, *Chlorella vulgaris*. In: Wong YS, Tam NFY (eds) Wastewater treatment with algae. Springer, Berlin, pp 17–36

Tam NFY, Wong YS (1989) Wastewater nutrient removal by *Chlorella pyrenoidosa* and *Scenedesmus* Sp. Environ Pollut 58:19–34

Tarlan E, Dilek FB, Yetis U (2002) Effectiveness of algae in the treatment of a wood-based pulp and paper industry wastewater. Bioresour Technol 84:1–5

Tian C, Li B, Liu Z, Zhang Y, Lu H (2014) Hydrothermal liquefaction for algal biorefinery: a critical review. Renew Sustain Energy Rev 38:933–950

Validi M (2001) Bioremediation. Overview Pure Appl Chem 73:1163–1172

Wang L, Li YC, Chen P, Min M, Chen YF, Zhu J et al (2010) Anaerobic digested dairy manure as a nutrient supplement for cultivation of oil-rich green microalgae Chlorella sp. Bioresour Technol 101:2623–2628

Ward AJ, Lewis DM, Green FB (2014) Anaerobic digestion of algae biomass: a review. Algal Res 5:204–214

Woertz I, Feffer A, Lundquist T, Nelson Y (2009) Algae grown on dairy and municipal wastewater for simultaneous nutrient removal and lipid production for biofuel feedstock. J Environ Eng 135:1115–1122

Xue HB, Stumm W, Sigg L (1988) The binding of heavy metals to algal surfaces. Water Resour 22:917–926

Zhang Y, Su H, Zhong Y, Zhang C, Shen Z, Sang W, Yan G, Zhou X (2012) The effect of bacterial contamination on the heterotrophic cultivation of Chlorella pyrenoidosa in wastewater from the production of soybean products. Water Res 46:5509–5516

Chapter 17
Phytoremediation of Textile Dye Effluents

Shanmugaprakash Muthusamy, Dhilipkumar Govindaraj and Karthik Rajendran

Abstract Water is the vital source to live and the textile dye effluent is one of the major contaminants present in the wastewater which is highly toxic to all form of lives. Though some effective various methods such as physical, chemical, and biological methods are available to remove the textile dye effluents, phytoremediation is the most economical, eco-friendly, easy to do to degrade the contaminates completely/partially present in effluent. The different plants are found with naturally inhabited metabolic pathways to utilize different dyes and some of the genetically engineered plants are also produced in order to effectively degrade the dyes and to sustain different environmental conditions. Symbiotic relationships between the plant and microbes are also used to help the plants to overcome different kinds of stress. The enzymes like oxidoreductases which are extracted from the plants have shown potent activity against dyes. The significant decrease in color, turbidity, conductivity, total suspended solids (TSS), total dissolved solids (TDS), chemical oxygen demand (COD), and biological oxygen demand (BOD) are taken as indicators of effectiveness of phytoremediation. Several researchers have done extensive studies in phytoremediation area in order to understand the exact mechanism to during treatment of effluents. This chapter mainly focusses on various phytoremediatic methods and its mechanism used in textile effluents treatments.

Keywords Textile dyes · Effluents · Phytoremediation · Wastewater Enzyme

S. Muthusamy (✉) · D. Govindaraj
Downstream Processing Laboratory, Department of Biotechnology, Kumaraguru College of Technology, Coimbatore 641049, India
e-mail: shanmugaprakash.m.bt@kct.ac.in; sunbioin@gmail.com

K. Rajendran
MaREI Center, Environmental Research Institute, University College Cork, Cork, Ireland

K. Rajendran
School of Engineering, University College Cork, Cork, Ireland

S. J. Varjani et al. (eds.), *Bioremediation: Applications for Environmental Protection and Management*, Energy, Environment, and Sustainability,
https://doi.org/10.1007/978-981-10-7485-1_17

17.1 Introduction

Today, more than 1,00,000 different dye chemicals are available and millions of numbers of dyestuffs produced annually. Due to its recalcitrant of dyes, textile effluents mainly contains dye, various salts, acids, bases, surfactants, dispersants, humectants, oxidants, and detergents effluents mainly contains dye, various salts, acids, bases, surfactants, dispersants, humectants, oxidants, and detergents, etc and it is esthetically unacceptable and unusable for human consumption. Moreover, textiles are well known for its mutagenic and carcinogenic activity to various ecosystems and living beings. Many dyes used in textile industry are difficult to decolorize because of its complex structure mainly due to acidic, basic, disperse, azo, diazo, anthraquinone-based, and metal complex dyes present in the chemical structure (Slimani 2014). The second highly polluted resource is our water next to air. Two million tons of wastewater is produced by the humans per day which are equivalent to the world's human population (6.8 billion). The water pollution is our major concern since it is coming under the basic needs of life (food, water, and shelter). The major cause of water pollution is the oil spills in the ocean. The oil spills cause the sticky feathers in marine birds and affect the respiration of marine mammals by masking surface area with oil. The oil spills can be cleared by using oil dispersion technology or by using super bugs.

Another major cause of water pollution is textile effluents. In freshwater pollutants, Textile effluents carry the first position. These textile effluents carry high chemical oxygen demand (COD) and highly affect the aquatic biological systems. China, European Union, and India hold the major shares of the textile market. The pollutants are commonly present in waters at trace concentrations, ranging from a few ng/L to several μg/L depending on its source. The "low concentration" and variety of pollutants are difficult to detect during analysis but also create challenges for water and wastewater treatment processes.

17.2 Characterization of Textile Effluents (Source and its Characterization)

17.2.1 Textile Effluents

Even the developing countries are aiming at the common effluent treating plants (CETP) for the zero discharge solution. We cannot stop producing textiles and people fond on different colors so the textile effluents become an unavoidable one. The wastewater textiles effluents are produced in many levels of textile production. Ten percent of the dyes produced are released to the environment. Among the effluents produced, the water from the dyeing unit has more COD and pH when compared to the European standards. For dyeing constantly 17000–34000 L/1000 kg of products is utilized. Among them, 7000–24000 L/1000 kg of water is excreted as effluents. In cities like Tirupur, the consumption of water is 90 million liters per day.

17.2.2 Characteristics of Textile Effluents

The characteristics of the textile effluents vary upon the different processes adopted in industries according to the different fabrics used. The dyes used in textiles can be classified into two based on the chemical type (azo, indigo, etc.) and the process of application (acid, reactive, directive, etc.). Generally, effluent from textile industries contains COD ranges from (601–745 mg/dm^3), BOD5 (90–158 mg/dm^3), TSS, alkalinity (201–219 mg $CaCO_3$/dm^3), SO_4^{2-} (mg/dm^3) and dark brown color. There are various methods available to treat the textile effluent and some are discussed below.

17.2.3 Adsorption

Adsorption is the surface phenomena, and it is a physical process. This process does not involve any chemicals added to the effluents. The adsorbents are only added to it. The properties of the adsorbents are smaller volume, rigid, and larger surface area which tremendously increases the adsorption of chemicals in the effluents. The examples of the effective adsorbent used in industries are activated charcoal, calcium carbonate, etc. The effluent is concentrated with the adsorbents to form sludge. The sludge is removed and filtered/dried. The dried sludge is used for making roads as a product from waste. This process effectively removes the waste components from the effluents. Many non-conventional low-cost adsorbents, including natural materials, biosorbents, and waste materials from various agriculture and related industry, have been used for the treatment of textile dye effluents by several researchers. For example, bamboo dust date pits, olive stones, furniture, groundnut shell, sewage char and tyres, vermiculata plant, coconut shell, rice husk and straw, jute fiber, zeolite, coconut husk, oil palm fiber, waste apricot, corncob, coir pith, pitch, olive-seed waste, firewood, rattan sawdust, bio-plant of *Euphorbia rigida*, vetiver roots, durian shell, oil palm shell, sugars, wheat bran, *Heveabrasiliensis* seed coat, peach stones, almond shell, walnut shell, hazelnut shell and apricot stones, and Rosa canina seeds, these adsorbents were used in wastewater treatment for the removal of dissolved pollutants. The raw agricultural solid wastes contain various organic compounds (lignin, cellulose, and hemicellulose) with polyphenolic groups that might be useful for binding pollutants through different mechanisms (Rafatullah et al. 2010).

Disadvantages

1. Adsorbents are very costly.
2. Toxic chemicals are only concentrated not completely treated.
3. Chemicals are leached out after rain from the roads put by using sludge.
4. Regeneration of adsorbent is difficult.

17.2.4 Flocculation

Flocculation or coagulation is the process in which the coagulants like aluminum chloride, ferrous sulfate, natural coagulant such as chitosan and ferric chloride are added to the effluents. Chemical coagulation in wastewater treatment is to change the physical state of dissolved and suspended solids and increase the sedimentation rate (Verma et al. 2012). Similarly, flocculation is a chemical process where the dissolved solid and suspended solids are neutralized by reducing the electrostatic repulsion existing between colloidal particles. When the effluent is mixed with the coagulants, small floccules are produced and quickly settled down under the influence of gravity. Several researches carried out using pre-hydrolysed coagulants such as Polyaluminium chloride (PACl), Polyaluminium ferric chloride (PAFCl), Polyferrous sulfate (PFS), and Polyferric chloride (PFCl) suggest to provide a better color removal efficiency even at low temperature and may also produce a lower volume of sludge. This because by reducing electrostatic repulsion between the coagulants and contaminants and it adsorb on adjacent particles.

Disadvantages

1. It is only useful for removing azo dyes (dyes with metal coordinates).
2. It only removes the dye and not degrades it.
3. Production of sludge.

17.2.5 Microbial Treatment

In most of the textile industries, microbial treatment is considered as the secondary treatment for decoloring the textile dyes. Many different microbes are found to be with a molecular system for degrading dyes. The main advantage of this treatment is that it removes the toxicity of the dyes by degrading it fully or partially by turning it into the harmless form. The genetically modified microbes are also produced to cleave the particular dye since the usage of dyes differs from industry to industry. Metabolize of textile dyes in wastewater using microbial community has been extensively studied. In general, azo dyes are not readily metabolized in aerobic condition (Robinson et al. 2001). However, under anaerobic conditions, many microorganisms affect the highly electrophilic azo bonds present in the dye molecule, by the enzyme cytoplasmic azoreductase, to give colorless aromatic amines.

Table 17.1 Advantages and disadvantages of the current methods of dye removal from industrial effluents

Physical/ chemical methods	Advantages	Disadvantages
Fenton's reagent	Effective decolorization of both soluble and insoluble dyes	Sludge generation
Activated carbon	Good removal of wide variety of dyes	Very expensive
Membrane filtration	Removes all dye types	Sludge production
Silica gel	Effective for basic dye removal	Side reactions prevent commercial application
Ozonation	Applied in gaseous state: no alteration of volume	Short half-life (20 min)
Electrokinetic coagulation	Economically feasible	High sludge production
Ion exchange	Regeneration: no adsorbent loss	Not effective for all dyes
Irradiation	Effective oxidation at laboratory scale	Requires a lot of dissolved O_2

These intermediate compounds are highly resistant to further anaerobic mineralization and can be toxic or mutagenic to living system. But, the resultant amino compounds are good substrates for aerobic biodegradation and it can be easily mineralized by aerobic system. Table 17.1 provides the advantages and disadvantages of the current methods of dye removal from industrial effluents.

17.2.5.1 Factors Affecting Color Removal Using Microbes

- Oxygen,
- pH temperature,
- Temperature,
- Dye concentration,
- Nature of the dye,
- Electron donor,
- Electron potential,
- Redox mediator.

Disadvantages

1. The specific reactors are required for carrying out this treatment.
2. The maintenance of the optimized conditions is very difficult.
3. The sudden decrease in the microbial count due to the varying temperature and pH of the effluent may happen.
4. Immobilization of microorganisms requires more cost.

17.3 Phytoremediation

Phytoremediation is the process of using plants to remove or reduce the contaminants present in the environment. The word phytoremediation literally means that *phyto*-plants (Greek word) and *remedium*-removal of evil (Latin root). This process was first proposed by Chaney during the year 1983. Some plants accumulate contaminants within themselves by absorbing them via roots but some plants metabolize the contaminants and they are said to be accumulating and nutrient contaminant, respectively. Other processes require high mechanical instruments, more input energy, and the high cost of implementing but phytoremediation requires no much cost for implementing, and moreover, it is a solar energy driven process.

Phytoremediation is an in situ and eco-friendly technology to remove contaminants. Plants are mostly used for cleaning heavy metals and textile dyes. The major advantage of this method is that it prevents contamination nearby air, rain, and soil. There are several mechanisms of removal of textile dye which includes rhizofiltration, phytoextraction, phytotransformation, phytostabilisation, and phytovolatilization. Though several methods are available to treat the textile effluent, phytoremediation is considered as one of the best methods to treat the textile dyes. Other methods require more cost and lead to the production of secondary contaminants due to the usage of chemicals for treating the effluents. Many plants are naturally found with the remediation characteristics and some genetically engineered plants are also produced to clean textile dye effluents. Table 17.2 shows the remediation efficacy of different plants toward the dye.

17.4 Mechanisms of Phytoremediation

Due to its high surface area of the root hairs, it absorbs and tends to accumulate the water and essential nutrients for growth and also with contaminants which is non essential for plant growth. Based on the nature of the mechanism, phytoremiediation involves seven mechanims in which plants affect soil, sediment, and water (Etim 2012). Figure 17.1 represents the classification of phytoremediation.

17.4.1 Phytoextraction

Phytoextraction is defined as uptake/absorption and translocation of contaminants by plant roots into the above-ground portions of the plants (shoots) that can be harvested and burned gaining energy and recycling the metal from the ash (Rew 2007).

Table 17.2 Phytoremediation performances of different wild/indigenous plants for textile dyes

Sr no.	Name of the plant	Dye/effluent	Concentration (mg/L)	Decolorization time (h)	% decolorization	References
1	*Alternanthera philoxeroides*	Remazol Red	70	72	100	(Rane et al. 2015)
2	*Pogonatherum crinitum*	Effluent	NA	288	74	(Watharkar et al. 2015)
3	*Nasturtium officinale*	Acid Blue 92	20	96	78	(Torbati et al. 2014)
4	*Ipomoea hederifolia*	Scarlet RR	50	60	96	(Rane et al. 2014)
5	*Typhaangustifolia*	Reactive Blue 19	75	144	70	(Mahmood et al. 2014)
6	*Bouteloua dactyloides*	Effluent	NA	24	92	(Vijayalakshmidevi and Muthukumar 2014)
7	*Azolla filiculoide*	Basic Red 46	20	144	90	(Vafaei et al. 2012)
8	*Lemna minor*	Acid Blue 92	2.5	144	77	(Khataee et al. 2012)
9	*Portulaca grandiflora*	Reactive Blue 172	20	40	98	(Khandare et al. 2012)

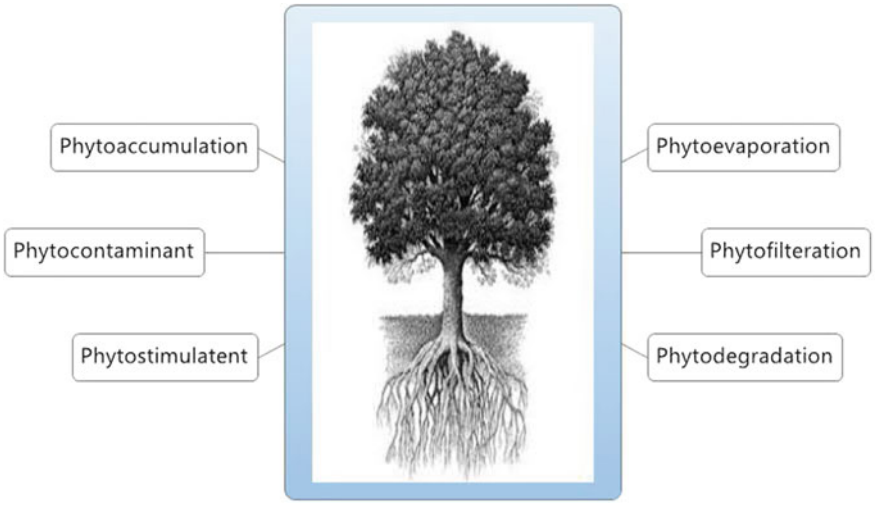


Fig. 17.1 Classification of phytoremediation

17.4.2 Phytostabilisation

Phytostabilization is the use of certain plant species to immobilize the contaminants in the soil and groundwater through absorption and accumulation in plant tissues, adsorption onto roots, or precipitation within the root zone preventing their migration in the soil, as well as their movement by erosion and deflation (Rew 2007).

17.4.3 Rhizofiltration

Rhizofiltration is the adsorption or precipitation onto plant roots or absorption into and sequestration in the roots of contaminants that are in solution surrounding the root zone by constructed wetland for cleaning up communal wastewater (Rew 2007).

17.4.4 Phytovolatilization

Phytovolatilization is the absorbing the elemental form of the contaminations from the soil and converts them into gaseous ions in biological means within the plants. It

occurs in growing trees and other plants that absorb water, nutrients along with the contaminants present in the soil. The low concentration of these contaminants can pass through the plants to the leaves by transpiration and disperse them into the atmosphere. Due to the high detection limits of analytical methods, it is difficult to determine the concentration of contaminants in phytovolatilization. Due the advancement in analytical facilities, it is possible to detect very low concentration of contaminants during emission. The factors like leaf surface area, contaminant concentration, nature of the contaminant species, water flow during transpiration, and cells viability are the major role for success of phytovolatilization. Some of the metal contaminants such as Se, Hg, and As are successfully removed from contaminated site using this method.

17.4.5 Phytodegradation or Phytotransformation

Phytodegradation is the process of the degradation of complex organic molecules to simple molecules or the incorporation of these molecules into plant tissues (Trapp et al. 2001). During phytodegradation, contaminants are fragmentized into small molecules which are not harmful to the plants. The various mechanisms such as degradation, adsorption, accumulation, and volatilization of the contaminant are involved in phytodegradation. The degradation rate depends on the solubility, acceptable range of contaminant concentration, and hydrophobic nature of contaminants. Various organic contaminants such as herbicides, chlorinated solvents, and organic pollutants were experimented for remediation by several researchers.

17.4.6 Rhizodegradation/Phytostimulation

Rhizodegradation refers to the microbial breakdown of contaminants within the plant root zone or rhizosphere. This is due to presence of high concentration of bacteria or other microorganisms present in the rhizosphere. Several researchers confirmed that huge diversity of microorganisms were found in rhizosphere. These microorganisms are used to assist plant to uptake the micronutrients present in the soil. Due to the high surface area of the root, microbes tend to grow on the root surface and it facilitates the high oxygen transfer rate to the environment. Because of the localized nature of microorganism, it is mainly useful for treating the contaminated soil, and there are several investigations were carried out to degrade the organic chemicals, including petroleum hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), chlorinated solvents, pesticides, polychlorinated biphenyls (PCBs),

benzene, toluene, ethylbenzene, and xylenes using rhizodegradation (EPA, 2000). It can also be seen as plant-assisted bioremediation, the stimulation of microbial and fungal degradation by the release of exudates/enzymes into the root zone (rhizosphere) (Zhuang et al. 2005).

17.4.7 Biotransformation of Pollutants by Plants

Many enzymes are capable of transforming organic contaminants present in the soil by catalyzing chemical reactions. Many enzymes present in the plant cells as the causative agents in the transformation of contaminants mixed with sediment and soil (Khaziev 1980). Those enzymes are dehalogenase, nitroreductase, nitrilases, peroxidase, and laccase. One of the important enzymes is peroxidase. These enzymes have been found on the external root surfaces of plants and having interaction with phenolic, aniline, and certain other aromatic contaminants present in the soil. The mechanism of peroxidase on pollutants is forming the polymerization pf the pollutant either onto the root surface or into the soil humic fraction. These polymeric complexes are often referred to as "bound residues"; this process is called as phytodecontamination category. Various factors affect the production of an enzyme such as age, season, stress induced, the concentration of contaminants, pH condition of the soil. Degradation capacities depend not only on the production of these enzymes but also on the activity of the enzyme, their release rates, the nature of the soil matrix, and the concentration of contaminant it encounters relative to other potential substrates.

17.5 Factors Affecting the uptake Mechanisms of Contaminants in Phytoremediation

- Selection of plant variety selection,
- nature/structural diversity and concentration of dyes,
- availability of dyes to plant roots,
- pH of contaminant,
- temperature and humidity of the system,
- presence of organic matter in soil, water, oxygen, and nutrients availability,
- types of microorganism,
- biomass of the remediating plants.

There are different approaches for the phytoremediation technologies which includes constructed wetlands, hydroponics, vertical flow beds, hybrid systems, and

Fenton's oxidation. Constructed wetlands are artificially or semi-artificially created to improve the water quality. Wetlands are easy to maintain to treat the organic matter, nutrients, and pathogens. It is one of the popular technologies across the globe in effective treatment of wastewaters. Similar to wetlands, vertical flow beds contain one or more vertical flow beds or reverse flow chambers which is highly effective in removing xenobiotic compounds. The vertical flow beds allow intense contact with the pollutants in the root surfaces which helps is efficient removal. Hydroponic systems help the plant metabolism in uptaking and degradation of pollutants. These systems are sustainable and help in climate mitigation and greenhouse gas reductions. Hybrid systems involve the combination of different technologies which helps in increasing the efficiency of pollutants removal.

17.6 Characterization of the Textiles Dyes and Effluents After Phytoremediation

Textile processing industries release these effluents containing highly complex nature of mixtures consists of acids, bases, detergents, surfactants, humectants, mordants, fasteners, fixers, softening agents and different class of textile dyes. Due to its presence of the above solute, these wastewaters acquire extremely high concentration of COD, BOD, TOC, TSS, TDS, turbidity, and conductivity. By treating this effluent by phytoremediation, these effluents are less toxic in nature. For instance, BOD and COD were reduced up to 40–70% using *E. Crassipe* for textile effluents at laboratory scale (Qaisar et al. 2005). A combinatorial vertical and horizontal flow laboratory scale reactor with *P. australis* plants also achieved reductions in BOD and COD up to 45%; a similar system at pilot scale showed an enhancement in the treatment efficacy (Bulc and Ojstršek 2008). Using static bioreactor planted with *T. angustifolia* was found efficient in treating textile effluent to maximum level and reduced the TDS, COD, and color by 86%, 59%, and 58%, respectively (Nilratnisakorn et al. 2007). In a vertical flow laboratory scale reactor with *G. pulchella* plants was also obtained to reduce the COD, TOC, and BOD by 70%, 74%, and 70%, respectively, within 60 h (Kabra et al. 2013). A photobioreactor is placed to *Portulaca grandiflora* and was found to be effective for treating textile effluents to produce less toxic levels and attained the COD, BOD, TOC, turbidity, TDS, and TSS reductions by 59%, 38%, 37%, 41%, 71%, and 60%, respectively, within 72 h (Khandare et al. 2013). Similarly, an another study was carried in phyto-tunnel constructed *with Portulaca grandiflora* plantation and found decrease in COD, BOD, TOC, turbidity, conductivity, TDS, and TSS of a textile effluent by 57%, 45%, 43%, 76%, 52%, 24%, and 77%, respectively, within 96 h (Khandare et al. 2014).

17.7 Toxicity Analysis of Dye Products in Dye Effluents (Kabra et al. 2013)

Toxicity studies of effluents are made by following analytical tools such as:

- Fourier transform infrared spectroscopy (FTIR),
- Thin layer chromatography (TLC),
- High-performance liquid chromatography (HPLC),
- High-performance thin layer chromatography (HPTLC),
- Gas chromatography–mass spectroscopy (GC–MS),
- Liquid chromatography–mass spectroscopy (LC–MS).

17.8 Various Physiochemical Factors Affecting the Phytoremediation of Textile Dyes and Effluents (Pilon-Smits 2005)

- Plants growth form,
- nature/structural diversity and concentration of dyes,
- availability of dyes to plant roots,
- pH of dye wastewater, temperature, and humidity,
- organic matter content in media, water, oxygen, and nutrients availability rhizospheric process,
- biomass of the remediating plants,
- the growth of microbes in the root zone,
- and mass transfer limitations of pollutant, etc.

17.9 Advantages Of Phytoremediation

- It has high effectiveness in reduction of contamination, applicable for wide range of contaminants, environmentally friendly.
- It has low cost compared to conventional physicochemical methods.
- It does not require expensive equipment or highly specialized personnel.
- The dye effluents are its effectiveness in reducing the concentration of contaminants to very low levels and lead to contaminant degradation.
- It is more cost-effective for handling large volumes of low concentration of contaminant and for large areas having low to moderately contaminated surface soils.

17.10 Disadvantages of Phytoremediation

- It is time-consuming process, and it may take at least several growing seasons to clean up a site. The intermediates compound formed from those organic and inorganic contaminants may be cytotoxic to plants.
- It may affect the plant growth rate.
- Excavation and disposal or incineration takes weeks to months to accomplish, while phytoextraction or degradation may need several years.
- Phytoremediation might be best suited for remote areas where human contact is limited or where soil contamination does not require an immediate response.
- Root contact is a primary limitation on phytoremediation applicability.
- Plant growth may inhibit the high concentration of contaminants. In presence of high organic contamination, rapid microbial degradation decreases the partial pressure of oxygen below the point where many plants can survive. These anaerobic zones are not appropriate targets for phytoremediation until the microbial degradation (and its effect **on** oxygen consumption) has subsided.
- The phytoremediation mechanism is not well understood.

17.11 Conclusions

Degradation of textile wastewater through phytoremediation and the factors that affect the processes have been discussed. This chapter also addressed the plant mechanism for textile treatment, factor affecting the process, reactors used for phytoremediation, different methods of characterization methods of textile effluents after phytoremediation. Finally, advantages and limitation of phytoremediation were discussed.

References

Bulc TG, Ojstršek A (2008) The use of constructed wetland for dye-rich textile wastewater treatment. J Hazard Mater 155(1):76–82

Etim E (2012) Phytoremediation and its mechanisms: a review. Int J Environ Bioenergy 2(3); 120–136

Ghodake GS, Talke AA, Jadhav JP, Govindwar SP (2009) Potential of brassica juncea in order to treat textile—effluent—contaminated sites. Int J Phytorem 11(4):297–312

Kabra AN, Khandare RV, Govindwar SP (2013) Development of a bioreactor for remediation of textile effluent and dye mixture: a plant–bacterial synergistic strategy. Water Res 47(3): 1035–1048

Khandare RV, Rane NR, Waghmode TR, Govindwar SP (2012) Bacterial assisted phytoremediation for enhanced degradation of highly sulfonated diazo reactive dye. Environ Sci Pollut Res 19(5):1709–1718

Khandare RV, Kabra AN, Kadam AA, Govindwar SP (2013) Treatment of dye containing wastewaters by a developed lab scale phytoreactor and enhancement of its efficacy by bacterial augmentation. Int Biodeterior Biodegradation 78:89–97

Khandare RV, Watharkar AD, Kabra AN, Kachole MS, Govindwar SP (2014) Development of a low-cost, phyto-tunnel system using Portulaca grandiflora and its application for the treatment of dye-containing wastewaters. Biotech Lett 36(1):47–55

Khataee A, Movafeghi A, Torbati S, Lisar SS, Zarei M (2012) Phytoremediation potential of duckweed (Lemna minor L.) in degradation of CI Acid Blue 92: artificial neural network modeling. Ecotoxicol Environ Saf 80:291–298

Khaziev F (1980) Soil enzyme activities. Science Press, Beijing

Mahmood Q, Masood F, Bhatti ZA, Siddique M, Bilal M, Yaqoob H, Farooq R, Ullah Z (2014) Biological treatment of the dye reactive blue 19 by cattails and anaerobic bacterial consortia. Toxicol Environ Chem 96(4):530–541

Nilratnisakorn S, Thiravetyan P, Nakbanpote W (2007) Synthetic reactive dye wastewater treatment by narrow-leaved cattails (Typha angustifolia Linn.): effects of dye, salinity and metals. Sci Total Environ 384(1):67–76

Paquin DG, Sun WH, Tang CS, Li QX (2006) A phytoremediation study: selection of tropical and other vascular plants for decolorization of Poly R-478 dye. Rem J 16(4):97–107

Pilon-Smits E (2005) Phytoremediation. Annu Rev Plant Biol 56:15–39

Qaisar M, Ping Z, Rehan SM, Ejaz ul I, Rashid AM, Yousaf H (2005) Anatomical studies on water hyacinth (Eichhornia crassipes (Mart.) Solms) under the influence of textile wastewater. J Zhejiang Univ-Sci B, 6(10):991–998

Rafatullah M, Sulaiman O, Hashim R, Ahmad A (2010) Adsorption of methylene blue on low-cost adsorbents: a review. J Hazard Mater 177(1):70–80

Rane NR, Chandanshive VV, Khandare RV, Gholave AR, Yadav SR, Govindwar SP (2014) Green remediation of textile dyes containing wastewater by Ipomoea hederifolia L. RSC Adv 4 (69):36623–36632

Rane NR, Chandanshive VV, Watharkar AD, Khandare RV, Patil TS, Pawar PK, Govindwar SP (2015) Phytoremediation of sulfonated Remazol Red dye and textile effluents by Alternanthera philoxeroides: An anatomical, enzymatic and pilot scale study. Water Res 83:271–281

Rew A (2007) Phytoremediation: an environmentally sound technology for pollution prevention, control and remediation in developing countries. Educ Res Rev 2(7):151–156

Robinson T, McMullan G, Marchant R, Nigam P (2001) Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative. Biores Technol 77(3):247–255

Slimani R, El Ouahabi I, Abidi F, El Haddad M, Regti A, Laamari MR, El Antri S, Lazar S (2014) Calcined eggshells as a new biosorbent to remove basic dye from aqueous solutions: thermodynamics, kinetics, isotherms and error analysis. Journal of the Taiwan Institute of Chemical Engineers 45(4):1578–1587

Torbati S, Khataee A, Movafeghi A (2014) Application of watercress (Nasturtium officinale R. Br.) for biotreatment of a textile dye: Investigation of some physiological responses and effects of operational parameters. Chem Eng Res Des 92(10):1934–1941

Trapp S, Köhler A, Larsen LC, Zambrano KC, Karlson U (2001) Phytotoxicity of fresh and weathered diesel and gasoline to willow and poplar trees. J Soils Sediments 1(2):71–76

Vafaei F, Khataee A, Movafeghi A, Lisar SS, Zarei M (2012) Bioremoval of an azo dye by Azolla filiculoides: Study of growth, photosynthetic pigments and antioxidant enzymes status. Int Biodeterior Biodegradation 75:194–200

Verma AK, Dash RR, Bhunia P (2012) A review on chemical coagulation/flocculation technologies for removal of colour from textile wastewaters. J Environ Manage 93(1):154–168

Vijayalakshmidevi S, Muthukumar K (2014) Phytoremediation of textile effluent pretreated with ultrasound and bacteria. J Environ Chem Eng 2(3):1813–1820

Watharkar AD, Khandare RV, Waghmare PR, Jagadale AD, Govindwar SP, Jadhav JP (2015) Treatment of textile effluent in a developed phytoreactor with immobilized bacterial augmentation and subsequent toxicity studies on Etheostoma olmstedi fish. J Hazard Mater 283:698–704

Zhuang P, Ye Z, Lan C, Xie Z, Shu W (2005) Chemically assisted phytoextraction of heavy metal contaminated soils using three plant species. Plant Soil 276(1–2):153–162

Chapter 18
Role of Biosurfactants in Enhancing the Microbial Degradation of Pyrene

Gauri Gupta, Avantika Chandra, Sunita J. Varjani, Chiranjib Banerjee and Vipin Kumar

Abstract Pyrene is a high molecular weight polycyclic aromatic hydrocarbon (PAH) with a symmetrical structure, commonly found as a pollutant of air, water, and soil. Being one of the most abundant high molecular weight pericondensed PAH and having its structure similar to several carcinogenic PAHs is being used as a model compound to study the degradation of high molecular weight PAHs. Therefore, its removal from the environment is a challenging task for scientists. Pyrene has been found to be toxic to the aquatic microinvertebrate *Gammarus pulex,* and its quinone metabolites are mutagenic and toxic to organisms in the environment. This chapter mainly focuses on the microbial degradation of ecologically toxic pyrene by pure microbial cultures and microbial consortium, simultaneously emphasizing the role of surfactants in enhancing the degradation process.

Keywords Pyrene · Biodegradation · Bacterial consortium · Biosurfactants Degradation

18.1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are organic compounds composed of two or more fused benzene rings that are arranged in linear, angular, or cluster configurations. Their toxic, carcinogenic, and mutagenic properties and persistent

G. Gupta · V. Kumar (✉)
Laboratory of Applied Microbiology, Department of Environmental Science & Engineering, Indian Institute of Technology (ISM), Dhanbad 826004, Jharkhand, India
e-mail: vipinmicro1@gmail.com

A. Chandra · C. Banerjee
Department of Environmental Science & Engineering, Indian Institute of Technology (ISM), Dhanbad 826004, Jharkhand, India

S. J. Varjani
Gujarat Pollution Control Board, Gandhinagar 382010, Gujarat, India

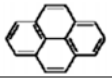
S. J. Varjani et al. (eds.), *Bioremediation: Applications for Environmental Protection and Management*, Energy, Environment, and Sustainability,
https://doi.org/10.1007/978-981-10-7485-1_18

nature make them environmental pollutants. These compounds can have harmful effect on the flora and fauna of contaminated sites and can result in health problems and genetic defects in human beings. Thus, environmental decontamination of PAHs becomes necessary. Although several conventional methods are available due to the economical and eco-friendly nature of biological methods, they are more prevalent for use. Bioremediation, use of microbes for decontamination, is one such biological method which is increasingly used. The process of bioremediation includes microbial degradation of soil PAHs into less complex metabolic compounds (Gupta et al. 2016). Compared to low molecular weight PAHs, the bioremediation of high molecular weight (HMW) is difficult to perform due to low bioavailability, hydrophobicity, and thermodynamically stable nature (Mihelcic et al. 1993). Several studies have been reported on the biological degradation of low molecular weight PAH using bacteria, fungi and algae, which have capabilities to decontaminate PAH-contaminated soil and water, but only a few genera have been observed to degrade the high molecular weight PAHs, such as pyrene (Peng et al. 2008). Due to its mutagenic and carcinogenic properties, its removal from the environment has been crucial (Das and Mukherjee 2007). In environment, the origination of PAHs occurs through natural and anthropogenic sources. Natural sources are forests and rangeland fires, trees and volcanic eruptions exudates. Some of the important anthropogenic sources include burning of fossil fuels, wood, coal tar, garbage, petroleum oil refining, electric power generation, home heating, internal combustion engines, municipal solid waste incineration, and petroleum spills (Haritash and Kaushik 2009). United State Environmental Protection Agency (USEPA) has ranked it as one of the top 129 hazardous pollutants (Kafilzadeh et al. 2012). During its degradation, its quinone-based metabolites are toxic and mutagenic to organisms in the environment (Bolton et al. 2000). Pyrene has several applications, e.g., pyrene and its derivatives are used in the manufacture of dyes and dye precursors (pyranine and naphthalene-1,4,5,8-tetracarboxylic acid). Pyrene derivatives are used as molecular probes via fluorescence spectroscopy having high quantum yield lifetime. Pyrene in its excited state becomes non-planar in structure which results in its highly sensitive fluorescence spectrum toward solvent polarity and thus used as probe in solvent environment determination. This work has emphasized on the biological removal of pyrene using pure microbial cultures and microbial consortium, with a simultaneous highlight on the role of surfactants in the enhancement of the degradation process.

18.2 Occurrence and Physical Properties of Pyrene

Pyrene, a tetracyclic compound, is most abundant high molecular weight PAH which possesses a symmetrical structure. Since pyrene has its structure similar to several carcinogenic PAH, it has been used as a model compound to study the degradation of high molecular weight PAHs (Kanaly and Harayama 2000). Pyrene is a four-ring PAH composed of fused rings which are grouped in a clustered

Table 18.1 Physical and chemical properties of pyrene. *Source* Mueller et al. (1996)

Properties	Structure/Values
Molecular structure	
Molecular formula	$C_{16}H_{10}$
Molecular weight ($gmol^{-1}$)	202.25
Melting point (°C)	156
Boiling point (°C)	360
Vapor pressure (mm Hg)	6.9×10^{-7}
Solubility in water ($mg\ L^{-1}$)	0.132
Carcinogenic potency	U

U unclassified as to carcinogenicity to humans

arrangement. In the environment, pyrene is commonly found as a pollutant of air, water, and soil and is highly persistence (IARC 1986). The production of pyrene is a result of gasification processes and other incomplete combustion processes (Ceyhan 2012). As the number of fused benzene rings increases, the solubility of PAHs decreases. Pyrene is highly hydrophobic in nature with low water solubility (Kim et al. 2007) and aqueous solubility of 0.132 mg L^{-1} with a vapor pressure value of 6.9×10^{-7} torr. The physical–chemical properties of pyrene are summarized in Table 18.1. Pyrene looks colorless crystal-like solid but can also look yellow. The maximum permissible concentration of pyrene in soil is 2300 mg kg^{-1} and in water is 830 µg L^{-1}. High molecular weight PAH-like pyrene does not volatilize and remains bound to soil organic particles which lead to decreased bioavailability for natural degradation (Gupta et al. 2017). The chemical structure of pyrene makes it recalcitrant and resistant to microbial degradation.

18.3 Toxic Effects Caused Due to Pyrene Exposure

IARC (1986) reported that though pyrene is not carcinogenic to humans, nonhuman primates, or laboratory animals, when applied along with benzo[a]pyrene on mouse skin, it enhanced the carcinogenic effects of benzo[a]pyrene (Wei et al. 2017). The metabolites of pyrene which are quinone based are toxic and mutagenic in nature (Saraswathy and Hallberg 2002). In an environment contaminated with PAHs, pyrene exposure occurs through skin, food, air, and water contaminated with pyrene. The common sources of pyrene which may lead to dermal exposure are heavy oils, coal tar, roofing tar, or creosote. Inside the body, PAHs target organs like kidneys, fat tissues, and liver. When mice injected with pyrene developed a kidney disease known as nephropathy. Several other symptoms were also observed. These include induction of tumors, increase in the liver weight and decrease in the weight of kidney weight, damage to skin, body fluids, and immune system. However, so far these effects have not been seen in humans (IARC 1986).

18.4 Pyrene Degradation by Single Microbial Species and Microbial Consortium

The ubiquitous distribution and genotoxic nature of pyrene result in environmental contamination (Kumari et al. 2013). Various physical and chemical methods like electrochemical remediation, landfilling, solvent extraction, air sparging, thermal destruction/incineration, photocatalytic remediation, and use of synthetic surfactants are employed for cleaning of such contaminated sites (Gupta et al. 2016). However, biological methods are more eco-friendly, economical, and efficient for degradation of high molecular weight pyrene. Bioremediation of high molecular weight PAHs has been widely studied over the past several years (Boonchan et al. 2000). Though bioremediation is the major process for pyrene degradation, there have been some unsuccessful attempts for removal of HMW pyrene. Bioremediation involves the use of certain microbial species which may partially degrade pyrene to less toxic substrates (Saraswathy and Hallberg 2002). These microorganisms include bacteria, algae, fungi, cyanobacteria, and heterotrophic bacteria, which contribute to PAHs degradation (Gupta et al. 2017). Most of these species are capable of using pyrene as the sole carbon and energy source and degrade it to its oxidized products (Kim et al. 2007). The chief bacterial species responsible for pyrene degradation are *Bacillus cereus* (Kazunga and Aitken 2000), *Pseudomonas fluorescens* (Boonchan et al. 1998), *Pseudomonas stutzeri* (Kazunga and Aitken 2000), *Sphingomonas paucimobilis* (Kastner et al. 1998), *Burkholderia cepacia* (Juhasz et al. 1997), *Stenotrophomonas maltophilia* (Boonchan et al. 2000) *Rhodococcus* sp. (Walter et al. 1991), and *Cycloclasticus* sp. P1 (Wang et al. 2008). Several fungal strains such as certain species of white rot fungi *Phanerochaete chrysosporium* (Hammel et al. 1986), *Pleurotus ostreatus* (Bezalel et al.1996), *Crinipellis stipitaria* (Lange et al. 1994), some micromycetes (Ravelet et al. 2000) and ectomycorrhizal fungi (Braun-Lullemann et al. 1999) have also been isolated for pyrene degradation. Several strains of *Pseudomonas* such as *Pseudomonas* sp. LP1 (Obayori and Salam 2010), *P. aeruginosa* strains LP5 and LP6 (Obayori et al. 2008) have been demonstrated to degrade pyrene. Several researchers have reported the incapability of *Pseudomonas* and some other genera do not utilize pyrene (Daugulis and McCracken 2003). Jorfi et al. (2013) studied pyrene degradation using bacterial sp. *Pseudomonas aeruginosa* SP4 and reported it to be 84.60%. Kafilzadeh et al. (2012) isolated Corynebacterium sp. from soil and demonstrated 79.4% of pyrene degradation during an incubation period of 10 days. Walter et al. (1991) reported 72% pyrene degradation in 14 days by a *Rhodococcus* sp. isolated from soil. Rehmann et al. (1998) reported the isolation of *Mycobacterium* sp. KR2 and observed its pyrene degradation efficiency to be 60% after an incubation time of 8 days. Table 18.2 presents the data gathered on the degradation rate of pyrene by mixed microbial species.

Pyrene degradation by both pure and mixed cultures has been reported (Kazunga and Aitken 2000). A microbial consortium (a mixture of various microbial species)

Table 18.2 Pyrene degradation rates by single microbial species. Modified from Gupta et al. (2017)

Single microbial species	Percent degradation (%)	Experiment time (days)	References
Source-soil			
Mycobacterium sp.	89.1	10	Kafilzadeh et al. (2012)
Leclercia adecarboxylata PS4040	61.5	20	Sarma et al. (2010)
Pseudomonas putida	97.4	42	Bishnoi et al. (2009)
Pseudomonas paucimobilis	95.5		
Ochrobactrum sp. P2	80.0	14	Kumari et al. (2013)
Pseudomonas sp. BP10	96.0		
Acinetobacter PSM11	75.0		
Native microbes	17.0	14	Kumari et al. (2013)
P. terrestre	75.0	28	Saraswathy and Hallberg (2002)
T. harzianum	65.0		
P. janthinellum	57.0		
Stenotrophomonas maltophilia BR 12	87.0	20	Singh et al. (2015)
Phanerochaete chrysosporium	92.2	42	Bishnoi et al. (2008)
Burkholderia cepacia			
VUN 10 001	100.0	7	Juhasz et al. (1997)
VUN 10 002	99.7		
VUN 10 003	95.3		
Source-water			
Proteus vulgaris	71.5	7	Ceyhan et al. (2012)

has been found to be more effective than pure cultures in the degradation process. The microbes within consortia together perform synergistic interactions among themselves, resulting in the highest possible degradation (Wu et al. 2013). Table 18.3 presents the data gathered on degradation of pyrene by microbial consortium. Lin and Cai (2008) studied pyrene degradation using two *Bacillus* sp. isolated from Mangrove sediment and observed its degradation efficiency for an incubation period of 21 days. When used as pure isolates, *Bacillus cereus* Py5 degraded pyrene around 65.8% and *Bacillus megaterium* Py6 degraded around 33.7% of pyrene. And on application of consortium of both these strains, the degradation efficiency was found to be 92.1%, which was much higher than pure isolates. Malik and Ahmed (2012) studied a consortium (*Bacillus* sp., *Micrococcus* sp., *Staphylococcus* sp. *Pseudomonas* sp., *Alcaligenes faecalis, Psychrobacter* sp.) and demonstrated a pyrene degradation efficiency of 46.17%.

Table 18.3 Pyrene degradation rates by microbial consortium. Modified from Gupta et al. (2017)

Microbial consortium	Percent degradation (%)	Experiment time	References
Source-soil			
Gamma proteobacterium AY972873.1, *Citrobacter* sp. S-77 AB668058.1, *Citrobacter* sp. AB668058.1	85.0	10 days	Wu et al. (2013)
Bacterium FJ037700.1 Bacterium JF733793.1 Bacterium JF&33804 *Raoultella ornithinolytica* HQ259705.1	75.0	30 days	Wu et al. (2013)
Consortium *Mycobacterium* sp. *Strain* A1-PYR *Sphingomonas* sp. *Strain* PHe	50.0	7 days	Zhong et al. (2011)
Bacterial fungal consortium *Bacillus* sp. PY1, *Sphingomonas* sp. PY2 *Fusarium* sp. PY3	96.1	9 days	Liu et al. (2013)
Mycobacterium fortuitum, Bacillus cereus, Microbacterium sp., *Gordonia polyisoprenivorans, Microbacteriaceae* bacterium, naphthalene utilizing bacterium	96.0	70 days	Jacques et al. (2008)
Source-mangrove sediment			
Rhodococcus sp., *Acinetobacter* sp., *Pseudomonas* sp.	90.0	4 weeks	Hang (2007)
Consortium YL *Bacillus cereus* Py 5 *Bacillus megaterium* Py 6	92.1	21 days	Lin and Cai (2008)

Moscoso et al. (2012) studied pyrene degradation at the flask and bioreactor scale and compared the degradation efficiency of consortium LB2 (*Staphylococcus warneri, Bacillus pumilus*) and a pure culture *Pseudomonas stutzeri*. Consortium was observed to have higher degradation rates than pure culture. By pure culture, 85% of pyrene was degraded at flask scale and 90% of pyrene removal was observed at bioreactor scale. Whereas using bacterial consortium, 75 and 100% of degradation were observed at flask and bioreactor scale, respectively. Subashchandrabose et al. (2017) studied the pyrene degradation by a soil microalga, *Chlorella* sp. MM3 and reported the accumulation of two major metabolites in culture medium. They also suggested that the alginate immobilized cells of *Chlorella* sp. MM3 in the presence of low concentrations of Tween 80 has great potential in remediating soils contaminated with pyrene. Several reporters have studied the applications of biosurfactants to enhance the degradation rates of several compounds (Barkay et al. 1999).

This leads the focus on isolation and characterization of biosurfactant-producing microbes which can utilize pyrene and other PAHs (Guo et al. 2005).

18.5 Pyrene Degradation Pathway by Microbes

There are several pathways proposed for microbial degradation of pyrene. Liang et al. (2006) studied the pyrene degradation by *Mycobacterium* sp. strain KMS and observed the enzymes and metabolites and produced during the process. During pyrene metabolism, the metabolites formed were identified using high-performance liquid chromatography. The study observed the identification of various important metabolites such as pyrene-4,5-dione, *cis*-4,5-pyrene-dihydrodiol, phenanthrene-4,5-dicarboxylic acid, and 4-phenanthroic acid were identified during pyrene degradation. Although Liang et al. (2006) could not identify one of the important metabolite *trans*-4,5-pyrene-dihydrodiol, the enzyme (epoxide hydrolase) which responsible for its formation was identified. They demonstrated that the metabolite pyrene-4,5-dione, which is a result of nonenzymatic autooxidation of 4,5-dihydroxypyrene, usually gets accumulated as a dead end product in some gram-negative bacterial cultures, was further consumed and degraded by *Mycobacterium* sp. strain KMS. Liang et al. (2006) for the first time exhibited the conversion of pyrene to quinone by a *Mycobacterium* sp.

Kumari et al. (2013) studied pyrene degradation using three bacterial strains BP10, P2, and PSM11 and found them to be the efficient degraders of pyrene. They also concluded that the higher activity of catechol 1,2-dioxygenase than catechol 2,3-dioxygenase shows that ortho cleavage represents the main pathway for initial pyrene degradation. It was also reported that several enzymes by bacteria BP10 play important role in pyrene degradation.

18.6 Surfactant-Enhanced Degradation of Pyrene

The low water solubility of PAHs limits their bioavailability to degrade microbes (Mihelcic et al. 1993). The interfacial surface area between water and oil restricts the bacterial growth on hydrocarbons (Shreve et al. 1995). This leads to decrease in hydrocarbon degradation. Biosurfactants help in desorption of PAH from soil organics making them more bioavailable for microbial attack, and thus enhancing the degradation rates (Barkay et al. 1999). Several microorganisms result in the production of biosurfactants while growing in water-immiscible substrates. Biosurfactants have diverse molecular structures. The use of biosurfactants over synthetic surfactants has proved to be more beneficial as chemically synthetic surfactants are extortionate, toxic in nature and are non-biodegradable (Gupta et al. 2016). Biosurfactants have amphiphilic properties which help in the compound mobilization by reducing the surface and interfacial tension and by accumulating

the hydrophobic compounds in the micelles formed by surface monomers. Surfactant molecule is composed of hydrophilic headgroup and one or two hydrophobic parts (Banat et al. 2000). In a system, at surfactant concentration beyond a certain threshold (the critical micelle concentration CMC), the surfactant monomers aggregate to form micelles. This leads to mobilization of hydrophobic substrates to micelles leading to their solubilization. Above CMC, the solubility of PAHs increases (Makkar and Rockne 2003).

During microbial degradation of PAHs, several surface-active agents are naturally produced, these are called as biosurfactants. Biosurfactants are cost-effective, biodegradable in nature and can be produced and used in extreme conditions. Biosurfactants are commonly used in oil and petroleum industries as they help in solubilization of hydrocarbons in petrol and oil industries. However, some reports have contradicted with the use of biosurfactants and reported that their presence inhibits biodegradation (Stelmack et al. 1999).

Table 18.4 compiles the effects of biosurfactants on degradation of pyrene. Jorfi et al. (2013) studied pyrene degradation using *Pseudomonas Aeruginosa* SP4 and reported the production of biosurfactant during the degradation process. The reported biosurfactant was glycolipid type. Nie et al. (2010) isolated *Pseudomonas aeruginosa* strain NY3 from petroleum contaminated soil. The strain was able to produce novel biosurfactant rhamnolipid and could efficiently degrade high molecular weight PAHs including pyrene. Das and Mukherjee (2007) reported the production of biosurfactants by *Bacillus subtilis* DM-04 and *Pseudomonas aeruginosa* M and NM strains during pyrene degradation. It was also shown that the biosurfactant produced by *B. subtilis* DM-04 was more efficient than *P. aeruginosa* M and NM strains in reducing the surface tension of growth medium. *Stenotrophomonas maltophilia* was reported to produce biosurfactant (BR-12) during pyrene degradation. About 87% of pyrene degradation was reported in 20 days (Singh et al. 2015).

Table 18.4 Literature shows positive effects of biosurfactants on biodegradation of pyrene

Biosurfactants	Microorganism	Source	Remarks	References
Rhamnolipid	*P. aeruginosa* 64	Soil	Leads to enhanced degradation	Straube et al. (1999)
Alasan	*Acinetobacter radioresistens* KA53	Liquid	Enhanced degradation rates	Barkay et al. (1999)
Proteolipid	*Ochrobactrum* sp. P2	Soil	Increased desorption thus enhanced degradation	Kumari et al. (2013)
Glycolipid	*Pseudomonas* sp. BP10 *Acinetobacter* PSM11	Soil	Increased desorption thus enhanced degradation	Kumari et al. (2013)

18.7 Conclusions and Future Scope

The use of biological methods in pyrene decontamination is gaining prevalence due to their economic and eco-friendly approach. However, the microbial efficiency has found to be lower for degradation of high molecular weight PAHs (pyrene) as compared to low molecular weight PAHs. This is one of the major scopes for future research for enhancement of microbial degradation of pyrene at contaminated sites. This study has elaborated the literature supporting the pyrene degradation using pure bacterial culture and bacterial consortium. During the degradation process, several physicochemical, biological, and environmental factors can serve as limiting agents. These may include pH, temperature, essential inorganic nutrients, lack of oxygen and can be the future scope of study. Current research trends such as bioaugmentation (involves the application of microbes with pyrene degradation potential to overcome the catabolic limitations of indigenous microorganisms) can be applied for enhancement of pyrene degradation at pyrene contaminated sites. Not much has been studied about pyrene metabolism pathway. Also, the metabolites produced during the process have not been expounded yet. Also, pyrene has low water solubility and low bioavailability; therefore, methods enhancing its bioavailability should be explored.

References

Banat I, Makkar RS, Cameotra S (2000) Potential commercial applications of microbial surfactants. Appl Microbiol Biotechnol 53:495–508

Barkay T, Navon-Venezia S, Ron E et al (1999) Enhancement of solubilization and biodegradation of polycyclic aromatic hydrocarbons by the bioemulsifier alasan. Appl Environ Microbiol 65:2697–2702

Bezalel L, Hadar Y, Cerniglia CE (1996) Mineralization of polycyclic aromatic hydrocarbons by the white rot fungus *Pleurotus ostreatus*. Appl Environ Microbiol 62:292–295

Bishnoi K, Kumar R, Bishnoi NR (2008) Biodegradation of polycyclic aromatic hydrocarbons by white rot fungi *Phanerochaete chrysosporium* in sterile and unsterile soil. J Sci Ind Res 67:538–542

Bishnoi K, Sain U, Kumar R et al (2009) Distribution and biodegradation of polycyclic aromatic hydrocarbons in contaminated sites of Hisar (India). Ind J Exp Biol 47:210–217

Bolton JL, Trush MA, Penning TM et al (2000) Role of quinones in toxicology. Chem Res Toxicol 13:135–160

Boonchan S, Britz ML, Stanley GA (1998) Surfactant-enhanced biodegradation of high molecular weight polycyclic aromatic hydrocarbons by *Stenotrophomonas maltophilia*. Biotechnol Bioeng 59:482–494

Boonchan S, Britz ML, Stanley GA (2000) Degradation and mineralization of high-molecular weight polycyclic aromatic hydrocarbons by defined fungal-bacterial co-cultures. Appl Environ Microbiol 66:1007–1019

Braun-Lullemann A, Huttermann A, Majcherczyk A (1999) Screening of ectomycorrhizal fungi for degradation of polycyclic aromatic hydrocarbons. Appl Microbiol Biotechnol 53:127–132

Ceyhan N (2012) Biodegradation of pyrene by a newly isolated *Proteus vulgaris*. Sci Res Essays 7:66–77

Das K, Mukherjee AK (2007) Differential utilization of pyrene as the sole source of carbon by *Bacillus subtilis* and *Pseudomonas aeruginosa* strains: role of biosurfactants in enhancing bioavailability. J Appl Microbiol 102:195–203

Daugulis AJ, McCracken CM (2003) Microbial degradation of high and low molecular weight polyaromatic hydrocarbons in a two-phase partitioning bioreactor by two strains of Sphingomonas sp. Biotechnol Lett 25:1441–1444

Guo CL, Zhou HW, Wong YS et al (2005) Isolation of PAH-degrading bacteria from mangrove sediments and their biodegradation potential. Mar Pollut Bull 51:1054–1061

Gupta G, Kumar V, Pal AK (2016) Biodegradation of polycyclic aromatic hydrocarbons by microbial consortium: a distinctive approach for decontamination of soil. Soil Sediment Contam 25:597–623

Gupta G, Kumar V, Pal AK (2017) Microbial degradation of high molecular weight polycyclic aromatic hydrocarbons with emphasis on pyrene. Polycycl Aromat Compd 6:1–3

Hammel KE, Kalayanaraman B, Krik TK (1986) Oxidation of PAHs and dibenzo[p]dioxins by *Phanerochaete chrysosporium* ligninase. J Biol Chem 261:16948–16952

Hang YS (2007) Bioremediation of polycyclic aromatic hydrocarbons (PAHs) by microorganisms in mangrove sediments. PhD thesis, City University of Hong Kong

Haritash AK, Kaushik CP (2009) Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): a review. J Hazard Mater 169:1–15

IARC (1986) Monographs on the evaluation of carcinogenic risk of chemicals to humans: an updating on IARC monographs. In: International agency for research on cancer 38 World Health Organization, Lyon, France

Jacques RJS, Okeke BC, Bento FM et al (2008) Microbial consortium bioaugmentation of a polycyclic aromatic hydrocarbons contaminated soil. Bioresour Technol 99:2637–2643

Jorfi S, Rezaee A, Mobeh-Ali G-A et al (2013) Application of biosurfactants produced by *Pseudomonas aeruginosa* SP4 for bioremediation of soils contaminated by pyrene. Soil Sediment Contam 22:890–911

Juhasz AL, Britz ML, Stanley GA (1997) Degradation of fluoranthene, pyrene, benz[a]anthracene and dibenz[a,h]anthracene by Burkholderia cepacia. J Appl Microbiol 83:189–198

Kafilzadeh F, Hoshyaripour F, Tahery Y et al (2012) Bioremediation of pyrene by isolated bacterial strains from the soil of the landfills in Shiraz (Iran). Ann Biol Res 3:486–494

Kanaly RA, Harayama S (2000) Biodegradation of high-molecular weight polycyclic aromatic hydrocarbons by bacteria. J Bacteriol 182:2059–2067

Kastner M, Breur-Jammali M, Mahro B (1998) Impact of inoculation protocols, salinity, and pH on the degradation of polycyclic aromatic hydrocarbons (PAHs) and survival of PAH-degrading bacteria introduced into soil. Appl Environ Microbiol 64:359–362

Kazunga C, Aitken MD (2000) Products from the incomplete metabolism of pyrene by polycyclic aromatic hydrocarbon-degrading bacteria. Appl Environ Microbiol 66:1917–1922

Kim SJ, Kweon O, Jones RC et al (2007) Complete and integrated pyrene degradation pathway in *Mycobacterium vanbaalenii* PYR-1 based on systems biology. J Bacteriol 189:464–472

Kumari B, Singh SN, Deeba F et al (2013) Elucidation of pyrene degradation pathway in bacteria. Adv Biores 4:151–160

Lange B, Kremer S, Sterner O et al (1994) Pyrene metabolism in *Crinipellis stipitaria*: identification of trans-4,5-dihydro-4,5-dihydroxypyrene and 1-pyrenylsulfate in strain JK364. Appl Environ Microbiol 60:3602–3607

Liang Y, Gardner DR, Miller CD et al (2006) Study of biochemical pathways and enzymes involved in pyrene degradation by *Mycobacterium* sp. strain KMS. Appl Environ Microbiol 72:7821–7828

Lin Y, Cai LX (2008) PAH-degrading microbial consortium and its pyrene-degrading plasmids from mangrove sediment samples in Huian, China. Mar Pollut Bull 57:703–706

Liu S, Hou Y, Sun G (2013) Synergistic degradation of pyrene and volatilization of arsenic by co-cultures of bacteria and a fungus. Front Environ Sci Eng 7:191–199
Makkar RS, Rockne KJ (2003) Comparison of synthetic surfactants and biosurfactants in enhancing biodegradation of polycyclic aromatic hydrocarbons. Environ Toxicol Chem 22:2280–2292
Malik ZA, Ahmed S (2012) Degradation of petroleum hydrocarbons by oil field isolated bacterial consortium. Afr J Biotechnol 11:650–658
Mihelcic JR, Lueking DR, Mitzell RJ et al (1993) Bioavailability of sorbed and separate-phase chemicals. Biodegrad 4:141–153
Moscoso F, Teijiz I, Deive FJ et al (2012) Efficient PAHs biodegradation by a bacterial consortium at flask and bioreactor scale. Bioresour Technol 119:70–276
Mueller JG, Cerniglia CE, Pritchard PH (1996) Bioremediation of environment contaminated by polycyclic aromatic hydrocarbons. In: Crawford RL, Crawford DL (eds) Bioremediation: principles and applications
Nie M, Yin X, Ren C et al (2010) Novel rhamnolipid biosurfactants produced by a polycyclic aromatic hydrocarbon-degrading bacterium *Pseudomonas aeruginosa* strain NY3. Biotechol Adv 28:635–643
Obayori OS, Ilori MO, Adebusoye SA et al (2008) Pyrene-degradation potentials of Pseudomonas species isolated from polluted tropical soils. World J Microbiol Biotechnol 24:2639–2646
Obayori OS, Salam LB (2010) Degradation of polycyclic aromatic hydrocarbons: role of plasmids. Sci Res Essays 5:4093–4106
Peng RH, Xiong AS, Xue Y et al (2008) Microbial biodegradation of polyaromatic hydrocarbons. FEMS Microbiol Rev 32:927–955
Ravelet C, Krivobok S, Sage L et al (2000) Biodegradation of pyrene by sediment fungi. Chemosphere 40:557–563
Rehmann K, Noll HP, Steinberg CE et al (1998) Pyrene degradation by *Mycobacterium* sp. strain KR2. Chemosphere 36:2977–2992
Saraswathy A, Hallberg R (2002) Degradation of pyrene by indigenous fungi from a former gasworks site. FEMS Microbiol Lett 210:227–232
Sarma PM, Duraja P, Deshpande S et al (2010) Degradation of pyrene by an enteric bacterium, Leclercia adecarboxylata PS4040. Biodegradation 21:59–69
Shreve GS, Inguva S, Gunnam S (1995) Rhamnolipid biosurfactant enhancement of hexadecane biodegradation by *Pseudomonas aeruginosa*. Mol Mar Biol Biotechnol 4:331–337
Singh A, Kumar K, Pandey AK et al (2015) Pyrene degradation by biosurfactant producing bacterium *Stenotrophomonas maltophilia*. Agric Res 4:42–47
Stelmack PL, Gray MR, Pickard MA (1999) Bacterial adhesion to soil contaminants in the presence of surfactants. Appl Environ Microbiol 65(1999):163–168
Straube W, Jones-Meehan J, Pritchard P et al (1999) Benchscale optimization of bioaugmentation strategies for treatment of soils contaminated with high molecular weight polyaromatic hydrocarbons. Resour Conserv Recycl 27:27–37
Subashchandrabose SR, Logeshwaran P, Venkateswarlu K et al (2017) Pyrene degradation by Chlorella sp. MM3 in liquid medium and soil slurry: possible role of dihydrolipoamide acetyltransferase in pyrene biodegradation. Algal Res 23:223–232
Walter U, Beyer M, Klein J et al (1991) Degradation of pyrene by *Rhodococcus* sp. UW1. Appl Microbiol Biotechnol 34:671–676
Wang B, Lai Q, Cui Z et al (2008) A pyrene-degrading consortium from deep-sea sediment of the west pacific and its key member *Cycloclasticus* sp. P1. Environ Microbiol 10:1948–1963
Wei K, Yin H, Peng H et al (2017) Characteristics and proteomic analysis of pyrene degradation by Brevibacillus brevis in liquid medium. Chemosphere 178:80–87
Wu M, Chen L, Tian Y et al (2013) Degradation of polycyclic aromatic hydrocarbons by microbial consortia enriched from three soils using two different culture media. Environ Pollut 178: 152–158

Yan J, Wang L, Fu PP et al (2004) Photo-mutagenicity of 16 polycyclic aromatic hydrocarbons from the US EPA priority pollutant list. Mutat Res Genet Toxicol Environ Mutagen 557: 99–108

Zhong Y, Luan T, Lin L et al (2011) Production of metabolites in the biodegradation of phenanthrene, fluoranthene and pyrene by the mixed culture of *Mycobacterium* sp. and *Sphingomonas* sp. Bioresour Technol 102:2965–2972

Author Biographies

Gauri Gupta graduated from the Banasthali University, Rajasthan in 2011. Subsequently she completed her Master's degree in Environmental Science from Lucknow University, Lucknow in 2013. She started her Ph.D. in 2014 at Indian Institute of Technology (Indian School of Mines), Dhanbad, India. Her research work focuses on microbial degradation of polycyclic aromatic hydrocarbons (PAHs).

Avantika Chandra has completed her master's degree in Environmental Science and Management from Patna University in 2010. She has completed her Ph.D. in Environmental Science from the Indian Institute of Technology (Indian School of Mines), Dhanbad, India. Her research work focuses on remediation of pollutants on aquatic and terrestrial ecosystems.

Dr. Sunita Varjani is Scientific Officer at Gujarat Pollution Control Board, Gandhinagar, Gujarat, India. Her major areas of research are Industrial and Environmental Microbiology/Biotechnology and Molecular biology. She has delivered talks as invited speaker in many international conferences including the countries India and China. She is recipient of visiting scientist fellowship from EPFL, Lausanne, Switzerland (2017).

Chiranjib Banerjee is a DST INSPIRE faculty at Department of Environmental Science and Engineering at Indian Institute of Technology (Indian School of Mines), Dhanbad, India. He completed his Post Graduation in Biotechnology and Doctorate from Birla Institute of technology, Mesra.

Vipin Kumar is an Assistant Professor in the Department of Environmental Science and Engineering at Indian Institute of Technology (Indian School of Mines), Dhanbad, India. He completed his Post Graduate in Microbiology and Doctorate in Environmental Biology. Dr. Kumar's research interest includes microbial remediation of pollutants, biological treatment of solid waste, etc. Earlier, he served as a Research Associate and Senior Research Fellow under the Department of Biotechnology (DBT) Government of India, New Delhi.

Chapter 19
Bioremediation of Nitrate-Contaminated Wastewater and Soil

K. S. Rajmohan, Margavelu Gopinath and Raghuram Chetty

Abstract Water utilization is in a steep hike due to urbanization and population increase. On the other hand, pollution of fresh water due to human activities is increasingly a major concern as it affects economy and growth of a nation. Among various water pollutants, nitrogen compounds form a significant role in wastewater contamination due to increase in anthropogenic sources like agriculture. Release of nitrate into fresh water poses severe problems including eutrophication, methemoglobinemia, and other health issues. Thus, nitrate contamination in water and soil has become a growing environmental concern. According to USEPA standards, the maximum contamination level for nitrate is 45 mg L^{-1}, and the same standard is adopted by the Bureau of Indian Standards (BIS). Among various technologies employed for treating nitrate-contaminated water, biological denitrification is one of the more versatile and promising methods widely being employed. The treatment of NO_3^- using bacteria referred as denitrification or bioremediation of nitrate has high separation efficiency. This chapter focuses on various biodenitrification processes, immobilization of microorganism, and different reactors employed for removing the nitrate from water. Different reactor designs ranging from fixed-bed reactors to biological aerated filters have been demonstrated for effective denitrification.

Keywords Biological denitrification · Autotrophic · Deammonification Denitrification nitrate · Methemoglobinemia

K. S. Rajmohan (✉) · R. Chetty
Department of Chemical Engineering, Indian Institute of Technology Madras, Chennai 600036, Tamil Nadu, India
e-mail: ksrajmohan@ddn.upes.ac.in

K. S. Rajmohan · M. Gopinath
Department of Chemical Engineering, University of Petroleum and Energy Studies, Dehradun 248007, Uttarakhand, India

S. J. Varjani et al. (eds.), *Bioremediation: Applications for Environmental Protection and Management*, Energy, Environment, and Sustainability,
https://doi.org/10.1007/978-981-10-7485-1_19

19.1 Introduction

According to WHO (2015) report, safe drinking water remains a dream for 663 million people in the world. Sustainable development goal (SDG) by World Health Organization has a target 6.3 which insists safe drinking water to everyone by 2030. The theme of world water day 2017 was "why waste water?" which insists on reducing water wastage, treat wastewater, and reuse water along with energy recovery and nutrient management. Nitrogen is an indispensable element of all living things. Earth's atmosphere contains rich nitrogen, and nitrogen is removed naturally from it. Nitrogen is a vital building block in protein synthesis, accounting for 13 mass %. When nitrogen and phosphorus are present in water or soil, they act as nutrients or biostimulant. The chemistry of nitrogen is complex as it exists in eight different oxidation states, and interestingly, change of state can be effected by living organisms. However, excess nitrogen may pose a severe problem to the environment including eutrophication, methemoglobinemia, and other health issues. Thus, nitrate contamination in water and soil has become a growing environmental concern. According to USEPA standards, the maximum contamination level for nitrate is 45 mg L^{-1}, and the same standard is adopted by the Bureau of Indian Standards (BIS). The adverse impact of nitrate on health and ecosystem and concerns over diminishing water quality have increased the interest in nitrate removal technologies. It is beneficial from a water quality viewpoint to exploit any process in the nitrogen cycle that acts as a basin for nitrogen in groundwater. Nitrogen cycle consists of various steps through which nitrogen is returned to the atmosphere. Denitrification is the last step in the nitrogen cycle. Microbes present in the soil and water use nitrate as an electron acceptor when oxygen is in deficit. However, natural denitrification is a slow process. In biological denitrification technique, the rate of denitrification is accelerated by continuously supplying the carbon source and maintaining the process parameters constant.

19.2 Sources of Nitrate-Contaminated Wastewater

Nitrate is a key contaminant present in effluent wastewater of various industries including fertilizer, metal ores, explosive, paper mills. Generally, nitrate level in the contaminated water varies from 200 to 500 mg L^{-1} based on the nature of source as listed in Table 19.1. Low-level waste from nuclear industries contains as high as 50000 mg NO_3^- L^{-1}. Agricultural runoffs rich in nitrate contaminate water and soil. Waste disposal site drainage and municipal waste are liable to get oxidized to nitrate that also augments pollution of groundwater with nitrate. Apart from chemical industries, nitrate waste is also formed in electronic, mining, and petroleum industries. All these wastewaters when disposed into the natural water bodies, they cause severe health and environmental concerns.

Table 19.1 Nitrate levels at various sources as reported in the literature

Nitrate level mg L^{-1}	Wastewater source	Reference
70–85	Domestic wastewaters/septic tanks	Oladoja and Ademoroti (2006) and Wu et al. (2007)
200	fertilizer, Diaries, metal finishing industries	Peyton et al. (2001)
1000	Brackish water	Dorante et al. (2008)
222	Tannery, Pisa, Italy	Munz et al. (2008)
325	Glasshouses waste	Park et al. (2009)
3600	Explosives factory, China	Shen et al. (2009)
50,000	Nuclear industry	Francis and Hatcher (1980)

19.3 Environmental and Health Concerns Due to Nitrate Contamination

Appropriate disposal of wastewater is vital in conserving the water and soil quality, such that we may use them without any hesitation. When the treatment of wastewater is insufficient, it poses several threats to the environment. When the pollutants such as nitrate and phosphate reach the water body, they act as the nutrient for the microorganisms such as algae which lead to the algae bloom. Algae bloom leads to reduced sunlight penetration into the water, detrimental to the submerged plants, low dissolved oxygen, and the demise of the aquatic living organisms which is collectively termed as eutrophication. Such water sources must be considered as potable water source only after appropriate treatment for denitrification (Breiza and Winter 2010).

When the nitrate-contaminated water is used for drinking purpose, it causes severe health concerns. Particularly, when pregnant women consume nitrate-contaminated water, the infants born to them suffer from methemoglobinemia. Hypertension, leukemia, goiter, brain tumors, stomach cancer, thyroid disorders, and nasopharyngeal have been reported in adults who were exposed to higher concentrations of nitrate. In cattle, muscular weakness and abdominal pains were reported.

19.4 Technologies Available for Nitrate Removal

Treatment of industrial waste polluted with the nitrate needs interdisciplinary approach, as nitrate is highly stable at low concentrations, does not undergo co-precipitation, affected by suspended solids present. Various techniques employed for nitrate remediation are shown in Fig. 19.1. Commercially, physico-chemical as well as chemical processes including ion exchange (IX), reverse

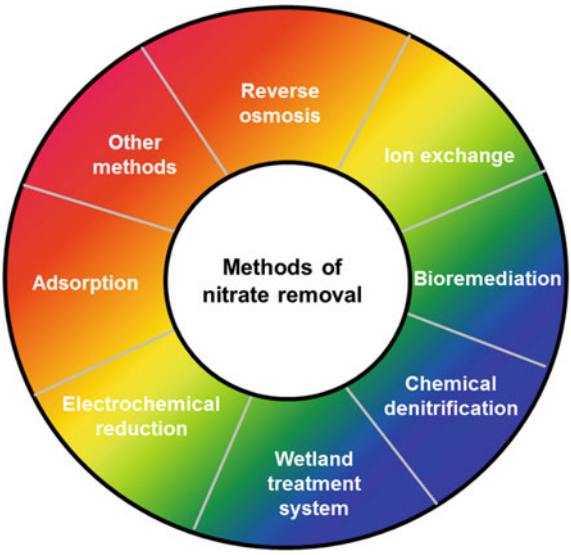


Fig. 19.1 Various technologies used for nitrate removal

osmosis (RO), chemical denitrification are employed for removing nitrate from water (Raboni et al. 2015).

The treatment of nitrate using bacteria referred as denitrification or bioremediation of nitrate is a promising method as it has high separation efficiency. Biological denitrification offers various advantages such as economic, no secondary effluent production, and no toxic by-products.

19.5 Biological Denitrification

In general, the biological methods used for wastewater treatment are broadly classified into five categories as shown in Fig. 19.2.

Denitrification is also referred to as biological denitrification or enzymatic denitrification (Tiedje et al. 1983; Paul and Clark 1989). Denitrification, a microbial-mediated process, is known to occur under anoxic or anaerobic environments because facultative microorganisms will first utilize dissolved oxygen as an electron acceptor and will not reduce nitrate. Hence, usually anaerobic and anoxic processes are used for denitrification. As shown in Fig. 19.3, denitrification is broadly classified into two methods, assimilatory and dissimilatory denitrification. In the case of assimilatory denitrification, nitrate is first converted into ammonia which is later oxidized to nitrogen or used for the synthesis of protein. Dissimilatory denitrification involves reduction of nitrate (NO_3) to nitrogen gas (N_2), through the production of nitrite (NO_2^-) and nitrous oxide (N_2O) and gaseous nitric oxide (NO) intermediates. Dissimilatory denitrification is further classified into two based on how the microorganisms are introduced into the unit process.

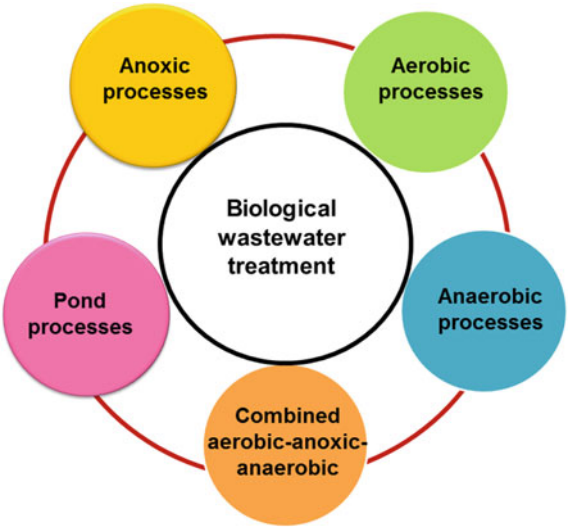


Fig. 19.2 Major categories of biological methods for wastewater treatment

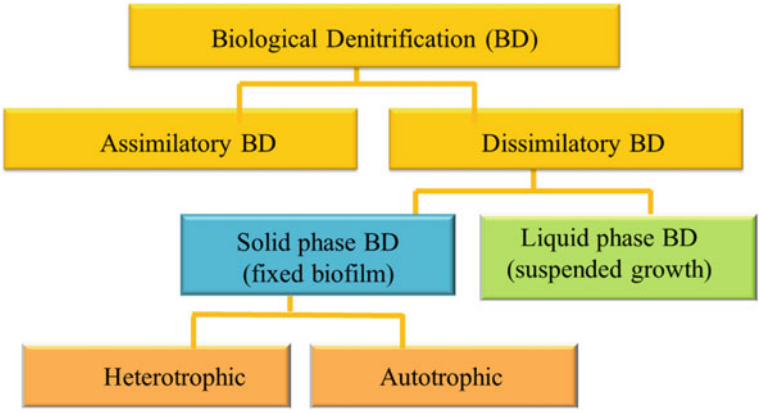


Fig. 19.3 Classification of biological denitrification

Suspended growth and attached growth are employed to introduce the microorganism into the system. Fixed biofilm involves attachment of microorganisms (immobilization of enzymes) to a surface as a biofilm. Suspended growth in activated sludge usually requires more skilled operators and more frequent maintenance than the attached-growth process (Fig. 19.4).

In this process, bacteria in an anaerobic environment use NO_3^- as a terminal electron (e-) acceptor in their metabolic processes. Thus, terminal e- acceptors

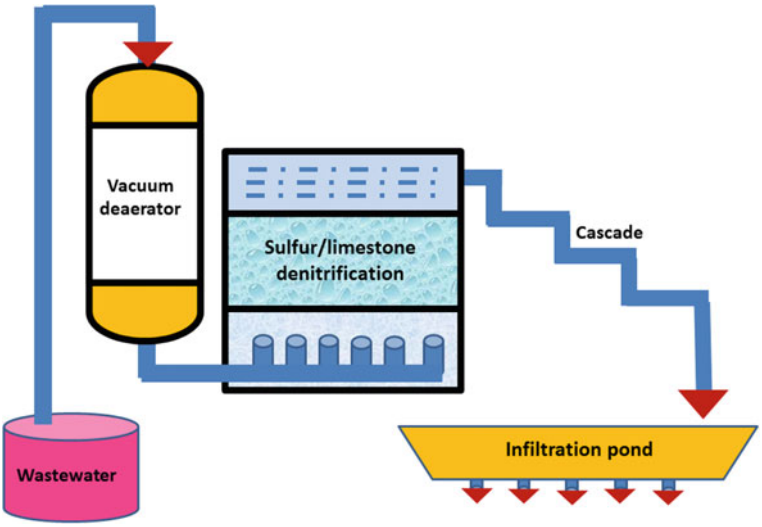


Fig. 19.4 Upflow fixed-bed bioreactor using sulfur/limestone built in Netherland

(N-oxides), bacteria with metabolic capacity, e- donors such as sucrose and methanol, and restricted O_2 supply are essential for denitrification to take place.

An anoxic environment is an environment in which it is very little to no free dissolved oxygen but where oxygen is present combined with other molecules, such as nitrate. Under anoxic conditions, at low dissolved oxygen concentration (below 0.2 mg L^{-1}), facultative bacteria disintegrates nitrate (NO_3^-) to extract oxygen (O_2), thus nitrate is converted to nitrous oxide (N_2O), eventually nitrogen gas (N_2) escapes into the atmosphere as gas bubbles.

19.6 Heterotrophic and Autotrophic Denitrification

Methanol, ethanol, acetic acid, methane, carbon monoxide have been studied as substrates for heterotrophic denitrification whereas hydrogen, sulfide, sulfur, and thiosulfates compounds have been utilized for autotrophic denitrification. Basically, they differ in the requirement of anaerobic conditions. Heterotrophic needs absence of oxygen whereas autotrophic requires oxygen. A comparison of heterotrophic and autotrophic denitrification is reported in Table 19.2 and discussed in the following sections. The microbial species available for autotrophic denitrification are limited and display slow growth leading to production of low solids and decreased efficiency.

Table 19.2 Comparison of autotrophic and heterotrophic denitrification

Parameter	Autotrophic denitrification	Heterotrophic denitrification
Requirement of oxygen	Aerobic	Anaerobic
Requirement of organic carbon source	No	Yes
Microorganism genera	Thiobacillus, Thiomicrospira, *Paracoccus,* and *Thiosphaera*	Pseudomonos, micrococcus, Achromobacter, spirillum, and bacillus
Examples of carbon and energy source	Sulfur and hydrogen, CO_2 and HCO_3, iron, or alloyed nanoparticles and uranium	Methanol, ethanol or acetic acid, glucose, acetone, aspartate, or formic acid wheat straw, plant prunings
Complexity	Complex due to three-phase process	Less complex
Bacterial growth rate	Slow	High
Nitrate removal rate/efficiency	Low	High
Alkalinity	Consumes alkalinity and produces sulfates	Produces alkalinity
Reactors used	hydrogenotrophic denitrification reactor	Activated sludge, Packed bed reactor, Fluidized-bed reactor, Nitrazur" and "Biodent" Tube reactor
End product	Harmless N_2	Harmless N_2, NO, N_2O
Microbial sludge	Low	High
Secondary pollution	Zero	Formaldehyde is formed when methanol is used as substrate. Production of brine
Cost	High investment and high operation cost	Medium investment and operation cost

19.7 Suspended Growth Process and Fixed Film Process

Immobilization of activated sludge was employed in several cases (Isaka et al. 2007). In comparison with pure bacterial strains, immobilization of activated sludge is capable of removing various pollutants mainly because of the biodiversity of the activated sludge (Breiza and Winter 2010). In the case of suspended biofilms, the biomass tends to get washed out and leads to the formation of hyper-concentrated cultures. Immobilized biomass of pure bacteria generally referred to as fixed films has been employed for wastewater treatment. Immobilization may take place naturally or artificially (Fierro et al. 2008; Hill and Khan 2008). Gel entrapment method is used for artificial immobilization on a suitable support which is non-toxic, photo-transparent, and mechanically stable and has the ability to retention of cellular viability.

Immobilization techniques can be employed to enhance the oxygen mass transfer in reactor systems with high biomass retention. Also, fixed film processes provide the opportunity of scaling up the laboratory processes to industrial applications (Yan and Yu 2009). The reactor size required is significantly reduced, displays resistance to temperature fluctuations and toxic contaminants and thereby enabling uninterrupted operation for a longer period (Morita and Tojo 2007). Among chitosan, sodium alginate, sodium carboxymethyl cellulose, and polyvinyl alcohol tested as support for gel entrapment of biomass, sodium alginate displayed higher adsorption efficiency (Yan and Yu 2009).

19.8 Denitrification Microbiology

Denitrification is a typical respiratory process wherein an energy source, electron donor, or oxidizable substrate is necessary. Denitrifying bacteria are ubiquitous in nature. *Pseudomonas, Spirillum, Halobacterium, Thiobacillus, Moraxella, Methanomonas, Paracoccus, Propionibacterium, and Xanthomonas* are the bacteria genera containing denitrifying species with most of them being a facultative organism using nitrate as electron acceptor under anaerobic conditions. However, denitrification is preceded by ammonia oxidation to nitrite. Beta subdivision of the proteobacteria is known to display high activity. Microorganisms known to oxidize ammonia to nitrite are *Nitrosomonas, Nitrosovibrio and Nitrosolobus, Nitrosococcus, and Nitrosopir, Nitrosomonas europaea and Paracoccus denitrificans* (Uemoto and saiki 2000).

Denitrifiers are common among the Gram-negative bacteria such as *Pseudomonas, Alcaligenes, Thiobacillus, and Paracoccus*. Some Gram-positive bacteria (such as Bacillus) and a few halophilic archaeal microorganisms (e.g., Haloferax denitrificans) also displayed denitrification ability (Kim et al. 2005). *Thiomicrospira denitrificans* and *Thiobacillus denitrificans* are widely used autotrophic denitrifiers (Breiza and Winter 2010).

Each step in the denitrification mechanism is catalyzed by different enzymes. Both nitrate and nitrite reductases are induced by the corresponding substrates. When both enzymes are present, nitrate reductase activity is preferentially increased, and once nitrite concentration reaches a minimum concentration, then nitrate reductase activity increases and the denitrification process proceeds.

19.9 Organic Compounds for Denitrification

Denitrifying bacteria require an adequate supply of carbon as they break down nitrate into oxygen and nitrogen gas. Methanol is one of the widely used carbon sources for biological denitrification. The general rule of thumb is that the wastewater to be denitrified should have a methanol-to-nitrogen (nitrate) ratio of 3:1. The denitrification follows the Eq. 19.2 given in Table 19.3.

Table 19.3 Stoichiometry equation for different energy sources for denitrification

Electron donor/energy source	Denitrification type	Stoichiometry equation	Equation no.
Methanol	Heterotrophic	$6NO_3^- + 5CH_3OH \rightarrow 3N_2 + 5CO_2 + 7H_2O + 6OH$	(19.2)
Ethanol	Heterotrophic	$5C_2H_5OH + 12\ NO_3^- \rightarrow 10\,HCO_3^- + 2\,OH^- + 9\ H_2O + 6\ N_2$	(19.3)
		$0.613\ C_2H_5OH + NO_2^- \rightarrow 0.102\ C_5H_7NO_2 + 0.714\ CO_2 + 0.286\ OH^- + 0.980\ H_2O + 0.449\ N_2$	(19.4)
Sulfide	Autotrophic	$2NO_3^- + 5H_2 \rightarrow N_2 + 4H_2O + 2OH^-$	(19.5)
		$5S^{2-} + 8NO_3^- + 8H^+ \rightarrow 5SO_4^{2-} + 4N2 + 4H_2O$	(19.6)

Different carbon source/electron donor follows different stoichiometry, and an example each for autotrophic and heterotrophic denitrification are listed in Table 19.3. *Paracoccus, Thiobacillus, Thiosphaera* are capable of using hydrogen, various reduced sulfur compounds.

The amount of ethanol requirement for denitrification depends on the dissolved oxygen (DO) and nitrate removal rate (NRR) as given in Eq. 19.1,

$$M_{\text{substrate}}\left(\text{mg L}^{-1}\right) = \text{NRR}\left(\text{mg L}^{-1}\right) * 0.475 + \text{DO}\left(\text{mg L}^{-1}\right) * 0.55 \quad (19.1)$$

19.10 Factors Affecting Nitrate Removal Efficiency

Various physical and biological parameters such as temperature, pH, dissolved oxygen, initial nitrate concentration, hydraulic retention time, grain size, bacterial populations, electron donor species, and presence of trace and heavy metals are known to influence the solid-phase denitrification rates in different microenvironments (Ashok and Hait 2015).

19.10.1 Effect of Hydraulic Residence Time (HRT)

The time spent by the wastewater in the biological reactor is usually referred to as hydraulic residence time (HRT). Generally, the time required depends on the growth rate of microorganism, the initial concentration of nitrate, co-existing species, and temperature.

This parameter is vital during the start-up of a new reactor system. A minimum HRT of 6 h is required when NO_3^- concentration is above 70 mg L^{-1}, and around 3–4 h HRT is necessary when NO_3^- concentration is below 40 mg L^{-1} (Zhou et al. 2011).

19.10.2 Effect of Temperature

The effect of temperature on denitrification was studied in a diverse ecosystem, and it has been inferred that the denitrification rate is higher at the higher temperatures. The temperature of effluents varies from 5 to 50 °C, depending on the local weather of different places. Denitrification rate increases with temperature and based on the type of organic source. For instance, a threefold increase in the denitrification rate was reported while increasing the temperature by 30 °C with starch/polyvinyl alcohol (PVA) as the carbon source (Li et al. 2013). The denitrification rate was doubled when the temperature was increased by 5 °C in a polycaprolactone filled

packed bed reactor. Low temperature does not favor the heterotrophic denitrification particularly during the start-up of a process. However, autotrophic denitrification can sustain low temperature by having high hydraulic retention time. Assumedly, these reaction rates get doubled for every 10 °C increase in temperature (Bouletreau et al. 2012). The denitrification reactor volume at 10 °C would be about four times the volume required at 20 °C to achieve the same degree of nitrification. It has been reported that high temperature range of 28–38 °C is known to favor nitrogen elimination via nitrite, and in the temperature range of 10–20 °C, the maintenance of high nitrite accumulation rate is difficult because of the fact that the specific growth rate of nitrite-oxidizing bacteria is higher than that of ammonia-oxidizing bacteria (van Dongen et al. 2001).

19.10.3 Effect of Grain Size

In the case of fixed biofilm denitrification processes, the grain size of the support also contributes to the overall denitrification rate. A large surface area should prevail at the support to have sufficient microbial growth rate and metabolic activity. Hence, larger the intra-granular space, higher will be the microbial growth and in turn higher will be the denitrification rate. In case of chemolithotrophic denitrification, surface area as well as particle size of elemental sulfur or iron particles influences the denitrification rate (Torrento et al. 2010)

19.10.4 Effect of Dissolved Oxygen (DO)

Microorganisms would like to oxidize organic carbon source using the electron acceptor that offers a maximum energy as shown in Table 19.4. If oxygen is present in the soil/water, microorganisms preferably use O_2 as the electron acceptor for the oxidation of organic carbon sources. Subsequently, other electron acceptors will be utilized. Thus, denitrification is essentially an anaerobic or anoxic process. Lower the oxygen concentration, maximum will be the denitrification (Viotti et al. 2016).

Table 19.4 Electron acceptors in saturated zones of water table with natural carbon sources and its Gibbs free energy

Electron acceptor	Process	End product	Gibbs free energy (kJ electron^{-1})
Dissolved oxygen	Aerobic oxidation	Water	−78.5
NO_3^-	Denitrification	N_2	−72.3
MnO_2	Mn reduction	$MnCO_3$	−50.3
FeOOH	Fe reduction	$FeCO_3$	04.6
SO_4^{2-}	Sulfate reduction	HS^-	21.4

The specific growth rate of denitrifying bacteria will decrease linearly from 0.3 mg L^{-1} and becomes zero at 1 mg L^{-1}.

19.10.5 Effect of Initial Nitrate Concentration (C_0)

Biological denitrification is favored by low initial nitrate concentration (C_0). Higher the C_0, lower is the nitrate removal efficiency. Denitrification mechanism involves multistep reduction to form harmless nitrogen gas. At lower C_0, N_2 selectivity is higher. N_2O is the major end product at higher C_0 since N_2O reduction to nitrogen is hindered at such higher concentrations. Autotrophic denitrification significantly reduces N_2O emissions in the effluent (Yang et al. 2016b).

Biodenitrification studies using very high nitrate concentration are rarely reported. An activated sludge process to degrade nitrate concentration of 40,000 mg L^{-1} in 6 h using a systematically acclimatized activated sludge to a synthetic wastewater was reported (Dhamole et al. 2007). In general, biological denitrification is ineffective when the initial nitrate concentration is above 1000 mg L^{-1}, and this limitation is overcome by combining it with other techniques such as electrochemical nitrate reduction. Electrochemical reduction of nitrate can be effected by using low-cost copper-based catalysts supported on CNTs which shows superior activity than activated carbon (Rajmohan and Chetty 2014; Rajmohan and Chetty 2017). When a biofilm is attached to the cathode, biodenitrification as well as electrochemical reduction, collectively termed as bioelectrochemical reduction takes place which will accelerate the process of denitrification. An overview of bioelectrochemical reactors system is reported elsewhere (Rajmohan et al. 2016).

19.10.6 Effect of Salinity

Toilet flushing using seawater is employed in few places in China, and increased salt concentration in the sludge had an impact on the denitrification rate, inducing salt stress to the microbial flora, and inhibited several enzyme activities and eventually led to plasmolysis. Sulfate reduction-Autotrophic denitrification-Nitrification Integrated (SANI) process was employed for saline sludge treatment with a focus on minimization of biological sludge and conservation of energy (Wang et al. 2009). However, when the sludge is exposed to salinity for a long term, it can produce salinity resistant bacteria which accelerate the denitrification rate (Sudarno et al. 2010). Annamox bacteria such as *Candidatus kuenenia stuttgartiensis, Candidatus Kuenenia stuttgartiensis,* and *Candidatus Scalindua wagneri* were effective under elevated salt concentrations, and studies in an Anammox fixed-bed reactor containing non-woven biomass carrier revealed that Anammox bacteria were effective up to a salt *concentration of* 30 g L^{-1} (Liu et al. 2009).

19.10.7 Effect of PH

Maintenance of pH is an essential operating parameter as it influences the enzyme activity of the bacteria, thereby affecting the final product and nature of the sludge. Strongly acidic pH (<5) inhibits the denitrification rate and affects the final product formed by arresting the denitrification chain. As heterotrophic denitrification produces hydroxyl ions, the alkalinity of the water is increased. For every mg of N_2 formed, alkalinity increase of 3.54 mg of $CaCO_3$ is produced. Since heterotrophic denitrification undergoes an alkalinity producing reaction; optimum pH to perform denitrification is 7–8.5 (Bhuvanesh et al. 2013). Autotrophic denitrification generally consumes alkalinity. For every mg of NO_3^- converted to N_2, 3.94 mg of alkalinity as $CaCO_3$ is consumed. For instance, while sulfur is used as an electron donor, alkalinity is consumed and sulfates are produced which can be disposed of easily into the sea.

19.10.8 Effect of Other Trace Elements

The presence of trace metals favors the microbial growth. However, few may inhibit the microbial activity and affect the denitrification rate.

19.10.9 Effect of Free Ammonia Concentration

Nitrite buildup takes place due to concentrations of dissolved oxygen, pH temperature, presence of CO2, and heavy metals. Another factor causing nitrite buildup is free ammonia. Presence of free ammonia in the water inhibits both ammonia oxidation and nitrite oxidation. *Nitrosomonas* growth rate and ammonia oxidation were inhibited when free ammonia concentration is above 10 mg L^{-1} whereas *Nitrobacter* growth rate and nitrite oxidation were inhibited above 0.1 mgL^{-1}.

19.11 Reactors for Denitrification

In 1983, Eragny in France witnessed the first commercial denitrification plant with 80 m^3 h^{-1} capacity reducing nitrate concentration from 68 to 25 mg L^{-1}. In this reactor, ethanol and phosphate were used. In the same year, a 50 m^3 h^{-1} capacity reducing nitrate concentration from 80 to 30 mg L^{-1} was constructed in Chateau Landon in France. Ideal plug flow and continuous stirred reactors are used for denitrification. Hydraulic retention time, the time water molecule spend in the reactor, varies between 0.2 and 2 h for a separate sludge denitrification systems

depending upon the water temperature, DO concentration, organic carbon concentration, and nitrate concentration. Different reactor designs ranging from fixed-bed reactors to biological-aerated filters have been demonstrated for effective denitrification.

Among various unit processes adapted for denitrification, fixed film reactors are widely employed. Fixed film processes such as fixed bed, fluidized bed reactor, and biofilter comprise a calcium carbonate, activated carbon, sand anthracite, or elemental sulfur. Continuous stirred tank reactors with microorganisms encapsulated in polymer (sodium alginate) beads have been reported.

To accelerate the biodenitrification process, a combination of other technologies such as membrane filters, application electric field has been explored, and the results are promising.

Combination of Anammox reaction and partial nitrification in a single reactor, entitled CANON has been widely used. The combination of the prior partial nitrification and the subsequent anaerobic ammonium oxidation is a promising technique for efficient nitrate elimination from wastewater. The cooperation of *Nitrosomonas*-like ammonium-oxidizing bacteria and Anammox bacteria of ammonium-oxidizing bacteria causes complete autotrophic nitrogen removal under oxygen-restricted conditions in one single reactor (Sliekers et al. 2003). First, the nitrifiers oxidize ammonium to nitrite where it consumes oxygen and creates anoxic conditions needed by the Anammox bacteria. Thus, formed nitrite is consumed with the remainder of the ammonium by Anammox bacteria and converted into N_2 gas (Nielsen et al. 2005). The advantages of Anammox reactor systems over conventional denitrification system are 40–45% energy saving, no extra carbon source requirement or only 10% carbon demand, 45% less alkalinity demand and less sludge production. However, Anammox process has three drawbacks, only 90% nitrate removal efficiency leaving 10% nitrate in the water, chemical oxygen requires higher than 100 mg L^{-1} and disrupts Anammox activity, the presence of nitrite-oxidizing bacteria will convert nitrite into nitrate affecting the growth of anaerobic ammonia oxidation bacteria.

Simultaneous partial nitrification, Anammox, and denitrification (SNAD) process overcomes the drawback of Anammox process as denitrifying bacteria boosts the nitrogen elimination efficiency and lessens the restraint of Anammox bacteria by organic matters (Wen et al. 2017). Simultaneous partial nitrification, Anammox and denitrification (SNAD) can simultaneously remove nitrogen along with carbon to overcome the problems of Anammox process (Wang et al. 2016). In SNAD reactor shown in Fig. 19.5, the Anammox reaction rate and denitrification reaction rate are found out to be 0.114 and 0.028 kg N m^{-3} d^{-1}, respectively. Thiosulfate is better electron donor than sulfide and sulfur, whereas ceramsite was the suitable carrier for denitrification (Zhang et al. 2017).

Gas transfer efficiencies are much greater than traditional bubble diffusers in membrane aeration. In a membrane-aerated bioreactor (MABR), the microporous membranes play two roles, one as the oxygen gas supplemental material and the other as the carrier for bacterial immobilization (Gong et al. 2007; Martin and Nerenberg 2012).

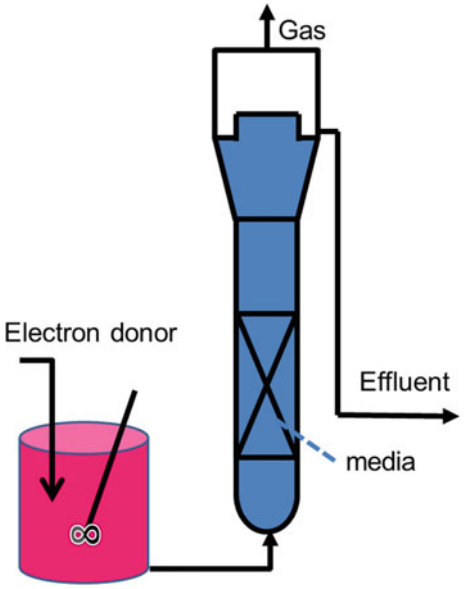


Fig. 19.5 Laboratory-scale upflow biofilter SNAD reactor

Airlift fixed-bed bioreactors were developed in which simultaneous nitrification and denitrification take place in outer and inner film, respectively. It was demonstrated that the reactor volume required is small, a low-odor sludge generation, and low suspended solids content in the effluent (Viotti et al. 2014).

After successful demonstrations in laboratory-scale and pilot-scale operations, a 35 m^3 h^{-1} capacity upflow filtration bioreactor plant using sulfur/limestone (in a mass ratio of 1:1) bed containing bacteria was commissioned. The raw water with a nitrate concentration of 70–80 mg L^{-1} is pumped from the storage tank and subjected to vacuum deaeration to prevent supersaturation of water with N_2 gas which may lead to channeling or clogging in the reactor. The water leaving the reactor is aerated and subjected to artificial recharge as shown in Fig. 19.4. Nitrate removal efficiency as high as 90% is achieved with 10–12 days residence time. Full-scale operations in France, Germany, and Great Britain have exemplified that biodenitrification is technically as well as economically feasible. Fixed-bed bioreactors offer the operational simplicity and the ease of process control in comparison with fluidized-bed reactors, and hence, most commercial applications use fixed-bed reactors. However, they differ in support system for fixed film, substrate, back washing techniques used (Mateju et al. 1992).

Thiobacillus denitrificans and sulfurimonas denitrificans are the most widely reported autotrophic denitrifiers with full genome sequencing. Thiobacillus. sp. denitrificans shares genes with aerobic, anaerobic, chemolithotrophic sulfur-oxidizing bacteria and phototrophic sulfur bacteria. Granular Sludge Autotrophic Denitrification showed threefold increases in denitrification rate (0.33 kg N

Table 19.5 Reactors used for biological denitrification reported in the literature

Reactor	Remarks	Reference
Rotating biological contactor	98 and 90% nitrate removal efficiency with acetic acid and ethanol, respectively	Mohseni-Bandpi and Elliott (1998)
Intensified biofilm-electrode reactor (IBER)		Zhao et al. (2011)
Upflow fixed-bed reactor	Acetic acid and phosphate were used. Nitrite buildup reduced after process equilibrium is achieved	Frick and Richard (1985)
Entrapped-mixed-microbial cells immobilization (EMMCI) process	A 10–20 g/L $NaHCO_3$ concentrations showed 96% denitrification efficiency	Yang et al. (1995)
Gas lift reactor	Nitrogen removal rate of 8.9 kg $N/m^3 \cdot day$	Sliekers et al. (2003)
Completely autotrophic nitrogen removal over nitrite (CANON)		Yan and Yu (2009) and Nielsen et al. (2005)
DEMON® process (DE-amMONnification)	Sequencing batch reactor (SBR) works under controlled DO and pH conditions	Wett (2007)
Membrane-aerated biofilm reactor (MABR)	Hydrophobic, gas-permeable membranes are used for bubbleless oxygen transfer	Martin and Nerenberg (2012) and Brindle and Stephenson (2000)
DeAmmon®process	Nitrogen removal efficiency is 70–85% at 25–30 °C	Plaza et al. (2011)
ANITA™Mox process	It uses a high-specific surface area support BiofilmChipM, and 80% nitrate removal efficiency is achieved	Christensson et al. (2011)
Terra-N® Process	Single-stage SBR uses bentonite in place of polymer, and 80–90% removal efficiency is achieved	WERF (2014)
Airlift upflow biofilm reactor	Simultaneous nitrification and denitrification occur. Less volume of reactor	Viotti et al. (2014)
Sulfate reduction-Autotrophic denitrification-Nitrification Integrated (SANI) process	Developed for treating sludge produced form saline water used for toilet flushing	Wang et al. (2009)
Granular sludge autotrophic denitrification (GSAD)	Threefold increase in denitrification rate than SANI process	Yang et al. (2016a, b)
Simultaneous partial nitrification Anammox and denitrification (SNAD)	Intermittent aeration with low DO restrains NOB growth and effects simultaneous nitrogen and carbon	Zhou et al. (2016) and Zhang et al. (2017)

m^{-3} d^{-1} than SANI process (0.1 N m^{-3} d^{-1}) The connections of the diverse genera of SOB have resulted in high sulfide and nitrogen removal efficiencies of the granular sludge (Pokorna and Zabranska 2015).

Downstream separation processes used include flotation, filtration, sedimentation, membranes, and high-rate clarifiers (WERF 2014) (Table 19.5).

19.12 Limitations of Denitrification

The treated water from biodenitrification plant contained 106–107 bacterial cells mL^{-1} and assimilable organic carbon in high concentration. Large biomass generation causes disposal problem. In the case of autotrophic denitrification, the reactor volume required is high due to slow denitrification rate. Moreover, low solubility of H_2 in water necessitates recirculation or operations under pressure. These forces higher investment and operations cost for autotrophic denitrification operations. Denitrification can leak N_2O which is a greenhouse gas and ozone-depleting substance that attributes to global warming (Fig. 19.6).

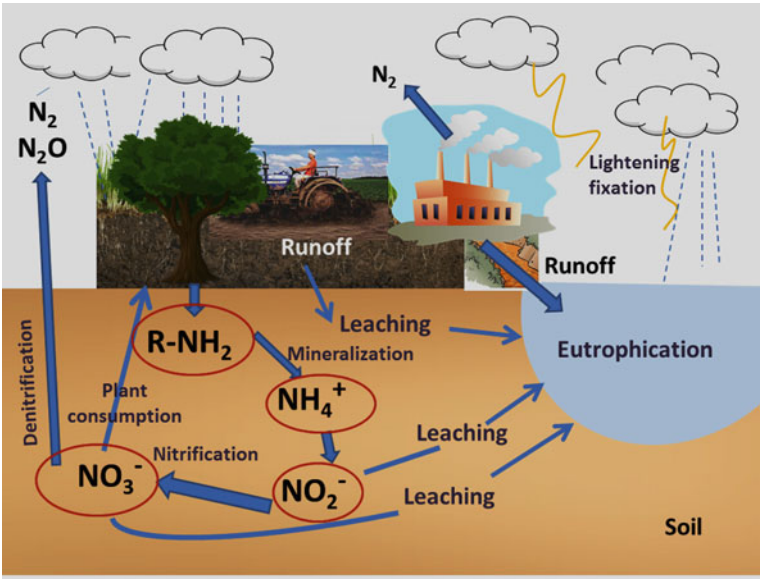


Fig. 19.6 Denitrification as a part of nitrogen cycle

19.13 Denitrification in Soil

As shown in Fig. 19.6, most of the reactions in soil are microbial aided or enzyme catalyzed. Korom (1992) showed that once nitrate is leached below the root zone of plants and trees, it may undergo four possible transformations. It may be denitrification, dissimilatory nitrate reduction into ammonium, assimilatory reduction into microbial biomass (DNRA), or soil retention. Among all, denitrification serves as a major N sink.

Wetlands have been employed for the removal of excess nitrogen in surface water. Denitrification is the main nitrogen removal process in the wetland soil due to anaerobic conditions during flooding (Riikauf et al. 2004).

Concentration of nitrate, adequate effective carbon and low oxygen partial pressure are essential for denitrification to take place. The rate of denitrification in wetlands depends on the soil type, pH, temperature, soil moisture, and salinity (Ligi et al. 2014; Dandie et al. 2011; Craft et al. 2009). Hydrological regime can control the duration of oxic and anoxic phases in soil, thus influencing the denitrification. Higher denitrification rates at upper soils were reported (Sun et al. 2017; Signor and Cerri 2013). Denitrification rates decrease with depth along soil profiles in both inland and coastal salt marsh soils (Shiau et al. 2016).

Effect of flooding pattern: The duration of oxic and anoxic phases in soil can be controlled by the hydrological regime (Pinay et al. 2007). Variation in flood frequency is known to affect the average denitrification rate. The effect of flood frequency on denitrification in soils is studied by Bai et al. (2017). Authors have reported higher average denitrification rate in TFW soils in comparison with those of the STFW and SFW soils, as given in Table 19.6. Effect of flooding frequency on the average denitrification rate in Xianghai, China inland wetlands was discussed therein (Bai et al. 2017). By changing the soil properties (soil organic matter, total nitrogen, pH, and salinity), the rate of denitrification can be altered, and in turn, nitrate level in the soil can be controlled.

Several studies on denitrification in upland soils, mainly in agricultural and forest soils, have been conducted, and annual denitrification rate as high as

Table 19.6 Effect of flooding frequency on the average denitrification rate in Xianghai, China, inland wetlands (Bai et al. 2017)

Wetland type	Frequency of flooding per year due to flow sediment regulation	Average denitrification rates ($mgkg^{-1}d^{-1}$)
Short-term flooding wetlands (STFW)	Approximately one month	139.49 ± 8.51
Seasonal flooding wetlands (SFW)	Approximately three months	146.49 ± 11.22
Tidal flooding wetlands (TFW)	Twice one day due to tidal cycles	153.92 ± 7.54

239 kg N/ha.year has been reported. Higher rate of denitrification is observed mainly in agricultural soils with annual denitrification rate of 13 kg N/ha.year whereas that of forest soils was 1.9 kg N/ha.year. Irrigated loam soils show higher denitrification rate since abundance of nitrate in the soil (Barton et al. 1999). An excellent review encompassing the advancements made in last 50 years in the field of soil denitrification studies was reported elsewhere (Simek and Cooper 2002).

Tropical soils that receive abundant rainfall as well as high temperatures are heavily weathered, desilicated, and enriched iron oxide content. Subtropical denitrification mechanism is dissimilar from several other soils due to its unique characteristics. For example, subtropical soils contain higher iron oxides than tropical soils (Zhang et al. 2009) resulting in higher redox potential that suppresses denitrification (Pu et al. 2002).

Several laboratory-scale studies have revealed that when the pH of the soil is lowered, the N_2O:N_2 ratio is increased, indicating higher denitrification rate. However, the relationship between pH and soil denitrification remains uncertain. Optimum pH for soil denitrification is yet to be established.

Because of diversity in the soil composition and weather conditions, soil denitrification remains a complicated research area. Research focus should be on determining the various factors (including dissolved oxygen) that are affecting the availability of electron donors in soil and their denitrification rates. Role of oxygen in the product distribution during soil denitrification needs to be explored.

19.14 Future Scope

Technology for heterotrophic denitrification is well developed in comparison with autotrophic denitrification. Inhibition effects of reaction intermediates or by-products needs to be investigated. Recent advancements in denitrification has focused on evaluating the negative impact of dissolved oxygen in the bioreactor, limiting its presence, evaluating the hydrodynamic behavior of the reactor system, effect of variation of nitrate concentration in the raw wastewater being treated.

Further development of in situ treatment including introducing substrates and nutrients into the aquifers and their long-term impact on ecosystem needs to be studied. Optimization of reaction conditions based on the nutrient, substrate, microorganism, pH, temperature, and dissolved oxygen needs to be studied for every wastewater source. Combination of biological denitrification with other technologies such as ion exchange, electrochemical methods, filtration, electrodialysis will pave a new way to overcome the challenges associated with the individual methods.

19.15 Summary

The nitrate concentration range in the wastewater may vary from low to high, which needs an apt method for treatment. Selection of a particular method depends upon the concentration of nitrate, volume of wastewater to be processed, land and power availability, environmental policy, and economic concerns. Biological denitrification can be employed to treat the nitrate-contaminated wastewater. Nitrite accumulation is the concern in biodenitrification which can be addressed by maintaining optimum pH, temperature, and hydraulic residence time. For higher concentrations (>1000 mg NO_3^- L^{-1}), biodenitrification can be combined with other technologies such as electrochemical reduction. Combination of traditional and innovative methods for denitrification for nitrate needs to be applied properly after careful evaluation. In particular, the challenges in biological denitrification can be addressed by combining it with other treatment methods to conserve our water resources.

References

Ashok V, Hait S (2015) Remediation of nitrate-contaminated water by solid-phase denitrification process—a review. Environ Sci Pollut Res 22(11):8075–8093

Bai J, Wang X, Jia J, Wang Y, Zhang S (2017) Denitrification of soil nitrogen in coastal and inland salt marshes with different flooding frequencies. Phys Chem Earth Parts A/B/C 97:31–36. https://doi.org/10.1016/j.pce.2017.01.015

Barton L, McLay CDA, Schipper LA, Smith CT (1999) Annual denitrification rates in agricultural and forest soils: a review. Soil Res 37(6):1073–1094

Bhuvanesh S, Maneesh N, Sreekrishnan T (2013) Start-up and performance of a hybrid anoxic reactor for biological denitrification. Biores Technol 129:78–84

Boulêtreau S, Salvo E, Lyautey E, Mastrorillo S, Garabetian F (2012) Temperature dependence of denitrification in phototrophic river biofilms. Sci Total Environ 416:323–328

Breisha GZ, Winter J (2010) Bio-removal of nitrogen from wastewaters-a review. J Am Sci 6(12): 508–528

Brindle K, Stephenson T (2000) The application of membrane biological reactors for the treatment of wastewaters. Biotechnol Bioeng 49(6):601–610

Craft C, Clough J, Ehman J, Joye S, Park R, Pennings S, Guo HY, Machmuller M (2009) Forecasting the effects of accelerated sea-level rise on tidal marsh ecosystem services. Front Ecol Environ 7:73–78

Dandie CE, Wertz S, Leclair CL, Goyer C, Burton DL, Patten CL, Zebarth BJ, Trevors JT (2011) Abundance, diversity and functional gene expression of denitrification communities in adjacent riparian and agricultural zones. FEMS Microbiol Ecol 77(1):69–82

Dhamole P, Nair R, D'Souza S, Lele S (2007) Denitrification of high strength nitrate waste. Biores Technol 98(2):247–252

Dorante T, Lammel J, Kuhlmann H, Witzke T and Olfs H W (2008) Capacity, selectivity, and reversibility for nitrate exchange of a layered double-hydroxide (LDH) mineral in simulated soil solutions and in soil. J Plant Nutr Soil Sci 171:777–784

Fierro S, del Pilar Sánchez-Saavedra M, Copalcúa C (2008) Nitrate and phosphate removal by chitosan immobilized Scenedesmus. Biores Technol 99(5):1274–1279

Francis CW, Hatcher CW (1980) Biological denitrification of high nitrate wastes generated in the nuclear industry. Bio Fluid Bed Treat Water and Waste 1:235–250
Frick B, Richard Y (1985) Experience with biological denitrification in a full scale drinking water treatment plant. Vom Wasser 64:145–154
Gong Z, Yang F, Liu S, Bao H, Hu S, Furukawa K (2007) Feasibility of a membrane-aerated biofilm reactor to achieve single-stage autotrophic nitrogen removal based on Anammox. Chemosphere 69(5):776–784
Hill C, Khan E (2008) A Comparative Study of Immobilized Nitrifying and Co-Immobilized Nitrifying and Denitrifying Bacteria for Ammonia Removal from Sludge Digester Supernatant. Water Air Soil Pollut 195(1–4):23–33
Isaka K, Sumino T, Tsuneda S (2007) High nitrogen removal performance at moderately low temperature utilizing anaerobic ammonium oxidation reactions. J Biosci Bioeng 103(5): 486–490
Kim J, Park K, Cho K, Nam S, Park T, Bajpai R (2005) Aerobic nitrification–denitrification by heterotrophic Bacillus strains. Biores Technol 96(17):1897–1906
Korom SF (1992) Natural denitrification in the saturated zone: a review. Water Resour Res 28(6):1657–1668
Li P, Zuo J, Xing W, Tang L, Ye X, Li Z, Yuan L, Wang K, Zhang H (2013) Starch/polyvinyl alcohol blended materials used as solid carbon source for tertiary denitrification of secondary effluent. J Environ Sci 25(10):1972–1979
Ligi T, Truu M, Truu J, Nõlvak H, Kaasik A, Mitsch WJ, Mander Ü (2014) Effects of soil chemical characteristics and water regime on denitrification genes (nirS, nirK, and nosZ) abundances in a created riverine wetland complex. Ecol Eng 72:47–55
Liu C, Yamamoto T, Nishiyama T, Fujii T, Furukawa K (2009) Effect of salt concentration in anammox treatment using non woven biomass carrier. J Biosci Bioeng 107(5):519–523
Martin K, Nerenberg R (2012) The membrane biofilm reactor (MBfR) for water and wastewater treatment: Principles, applications, and recent developments. Biores Technol 122:83–94
Matějů V, Čižinská S, Krejčí J, Janoch T (1992) Biological water denitrification—a review. Enzyme Microb Technol 14(3):170–183
Mohseni-Bandpi A, Elliot D (1998) Groundwater denitrification with alternative carbon sources. Water Sci Technol 38(6):237–243
Morita Y, Tojo M (2007) Modifications of PARP medium using fluazinam, miconazole, and nystatin for detection of Pythium spp. in Soil. Plant Disease 91(12):1591–1599. https://doi.org/10.1094/PDIS-91-12-1591
Munz G, Gori R, Cammilli L, Lubello C (2008) Characterization of tannery wastewater and biomass in a membrane bioreactor using respirometric analysis. Biores Technol 99(18): 8612–8618
Nielsen M, Bollmann A, Sliekers O, Jetten M, Schmid M, Strous M, Schmidt I, Larsen LH, Nielsen LP, Revsbech NP (2005) Kinetics, diffusional limitation and microscale distribution of chemistry and organisms in a CANON reactor. FEMS Microbiol Ecol 51(2):247–256
Oladoja NA, Ademoroti CMA (2006) The use of fortified soil-clay as on-site system for domestic wastewater purification. Water Res 40(3):613–620
Park JBK, Craggs RJ, Sukias JPS (2009) Removal of nitrate and phosphorus from hydroponic wastewater using a hybrid denitrification filter (HDF). Biores Technol 100(13):3175–3179
Paul EA, Clark FE (1989) Soil microbiology and biochemistry, 2nd edn. Academic Press, San Diego, California, England, p 340
Peyton BM, Mormile MR, Petersen JN (2001) Nitrate reduction with Halomonas campisalis: kinetics of denitrification at pH 9 and 12.5% NaCl. Water Res 35(17):4237–4242
Pinay G, Gumiero B, Tabacchi E, Gimenez O, Tavacchi-Planty AM, Hefting MM, Burt TP, Black VA, Nilsson C, Iordache V, Bureau F, Vought L, Petts GE, Ddcamps H (2007) Patterns of denitrification rates in European alluvial soils under various hydrological regimes. Freshw Biol 52:252–266

Plaza E, Stridh S, Ornmark J, Kanders L, Trela J (2011) Swedish experience of the deammonification process in a biofilm system. Proceedings of the Water Environment Federation, 2011(1):1067–1079

Pokorna D, Zabranska J (2015) Sulfur-oxidizing bacteria in environmental technology. Biotechnol Adv 33(6):1246–1259

Pu G, Xu Z, Saffigna PG (2002) Fate of 15 N-labelled nitrate in a wet summer under different residue management regimes in young hoop pine plantations. For Ecol Manag 170(1):285–298

Raboni M, Gavasci R, Viotti P (2015) Influence of denitrification reactor retention time distribution (RTD) on dissolved oxygen control and nitrogen removal efficiency. Water Sci Technol 72(1):45–51

Rajmohan KS, Gopinath M, Chetty R (2016) Review on challenges and opportunities in the removal of nitrate from wastewater using electrochemical method. J Environ Biol 37(6):1519

Rajmohan KS, Chetty R (2017) Enhanced nitrate reduction with copper phthalocyanine-coated carbon nanotubes in a solid polymer electrolyte reactor. J Appl Electrochem 47(1):63–74

Rajmohan KS, Chetty R (2014) Nitrate reduction at electrodeposited copper on copper cathode. ECS Trans 59(1):397–407

Riickauf U, Augustin J, Russow R, Merbach W (2004) Nitrate removal from drained and reflooded fen soils affected by soil N transformation processes and plan. Soil Biol Biochem 36(1):77–90

Shen J, He R, Han W, Sun X, Li J, Wang L (2009) Biological denitrification of high-nitrate wastewater in a modified anoxic/oxic-membrane bioreactor (A/O-MBR). J Hazard Mater 172(2):595–600

Shiau YJ, Dham V, Tian G, Chiu CY (2016) Factors influencing removal of sewage nitrogen through denitrification in mangrove soils. Wetlands 36(4):621–630

Signor D, Cerri EP (2013) Nitrous oxide emissions in agricultural soils: a review. Pesq Agropec Trop 43(3):322–338.http://www.scielo.br/pdf/pat/v43n3/a14.pdf

Simek M, Cooper JE (2002) The influence of soil pH on denitrification: progress towards the understanding of this interaction over the last 50 years. Eur J Soil Sci 53(3):345–354

Sliekers AO, Third KA, Abma W, Kuenen JG, Jetten MSM (2003) CANON and Anammox in a gas-lift reactor. FEMS Microbiol Lett 218(2):339–344

Sudarno U, Bathe S, Winter J, Gallert C (2010) Nitrification in fixed-bed reactors treating saline wastewater. Appl Microbiol Biotechnol 85(6):2017–2030

Sun M, Ye M, Liu K, Schwab A P, Liu M, Jiao J, Li H (2017) Dynamic interplay between microbial denitrification and antibiotic resistance under enhanced anoxic denitrification condition in soil. Environ Pol 222:583–591

Tiedje JM, Sexstone AJ, Myrold DD, Robinson JA (1983) Denitrification: ecological niches, competition and survival. Antonie Van Leeuwenhoek 48(6):569–583

Torrentó C, Cama J, Urmeneta J, Otero N, Soler A (2010) Denitrification of groundwater with pyrite and Thiobacillus denitrificans. Chem Geol 278(1):80–91

Uemoto H, Saiki H (2000) Nitrogen removal reactor using packed gel envelopes containing Nitrosomonas europaea and Paracoccus denitrificans. Biotechnol Bioeng 67(1):80–86

Viotti P, Luciano A, Mancini G, Torretta V (2014) A wastewater treatment using a biofilm airlift suspension reactor with biomass attached to supports: a numerical model. Int J Environ Sci Technol 11(3):571–588

Viotti P, Collivignarelli MC, Martorelli E, Raboni M (2016) Oxygen control and improved denitrification efficiency by dosing ferrous ions in the anoxic reactor. Desalination Water Treat 57(39):18240–18247

Van Dongen UGJM, Jetten MS, Van Loosdrecht MCM (2001) The SHARON®-Anammox® process for treatment of ammonium rich wastewater. Water Sci Technol 44(1):153–160

Wang J, Lu H, Chen GH, Lau GN, Tsang WL, van Loosdrecht MC (2009) A novel sulfate reduction, autotrophic denitrification, nitrification integrated (SANI) process for saline wastewater treatment. Water Res 43(9):2363–2372

Wang G, Xu X, Gong Z, Gao F, Yang F, Zhang H (2016) Study of simultaneous partial nitrification, ANAMMOX and denitrification (SNAD) process in an intermittent aeration membrane bioreactor. Process Biochem 51(5):632–641

Water environment research foundation—WERF (2014). Deammonification. Alexandria, p 22
Wen X, Gong B, Zhou J, He Q, Qing X (2017) Efficient simultaneous partial nitrification, anammox and denitrification (SNAD) system equipped with a real-time dissolved oxygen (DO) intelligent control system and microbial community shifts of different substrate concentrations. Water Res 119:201–211
Wett B (2007) Development and implementation of a robust deammonification process. Water Sci Technol 56(7):81–88
WHO (2015) Guidelines for drinking-water quality. Geneva, World Health Organization
Wu C, Chen Z, Liu X, Peng Y (2007) Nitrification–denitrification via nitrite in SBR using real-time control strategy when treating domestic wastewater. Biochem Eng J 36(2):87–92
Yan J, Hu YY (2009) Partial nitrification to nitrite for treating ammonium-rich organic wastewater by immobilized biomass system. Biores Technol 100(8):2341–2347
Yang PY, Nitisoravut S, Wu JS (1995) Nitrate removal using a mixed-culture entrapped microbial cell immobilization process under high salt conditions. Water Res 29(6):1525–1532
Yang W, Lu H, Khanal SK, Zhao Q, Meng L, Chen GH (2016a) Granulation of sulfur-oxidizing bacteria for autotrophic denitrification. Water Res 104:507–519
Yang W, Zhao Q, Lu H, Ding Z, Meng L, Chen GH (2016b) Sulfide-driven autotrophic denitrification significantly reduces N_2O emissions. Water Res 90:176–184
Zhao Y, Feng C, Wang Q, Yang Y, Zhang Z, Sugiura N (2011) Nitrate removal from groundwater by cooperating heterotrophic with autotrophic denitrification in a biofilm–electrode reactor. J Hazard Mater 192(3):1033–1039
Zhou W, Liu X, Dong X, Wang Z, Yuan Y, Wang H, He S (2016) Sulfur-based autotrophic denitrification from the micro-polluted water. J Environ Sci 44:180–188
Zhang J, Cai Z, Cheng Y, Zhu T (2009) Denitrification and total nitrogen gas production from forest soils of Eastern China. Soil Biol Biochem 41(12):2551–2557
Zhang F, Peng Y, Miao L, Wang Z, Wang S, Li B (2017) A novel simultaneous partial nitrification Anammox and denitrification (SNAD) with intermittent aeration for cost-effective nitrogen removal from mature landfill leachate. Chem Eng J 313:619–628

Author Index

A
Agarwal, Avinash Kumar, 1
Aiswarya, R., 331

B
Banerjee, Chiranjib, 375
Bharathiraja, B., 243

C
Chandra, Avantika, 375
Chatterjee, Soumya, 277
Chetty, Raghuram, 387

F
Femina Carolin, C., 197

G
Ghosh, Pooja, 223
Gnansounou, Edgard, 1
Gopinath, Margavelu, 7, 301, 387
Govindaraj, Dhilipkumar, 359
Grace Pavithra, K., 127
Gunasundari, E., 165
Gupta, Gauri, 375
Gurunathan, Baskar, 1, 53, 77, 301, 331

H
Harshiny, Muthukumar, 99

I
Iyyappan, J., 243

J
Jayamuthunagai, J., 243

K
Kumari, Moni, 223
Kumar, Narasimhan Manoj, 53
Kumar, Vipin, 375

M
Matheswaran, Matheswaran, 99, 115
Mazumder, Pranab Behari, 277
Muthukumaran, C., 53, 301
Muthusamy, Shanmugaprakash, 359

N
Nakkeeran, Ekambaram, 253

P
Prasad, Rajesh Kumar, 277
Praveenkumar, R., 243
Pulla, Rose Havilah, 301

R
Rajendran, Karthik, 359
Rajmohan, K.S., 301, 387
Raju, P. Srinavas, 277
Rathna, Ravichandran, 253
Renganthan, S., 331

S
Samsudeen, N., 115
Sekar, Aiswarya Devi, 99
Sellamuthu, Periyar Selvam, 77
Selvakumari, I. Aberna Ebenezer, 331
Senthil Kumar, P., 29, 127, 165, 197
Sharma, Sonika, 277
Sharmila, Govindasamy, 53
Suganya, S., 29

T
Tamilarasan, Krishnamurthi, 77
Thakur, Indu Shekhar, 223

V
Vairale, Mohan G., 277
Varjani, Sunita J., 1, 29, 197, 375
Vijay, Parthasarthy, 7, 301

S. J. Varjani et al. (eds.), *Bioremediation: Applications for Environmental Protection and Management*, Energy, Environment, and Sustainability,
https://doi.org/10.1007/978-981-10-7485-1