Microorganisms for Sustainability 16

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Microbes and Enzymes in Soil Health and Bioremediation

Springer

Microorganisms for Sustainability

Volume 16

Series editor
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Microbes and Enzymes in Soil Health and Bioremediation

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ISSN 2512-1901 ISSN 2512-1898 (electronic)
Microorganisms for Sustainability
ISBN 978-981-13-9116-3 ISBN 978-981-13-9117-0 (eBook)
https://doi.org/10.1007/978-981-13-9117-0

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The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Preface

This book was encouraged by the aspiration that daily human activities in the modern world add to the huge amount of waste into the environment. In order to meet the food demand and requirement, chemical fertilizers in excess have been added to agricultural land and harmful pesticides sprayed onto the crops and vegetables which altered the normal soil microflora and fauna. Plastic pollution becomes the biggest threat in the twenty-first century. Microplastic particles and fibers, crude oil, paints, varnishes, and other daily used stuff in modern life have created the threat to human life. This book had been written to provide a framework for the role of microbes and enzymes to maintain the health of the soil. This book mainly emphasizes the interaction of various pollutants with the soil and water on the Earth's surface.

Researchers across the globe have been trying to generate alternative ways from bio-based products which are more eco-friendly and biodegradable. The chemical products, such as oil, grease, adhesives, and detergents, could be replaced with bio-oil, bio-lubricants, bioadhesives, and biosurfactants which are sensitive to microbial attack and eco-friendly. But there are various challenges for the complete replacement with bio-based products which make it not feasible. A remarkable development in the genomic technologies in the last three decades has enabled the development of various engineered microbial strains and recombinant enzymes which are capable to attack the highly stable bond network in the polymers, oxides, and various complex organic compounds.

We compiled the chapters written by various experienced researchers working in the environmental biotechnology and relevant areas. This book will be helpful to people working in industry and academia, young professionals, and students. The first four chapters will introduce the various pollutants entering in nature and how the microbial population is getting affected. Various microbes important for the growth and development of the plant have also been discussed.

In fundamental nature, the rationale of this book is to provide a toolbox from which researchers, students, and environmentalists working in earth science will be benefited. Another major reason for editing the book was the topic of the research area of our interest. Generally, we spend many hours to collect the information on a wide range of topic and able to get little information or puzzling results. Thus, in the book, we compiled the chapters on all the important issues which need to be solved urgently. Chapter 1 describes the various environmental challenges and implications. Chapter 2 deals with microbes and processes in the bioremediation of soil and provides basic information about the bioremediation processes. Chapters 3, 4 and 5 deal with the effect of pollution on the physical and chemical properties of soil and the roles of microbes in plant growth and development. Chapters 6, 7 and 8 described the role of microbial enzymes in the degradation and removal of various pollutants. These chapters are emphasizing on the hydrolases, laccases, esterases, and other various microbial enzymes. Chapters 9 and 10 describe the microbial interventions for sustainable bioremediation strategies and degradation of chemical pesticides, while Chaps. 11, 12 and 13 discuss about phenolic compounds. Chapters 14 and 16 discuss on soil that has also been contaminated with heavy metals and pharmaceutical products. Chapter 15 describes the role of biosurfactants in soil health and bioremediation. We definitely hope that the present book will be beneficial for all the early-stage researchers and industrialists.

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About the Series Editor

Naveen Kumar Arora, Ph.D., microbiology, Professor in the Department of Environmental Science, Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow, Uttar Pradesh, India, is a renowned researcher in the field of environmental microbiology and biotechnology. His specific area of research is rhizosphere biology and PGPRs. He has more than 50 research papers published in premium international journals and several articles published in magazines and dailies. He is editor of 10 books, published by Springer. He is a member of several national and international societies, Secretary General of Society for Environmental Sustainability, in editorial board of four journals, and reviewer of several international journals. He is also the Editor in Chief of the journal *Environmental Sustainability* published by Springer Nature. He has delivered lectures in conferences and seminars around the globe. He has a long-standing interest in teaching at the PG level and is involved in taking courses in bacteriology, microbial physiology, environmental microbiology, agriculture microbiology, and industrial microbiology. He has been an advisor to 118 postgraduate and 8 doctoral students. Recently, he was awarded for excellence in research by the Honorable Governor of Uttar Pradesh. Although an academician and researcher by profession, he has a huge obsession for the wildlife and its conservation and has authored a book, *Splendid Wilds*. He is the President of Society for Conservation of Wildlife and has a dedicated website www.naveenarora.co.in for the cause of wildlife and environment conservation.

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Dr. Swati Sharma is working as an Assistant Professor at University Institute of Biotechnology (UIBT), Chandigarh University, Mohali, Punjab, India. She has completed her Ph.D. from the Department of Chemical and Natural Resources Engineering, Universiti Malaysia Pahang , Malaysia. She worked as a Visiting Researcher at the College of Life and Environmental Sciences at Konkuk University, South Korea. She has completed her master's degree (M.Sc.) at Dr. Yashwant Singh Parmar University of Horticulture and Forestry, India, and also worked as a Program Co-coordinator at the Himalayan Action Research Center, Dehradun, and Senior Research

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Chapter 1
Let's Protect Our Earth: Environmental Challenges and Implications

**Ashok Kumar, Tanvi Sharma, Sikandar I. Mulla, Hesam Kamyab,
Deepak Pant, and Swati Sharma**

Abstract Microbial enzymes play a vital role to maintain the soil health and removal of pollutants from the contaminated land. Soil microflora is closely associated to maintain the fertility of the soil. Use of chemical pesticides, fertilizers and other volatile sprays in the agricultural practices threatening the healthy microbial population in the soil. Every single particle of healthy soil is loaded with millions of bacteria which interact with the nutrients available in the surrounding and sustain the nutrient cycle, and this microflora is an essential component of life on earth. The rapid increase in the industrialization and urbanization polluted the water and air heavily which affected the microbial populations and their existence too. Some microbes have been evolved to breakdown the complex toxic pollutants entering the soil into non-harmful components and helping to maintain the soil fertility. Thus, it is urgently needed to identify these microorganisms and enzymes which are involved in restoring the remediation of toxic substances and restoration of microflora required for a normal life.

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A. Kumar, S. Sharma (eds.), *Microbes and Enzymes in Soil Health and Bioremediation*, Microorganisms for Sustainability 16,
https://doi.org/10.1007/978-981-13-9117-0_1

Keywords Enzymes · Soil health · Pesticides · Microflora · Industrialization · Pollutants

1.1 Introduction

In recent years, an increase in population growth and rapid industrialization not only ameliorate the standard of life but also affected the quality of the environment. Due to the release of harmful pollutants into the ecosystem such as plastics, pharmaceutical ingredients, greenhouse gases, pesticides, and synthetic dyestuffs, every part of the earth has severely affected. These pollutants not merely caused the teratogenic, carcinogenic, mutagenic, and toxic effects on humans beings or organisms but also created a serious risk to the environment (Jacob et al. 2018; Liu et al. 2019). The heavy metal ions from the contaminated land enter in the crop products and edible vegetable fruits or in the fish or aquatic organism from contaminated water which ultimately reaches in the human body. The continuous flow of these poisonous substances or metal impurities allows them to accumulate inside the human body and alter the normal microflora. All microbial genera, bacteria, fungi, algae, nematode, and protozoa play a significant role in bioremediations and maintaining soil health. To meet the energy crisis and food demand with the growing population, it is very important to save the agricultural land from contamination and maintain productivity.

These pollutants are broadly dispersed everywhere in the environment because of various human activities and industrial processes (Bilal et al. 2019b; Rasheed et al. 2019). Numerous methods such as filtration, reverse osmosis, incineration, lagooning treatment, landfill deposition, and bioremediation using microbes and their enzymes have been applied to treat harmful pollutants (Bilal et al. 2019a; Kuppusamy et al. 2017). The major advantages of using microbial enzymes for the degradation of environmental pollutants are high efficiency, minimum by-products, no secondary pollution, economic feasibility, and environmentally safe (Garcia-Garcia et al. 2016).

Various bacterial genera were used in bioremediation that can convert pollutant into less toxic compounds: *Pseudomonas* sp., *Achromobacter* sp., *Burkholderia* sp., *Rhodococcus* sp., *Ralstonia* sp., *Alcaligenes* sp., *Sphingomonas* sp., *Dehalococcoides* sp., and *Comamonas* sp. that ultimately reduce pollutants (Lloyd et al. 2003). However, highly diverse and specific microorganisms present in nature efficiently remove the several pollutants. But microbial-based remediation is usually slow, as compared to the daily production of a huge amount of waste which causes the pollutants to accumulate in the environment. Nevertheless, molecular biology allows producing novel strains of the microorganism with desirable features for the bioremediation process, thus considerably improving the degradation capability of pollutants (Zhao et al. 2017). Microbes play a very important role in nutrient cycles by intracellular digestion of complex macromolecules and converting these into smaller units in their metabolic activities. Secondly, the enzyme secreted in the extracellular environment facilitates the conversion of complex macromolecules into micromolecules which can be easily absorbed by other living species.

1.2 Major Environmental Challenges

1.2.1 Global Warming and Climate Change

Global warming is defined as the rise in earth temperature due to the increased level of carbon dioxide (CO_2) and other greenhouse gases (GHG). It is directly connected to the percentage of CO_2 present in the earth's atmosphere. The consequences of global warming are a rise in sea level, acidic oceans, increased air pollution, deviations in the cropping, and disease patterns. CO_2 is considered as the primary GHG that imparts to climate change, produced by the burning of fossil fuels such as coal, natural gas, and oil (He et al. 2018). A biological method like photosynthesis occurring in the plants convert CO_2 and water into organic compounds and maintains the equilibrium by fixing atmospheric CO_2 on the earth (Mondal et al. 2016). In the literature, many examples are already described for CO_2 conversion using microbes and their enzymes. Various algal species such as *Chlorella vulgaris*, *Nannochloropsis* sp., *Scenedesmus quadricauda*, *Chlamydomonas reinhardtii*, and *Nannochloris* sp. have been studied to sequester CO_2 (Eloka-Eboka and Inambao 2017). During photosynthesis, the RuBisCO enzyme in a photosynthetic organism is responsible for converting CO_2 into inorganic carbon. The major limitation of RuBisCO is a low affinity for CO_2 (Pavlik et al. 2017). However, CO_2 removal using biological methods is not suitable for region specific large-scale CO_2 sequestration such as industries outlets and polluted cities.

In a recent study, *Methylobacterium extorquens* formate dehydrogenase was reported for the conversion of CO_2 to formate (Jang et al. 2018). Carbonic anhydrase is an enzyme that is mostly used for conversion of CO_2 to bicarbonate (Sharma et al. 2018). Many bacterial species having CA enzyme are *Aeribacillus pallidus, Lactobacillus delbrueckii, Bacillus* sp., *Pseudomonas fragi, Serratia* sp., have been studied for conversion of CO_2 into calcium carbonate (Bose and Satyanarayana 2017; Li et al. 2015; Sharma et al. 2009; Srivastava et al. 2015; Sundaram and Thakur 2018). CO_2 sequestration using microbes offers a reduction of the major greenhouse gas CO_2 and, hence, ameliorates global warming.

1.2.2 Plastic Pollution on Earth

Synthetic plastics represent the main anthropogenic waste entering and accumulating into the environment. Indeed, plastic pollution is now considered a global environmental threat, together with ozone depletion, ocean acidification, and climate changes (Barboza et al. 2018). Plastic is used in everyday life such as packaging material, clothes, water bottles, and carpets. Plastics such as polyethylene terephthalate (PET), polypropylene, and polyethylene present a serious risk to plant and animals growing in the marine ecosystem. The chemical bonds between the plastic monomer are stronger, so they are resistant to natural degradation.

While microplastics are plastic particles less than five millimeter, are of special concern due to their small size, high surface/volume ratio, long environmental persistence, and their ability to enter into the cells and cause adverse effects (Peixoto et al. 2019). The abiotic degradation of man-made plastic by temperature, oxygen, UV radiation, and physical stress (Gewert et al. 2015) slowly degrades plastic and generates microplastic which can spread into the environment by wind (Urbanek et al. 2018). Due to the agglomerations of plastics in the environment, microorganisms are evolving catabolic pathways and enzymes to partly degrade plastic (Yang et al. 2015).

In the literature biodegradation of polyethylene by different microbial strains such as *B. subtilis, Acinetobacter baumanni, Arthrobacter* sp., *Staphylococcus epidermidis and Flavobacterium* sp. was reported (Restrepo-Flórez et al. 2014; Vimala and Mathew 2016). A newly discovered bacteria *I. sakaiensis* enzyme PETase was reported that uses polyethylene terephthalate (PET) as a major energy and carbon source for growth and converts into nontoxic form (Yoshida et al. 2016). This enzyme thus offers a platform for further modification using directed evolution and protein engineering strategy to boost the efficiency of the enzyme, toward the persistent challenge of highly crystalline polymer degradation (Austin et al. 2018). Thermoset plastics such as polyester polyurethane and aliphatic polyester are simply attacked by microbes because of easily digestible ester and urethane bonds in their structures. Other enzymes secreted by microbes that show biodegradable activity include the esterases, lipases, dehydratases, depolymerases, cutinases, ureases, and proteinases (Dang et al. 2018; Masaki et al. 2005; Sood et al. 2016; Zheng et al. 2005). Nowadays bioplastic made from renewable natural resources has received a lot of attention and can be used to replace the plastic (Mostafa et al. 2018). However, bioplastic has not completely replaced the petroleum-based plastic due to various economic and manufacturing challenges.

1.2.3 Chemical Pesticides as a Pollutant on Earth

Currently, pesticides are applied in agricultural production to halt the growth of pests and associated diseases. The most commonly used pesticides include atrazine, lindane, chlordane, DDT, aldrin, cypermethrin, and heptachlor (Pereira et al. 2015). The rise in the use of pesticides/chemical fertilizers in agriculture practices has led to contamination of water, air, and land and adverse effect on human health

(Craig 2019; Li 2018). Although pesticides play a vital role in agriculture, these are recalcitrant to biodegradation and persist in the ecosystem for many years (Kumar et al. 2018; Nicolopoulou-Stamati et al. 2016).

Various microbial species such as *Arthobacter*, *Aspergillus*, *Chorella*, *Penicillium*, *Pseudomonas*, *and Flavobacterium* have shown a capability to degrade pesticides into a less toxic product by using enzymes (Kumar et al. 2018). Various enzymes have been isolated from microorganism such as diisopropyl fluorophosphatase, parathion hydrolase, phosphotriesterase, esterase, and paraoxonase, to study the pathways involved in the biotransformation of these xenobiotic compounds (Cycoń and Piotrowska-Seget 2016; Lu et al. 2013; Singh and Walker 2006; Zuo et al. 2015). These indigenous microbes have limited degradation efficiency, so at present several bacteria containing pesticide-degrading gene can be used for constructing genetically engineered bacteria (Hong et al. 2010). For environmental sustainability, the development of biological pesticides has become the key to safeguard the health of human and agricultural development.

1.2.4 Pharmaceutical Pollution and Increased Antimicrobial Resistance

The excessive use of antibiotics in animal and human medicine, as well as in agriculture, has not only led to their accumulation in the environment but also developed the broad range of highly antibiotic-resistant microorganisms (Almakki et al. 2019). Most bacteria develop resistance against commonly used antibiotics like methicillin-resistant *S. aureus, erythromycin-resistant Streptococcus, penicillin-resistant Pneumococcus, and tetracycline-resistant Shigella* (*Sengupta* et al. 2013; Ventola 2015). Wastewater effluents from pharmaceutical industry enter into the rivers, rich in antibiotics, steroids, hormones, and analgesic components, which have adversely affected the microbial ecology (Ding and He 2010). The continuous exposure of terrestrial and aquatic microorganisms to pharmaceuticals products has affected the genetic composition of microbial genera and developed the antimicrobial resistant genes.

However, biological methods for converting pharmaceutical pollutant into non-toxic forms are attractive because they are inexpensive and environment-friendly (Zur et al. 2018). Many bacterial and fungal strains such as *Klebsiella, Penicillium, Pseudomonas, Aspergillus, Sphingomonas* sp., *Bacillus, Enterobacter, Aeromonas, and Streptomyces* have been reported for biotransformation of pharmaceutical pollutant (Rana et al. 2017). Biotransformation results in the formation of the end product that is less toxic and more stable than the initial compound.

1.2.5 *Heavy Metal Pollution on Earth*

Heavy metals are metalloids that have density more than 5 g/cm^3 such as mercury, arsenic, and lead (Tchounwou et al. 2012). Heavy metals occur naturally in the earth's crust and anthropogenic activities such as smelting, mining, petroleum combustion, and burning of coal in power plant, and use of fertilizer increases its existence in the environment (He et al. 2005). In natural systems, heavy metals affect cellular organelles like endoplasmic reticulum, lysosome, cell membrane, nuclei, mitochondria, and various enzymes involved in detoxification, metabolism, and damage repair. Metal ions interact with nuclear proteins and DNA, causing conformational changes and DNA damage that leads to apoptosis or carcinogenesis (Wang and Shi 2001). Due to the persistence of metal in the terrestrial environment, heavy metal pollution poses a risk to animal, plant, and human health (Mishra 2017).

Heavy metal bioremediation by microorganism is emerging as an efficient technique. Different mechanisms used by microorganisms to tolerate the metal toxicity are extrusion, biotransformation, use of enzymes, and synthesis of metallothioneins and biosurfactants (Igiri et al. 2018; Ramasamy et al. 2007). *P. putida* is cadmium-tolerant strain and has the intracellular ability to sequester zinc, copper, and cadmium, by using cysteine-rich low molecular weight proteins (Higham et al. 1986). *Bacillus pumilus*, *Alcaligenes faecalis*, *Brevibacterium iodonium*, and *Pseudomonas aeruginosa* were reported for the removal of cadmium and lead (De et al. 2008). Microbial bioremediation is a cost-effective and eco-friendly technology for the clean-up of heavy metals.

1.3 Restoration of Soil Health Using Microbes

Soil respiration, microbial biomass, enzyme activities, and microbial diversity are the major biological indicators of soil health. Healthy soils are necessary for the integrity of terrestrial ecosystem or to recover from trouble, such as climate change, pest infestation, drought, pollution, and human exploitation including agriculture (Ellert et al. 1997). Moreover, with the continuous increase in the world's population, the demand for food production has increased (Fageria et al. 2008). But nowadays agricultural practices include the use of potentially dangerous chemical fertilizers that affects the soil and human health (Glick 2018). Protection of soil is therefore of high priority, and a thorough understanding of ecosystem processes is a critical factor in assuring that soil remains healthy. These enzymes catalyze many vital reactions necessary for the life processes of soil microorganisms and also help in the stabilization of soil structure. Although microorganisms are the primary source of soil enzymes, plants and animals also contribute to the soil enzyme pool. Soil enzymes respond rapidly to any changes in soil management practices and environmental conditions. Their activities are closely related to the physiochemical and biological properties of the soil. Hence, soil enzymes are used as sensors for soil microbial status, for soil physiochemical conditions, and for the influence of soil treatments or climatic factors on soil fertility. Overall the microbe and enzyme profile in the soil must be stabilized in order to maintain the health of the soil, its fertility and to sustain agricultural growth. At present, the biogeochemical cycles in the agricultural ecosystem have been disturbed as a result of increased pollution and toxic level in the environment. The rate of organic content decomposition becomes lesser due to the extinction of various soil microflora. Therefore, it becomes necessary to find the suitable troubleshoot methods to prevent the increase in soil pollution to restore the decreased fertility of soil using biological approaches.

1.4 Conclusion

The soil microflora and enzyme quantity vary with external factors, physical and chemical conditions. Whichever may be the source of pollution either industries, fertilizers, pesticides, or urbanization and automobiles, each source has seriously damaged the microbial population and enzyme activity of the soil. This is also very complex to determine the exact level of microbial existence and enzyme activity in various geographical areas. Undoubtedly, molecular biology techniques, such as directed evolution and recombinant DNA technology, have revolutionized the speed of enzyme and microbe engineering which could be a milestone to restore the microbial population and enzyme activity in the soil. In this book chapter, we have given an overview of various types of pollutants which are affecting the soil or water bodies on the earth and how various microbes and enzymes are correlated with the existence or increasing level of this pollution.

Acknowledgments The financial support from the Jaypee University of Information Technology, Waknaghat, to undertake this study is thankfully acknowledged. Further, the authors have no conflict of interest either among themselves or with the parent institution.

References

Almakki A, Jumas-Bilak E, Marchandin H, Licznar-Fajardo P (2019) Antibiotic resistance in urban runoff. Sci Total Environ 667:64–76

Austin HP, Allen MD, Donohoe BS, Rorrer NA, Kearns FL, Silveira RL, Pollard BC, Dominick G, Duman R, El Omari K, Mykhaylyk V, Wagner A, Michener WE, Amore A, Skaf MS, Crowley MF, Thorne AW, Johnson CW, Woodcock HL, McGeehan JE, Beckham GT (2018) Characterization and engineering of a plastic-degrading aromatic polyesterase. Proceedings of the National Academy of Sciences of the United States of America, Medium: ED; Size: Article No. 201718804

Barboza LGA, Dick Vethaak A, Lavorante BRBO, Lundebye A-K, Guilhermino L (2018) Marine microplastic debris: an emerging issue for food security, food safety and human health. Mar Pollut Bull 133:336–348

Bilal M, Adeel M, Rasheed T, Zhao Y, Iqbal HMN (2019a) Emerging contaminants of high concern and their enzyme-assisted biodegradation – a review. Environ Int 124:336–353

Bilal M, Rasheed T, Nabeel F, Iqbal HMN, Zhao Y (2019b) Hazardous contaminants in the environment and their laccase-assisted degradation – a review. J Environ Manag 234:253–264

Bose H, Satyanarayana T (2017) Utility of thermo-alkali-stable gamma-CA from polyextremophilic bacterium Aeribacillus pallidus TSHB1 in biomimetic sequestration of CO2 and as a virtual peroxidase. Environ Sci Pollut Res Int 24(11):10869–10884

Craig K (2019) A review of the chemistry, pesticide use, and environmental fate of sulfur dioxide, as used in california. Rev Environ Contam Toxicol 246:33–64

Cycoń M, Piotrowska-Seget Z (2016) Pyrethroid-degrading microorganisms and their potential for the bioremediation of contaminated soils: a review. Front Microbiol 7:1463

Dang TCH, Nguyen DT, Thai H, Nguyen TC, Hien Tran TT, Le VH, Nguyen VH, Tran XB, Thao Pham TP, Nguyen TG, Nguyen QT (2018) Plastic degradation by thermophilic Bacillus sp. BCBT21 isolated from composting agricultural residual in Vietnam. Adv Nat Sci Nanosci Nanotechnol 9(1):015014

De J, Ramaiah N, Vardanyan L (2008) Detoxification of toxic heavy metals by marine bacteria highly resistant to mercury. Mar Biotechnol (NY) 10(4):471–477

Ding C, He J (2010) Effect of antibiotics in the environment on microbial populations. Appl Microbiol Biotechnol 87(3):925–941

Ellert BH, Clapperton MJ, Anderson DW (1997) An ecosystem perspective of soil quality. In: Gregorich G, Carter MR (ed) Soil quality for crop production and ecosystem health. Elsevier, Amsterdam

Eloka-Eboka AC, Inambao FL (2017) Effects of CO2 sequestration on lipid and biomass productivity in microalgal biomass production. Appl Energy 195:1100–1111

Fageria NK, Baligar V, Li Y (2008) The role of nutrient efficient plants in improving crop yields in the twenty first century. J Plant Nutr 31:1121–1157

Garcia-Garcia JD, Sanchez-Thomas R, Moreno-Sanchez R (2016) Bio-recovery of non-essential heavy metals by intra- and extracellular mechanisms in free-living microorganisms. Biotechnol Adv 34(5):859–873

Gewert B, Plassmann MM, MacLeod M (2015) Pathways for degradation of plastic polymers floating in the marine environment. Environ Sci: Processes Impacts 17(9):1513–1521

Glick BR (2018) Soil microbes and sustainable agriculture. Pedosphere 28(2):167–169

He ZL, Yang XE, Stoffella PJ (2005) Trace elements in agroecosystems and impacts on the environment. J Trace Elem Med Biol 19(2–3):125–140

He Q, Ji L, Yu B, Yan S, Zhang Y, Zhao S (2018) Renewable aqueous ammonia from biogas slurry for carbon capture: chemical composition and CO2 absorption rate. Int J Greenhouse Gas Control 77:46–54

Higham DP, Sadler PJ, Scawen MD (1986) Cadmium-binding proteins in Pseudomonas putida: pseudothioneins. Environ Health Perspect 65:5–11

Hong Y, Zhou J, Hong Q, Wang Q, Jiang J, Li S (2010) Characterization of a fenpropathrin-degrading strain and construction of a genetically engineered microorganism for simultaneous degradation of methyl parathion and fenpropathrin. J Environ Manag 91(11):2295–2300

Igiri BE, Okoduwa SIR, Idoko GO, Akabuogu EP, Adeyi AO, Ejiogu IK (2018) Toxicity and bioremediation of heavy metals contaminated ecosystem from tannery wastewater: a review. J Toxicol 2018:1–16

Jacob JM, Karthik C, Saratale RG, Kumar SS, Prabakar D, Kadirvelu K, Pugazhendhi A (2018) Biological approaches to tackle heavy metal pollution: a survey of literature. J Environ Manag 217:56–70

Jang J, Jeon BW, Kim YH (2018) Bioelectrochemical conversion of CO2 to value added product formate using engineered Methylobacterium extorquens. Sci Rep 8(1):7211

Kumar S, Kaushik G, Dar MA, Nimesh S, LÓPez-Chuken UJ, Villarreal-Chiu JF (2018) Microbial degradation of organophosphate pesticides: a review. Pedosphere 28(2):190–208

Kuppusamy S, Thavamani P, Venkateswarlu K, Lee YB, Naidu R, Megharaj M (2017) Remediation approaches for polycyclic aromatic hydrocarbons (PAHs) contaminated soils: Technological constraints, emerging trends and future directions. Chemosphere 168:944–968

Li Z (2018) Health risk characterization of maximum legal exposures for persistent organic pollutant (POP) pesticides in residential soil: an analysis. J Environ Manag 205:163–173

Li C-X, Jiang X-C, Qiu Y-J, Xu J-H (2015) Identification of a new thermostable and alkali-tolerant α-carbonic anhydrase from Lactobacillus delbrueckii as a biocatalyst for CO2 biomineralization. Bioresour Bioprocess 2(1):44

Liu L, Bilal M, Duan X, Iqbal HMN (2019) Mitigation of environmental pollution by genetically engineered bacteria — current challenges and future perspectives. Sci Total Environ 667:444–454

Lloyd JR, Lovley DR, Macaskie LE (2003) Biotechnological application of metal-reducing microorganisms. Adv Appl Microbiol 53:85–128

Lu P, Li Q, Liu H, Feng Z, Yan X, Hong Q, Li S (2013) Biodegradation of chlorpyrifos and 3,5,6-trichloro-2-pyridinol by Cupriavidus sp. DT-1. Bioresour Technol 127:337–342

Masaki K, Kamini NR, Ikeda H, Iefuji H (2005) Cutinase-like enzyme from the yeast Cryptococcus sp. strain S-2 hydrolyzes polylactic acid and other biodegradable plastics. Appl Environ Microbiol 71(11):7548–7550

Mishra GK (2017) Microbes in heavy metal remediation: a review on current trends and patents. Recent Pat Biotechnol 11(3):188–196

Mondal M, Khanra S, Tiwari ON, Gayen K, Halder GN (2016) Role of carbonic anhydrase on the way to biological carbon capture through microalgae—a mini review. Environ Prog Sustain Energy 35(6):1605–1615

Mostafa NA, Farag AA, Abo-dief HM, Tayeb AM (2018) Production of biodegradable plastic from agricultural wastes. Arab J Chem 11(4):546–553

Nicolopoulou-Stamati P, Maipas S, Kotampasi C, Stamatis P, Hens L (2016) Chemical pesticides and human health: the urgent need for a new concept in agriculture. Front Public Health 4:148–148

Pavlik D, Zhong Y, Daiek C, Liao W, Morgan R, Clary W, Liu Y (2017) Microalgae cultivation for carbon dioxide sequestration and protein production using a high-efficiency photobioreactor system. Algal Res 25:413–420

Peixoto D, Pinheiro C, Amorim J, Oliva-Teles L, Guilhermino L, Vieira MN (2019) Microplastic pollution in commercial salt for human consumption: a review. Estuar Coast Shelf Sci 219:161–168

Pereira L, Franco-Bernardes M, Pazin M, Dorta D (2015) Impact of pesticides on environmental and human health, Cells, drugs and environment, Ana Cristina Andreazza and Gustavo Scola, Intechopen 195–234

Ramasamy K, Kamaludeen, Banu SP (2007) Bioremediation of metals: microbial processes and techniques In: Singh SN, Tripathi RD (eds) Environmental Bioremediation Technologies. Springer, Berlin, Heidelberg.

Rana RS, Singh P, Kandari V, Singh R, Dobhal R, Gupta S (2017) A review on characterization and bioremediation of pharmaceutical industries' wastewater: an Indian perspective. Appl Water Sci 7(1):1–12

Rasheed T, Bilal M, Nabeel F, Adeel M, Iqbal HMN (2019) Environmentally-related contaminants of high concern: potential sources and analytical modalities for detection, quantification, and treatment. Environ Int 122:52–66

Restrepo-Flórez J-M, Bassi A, Thompson MR (2014) Microbial degradation and deterioration of polyethylene – a review. Int Biodeterior Biodegradation 88:83–90

Sengupta S, Chattopadhyay MK, Grossart HP (2013) The multifaceted roles of antibiotics and antibiotic resistance in nature. Front Microbiol 4:47

Sharma A, Bhattacharya A, Singh S (2009) Purification and characterization of an extracellular carbonic anhydrase from Pseudomonas fragi. Process Biochem 44(11):1293–1297

Sharma A, Sharma T, Meena KR, Kumar A, Kanwar SS (2018) High throughput synthesis of ethyl pyruvate by employing superparamagnetic iron nanoparticles-bound esterase. Process Biochem 71:109–117

Singh BK, Walker A (2006) Microbial degradation of organophosphorus compounds. FEMS Microbiol Rev 30(3):428–471

Sood S, Sharma A, Sharma N, Kanwar S (2016) Carboxylesterases: sources, characterization and broader applications. Insight Enzymme Res 1(1): 1–11

Srivastava S, Bharti RK, Verma PK, Thakur IS (2015) Cloning and expression of gamma carbonic anhydrase from Serratia sp. ISTD04 for sequestration of carbon dioxide and formation of calcite. Bioresour Technol 188:209–213

Sundaram S, Thakur IS (2018) Induction of calcite precipitation through heightened production of extracellular carbonic anhydrase by CO2 sequestering bacteria. Bioresour Technol 253:368–371

Tchounwou PB, Yedjou CG, Patlolla AK, Sutton DJ (2012) 2012. Heavy metal toxicity and the environment. Experientia Suppl 101:133–164

Urbanek AK, Rymowicz W, Mironczuk AM (2018) Degradation of plastics and plastic-degrading bacteria in cold marine habitats. Appl Microbiol Biotechnol 102(18):7669–7678

Ventola CL (2015) The antibiotic resistance crisis: part 1: causes and threats. Pharm Ther 40(4):277–283

Vimala PP, Mathew L (2016) Biodegradation of polyethylene using bacillus subtilis. Proced Technol 24:232–239

Wang S, Shi X (2001) Molecular mechanisms of metal toxicity and carcinogenesis. Mol Cell Biochem 222(1–2):3–9

Yang Y, Yang J, Wu W-M, Zhao J, Song Y, Gao L, Yang R, Jiang L (2015) Biodegradation and mineralization of polystyrene by plastic-eating mealworms: part 1. Chemical and physical characterization and isotopic tests. Environ Sci Technol 49(20):12080–12086

Yoshida S, Hiraga K, Takehana T, Taniguchi I, Yamaji H, Maeda Y, Toyohara K, Miyamoto K, Kimura Y, Oda K (2016) A bacterium that degrades and assimilates poly(ethylene terephthalate). Science 351(6278):1196–1199

Zhao Q, Bilal M, Yue S, Hu H, Wang W, Zhang X (2017) Identification of biphenyl 2, 3-dioxygenase and its catabolic role for phenazine degradation in Sphingobium yanoikuyae B1. J Environ Manag 204(Pt 1):494–501

Zheng Y, Yanful EK, Bassi AS (2005) A review of plastic waste biodegradation. Crit Rev Biotechnol 25(4):243–250

Zuo Z, Gong T, Che Y, Liu R, Xu P, Jiang H, Qiao C, Song C, Yang C (2015) Engineering Pseudomonas putida KT2440 for simultaneous degradation of organophosphates and pyrethroids and its application in bioremediation of soil. Biodegradation 26(3):223–233

Zur J, Pinski A, Marchlewicz A, Hupert-Kocurek K, Wojcieszynska D, Guzik U (2018) Organic micropollutants paracetamol and ibuprofen-toxicity, biodegradation, and genetic background of their utilization by bacteria. Environ Sci Pollut Res 25(22):21498–21524

Chapter 2
Microbes and Processes in Bioremediation of Soil

Tarun Gangar, Kamal Kumar Bhardwaj, and Reena Gupta

Abstract Environmental pollution has been increasing at an alarming rate since the beginning of the twenty-first century. There is an enormous increase in the production and use of xenobiotic compounds that have created new sites of environmental contamination and problem worsens as many of such xenobiotic compounds are either persistent or recalcitrant to microbial breakdown. The presence of anthropogenic organic compounds/chemicals in the environment is a matter of significant concern because of their potential toxicity, mutagenicity, and bioconcentration (biomagnification) in higher organisms. This is of immense concern and hence provides impetus to the development of certain remediation techniques. Various microorganisms play a key role in the bioremediation of soil and may range from bacteria majorly to a few actinomycetes and fungi. Bioremediation can be carried out *via* two main approaches, ex situ and in situ, and choice of method depends largely on site characteristics, concentration, and type of pollutants present. To enhance the remediation process, a more recent approach called bioaugmentation is also practiced. Bioaugmentation trials have met varying degrees of success. This chapter will largely focus on various microorganisms which are potent biomediators and also the processes involved in the same.

Keywords Bioremediation · Xenobiotic compounds · Bioconcentration · Bioaugmentation

2.1 Introduction

Increasing the standard of living and urbanization has posed a great degradative threat to the environment and ecosystem. A typical example is the monstrous sized heaps of waste dumped daily into the dumping yards of cities. Also, the

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A. Kumar, S. Sharma (eds.), *Microbes and Enzymes in Soil Health and Bioremediation*, Microorganisms for Sustainability 16,
https://doi.org/10.1007/978-981-13-9117-0_2

advancements in science, technology, and industrial sector have led to the production of waste ranging from municipal sewage to nuclear waste, and also this has rendered our ecosystem unfit for the survival of life forms on earth (Lin et al. 2018; Ontanon et al. 2018; Parewa et al. 2018). Previously, the use of conventional techniques was practiced such as waste disposal by landfilling, dumping in open grounds, etc. With rapid and ever-growing waste disposal problems, conventional methods failed to cope up with this issue. New methods like incineration and chemical decomposition are being developed, but the use of such methods is either uneconomical or not environment-friendly. Such problems lead to the development of newer and better technologies which may better solve the purpose. Modern-day bioremediation is one such method (Karigar and Rao 2011).

The literal meaning of "bioremediation" is "biological treatment". So, bioremediation by definition means the use of biological agents such as microorganisms (majorly bacterial and fungi) and/or plants (in case of phytoremediation) for the treatment of contaminated soil and water, so as to make it fit for reuse by all the biological entities. Some of the remediation processes used for the treatment of contaminated area include natural attenuation, composting, biopiling, bioventing, landfarming, thermal desorption, landfilling, soil washing, and incineration (USEPA 2014). Till now, majorly the success to bioremediation and biodegradation has been provided by the indigenous microbes thriving in that very environment, and this is highly dependent upon the growth characteristics and nutritional requirements of the microbes used for the purpose (Verma and Jaiswal 2016). There are several factors that define the choice of bioremediation techniques, e.g., nature of pollutant, degree of pollution, geographical location, the cost involved in the process, etc. (Frutos et al. 2012; Smith et al. 2015). Biological treatment of soil using various biological agents primarily plants and microorganisms is considered as one of the cheapest and safest methods to remove the hazardous contaminants from the soil. Plants have the capability to neutralize various types of harmful chemicals in the soil by direct utilization, followed by the biotransformation of such compounds into nontoxic products which are harmful neither to the environment nor to any other form of life (Macek et al. 2008).

The major focus of this chapter is the microbes which play a vital role in effective bioremediation of soil and also on processes involved in the biodegradation. Microorganisms have this inherent capability to catalyze the degradation and mineralization of various contaminating xenobiotic compounds, thus converting them into nontoxic by-products (Seshadri and Heidelberg 2005; Head et al. 2006; Gomez et al. 2017). Such a conversion process is often a result of consortia of microorganisms. Recently, biodegradation of total petroleum hydrocarbons was investigated in slurry phase bioreactor using aged refuse (Fu Chen et al. 2019). Bioremediation can only be effective when the environmental conditions permit the microbial growth and activity, or conversely, there is a need to manipulate certain environmental parameters to allow the growth of microbes so that degradation could proceed at a faster pace (Vidali 2001). Most of the bioremediation procedures run under complete aerobic environment, but to treat certain recalcitrant molecules, the system may run under anaerobic environment (Colberg and Young 1995).

2.2 The Basic Approach to Bioremediation

Mostly bioremediation proceeds through a process of oxidation-reduction reactions (redox), whereby a chemical species donates an electron to a different species that accepts an electron. Bioremediation procedures can be broadly classified as aerobic and anaerobic bioremediation.

2.2.1 Aerobic Bioremediation

Aerobic bioremediation is the most practiced and most prevalent form of oxidative bioremediation. As the name suggests here oxygen acts as the terminal electron acceptor for the oxidation of various contaminants such as polyaromatic hydrocarbons (PAHs), phenols, petroleum, etc. Preference of oxygen points toward higher oxidative potential of oxygen and its requirement by some enzyme systems to initiate the degradation process.

Under ideal conditions, the biodegradation rates of aliphatic, alicyclic, and aromatic compounds (low to moderate molecular weight) can be very high. With an increase in the molecular weight of the compound, its resistance toward biodegradation increases (Norris 1993).

There are several physical methods for aerating the soil above water table, e.g., landfarming, composting, and bioventing (Frutos and Fernandez 2010). Approaches for aeration of soil below water table include flushing aerated water through treatment zone, air sparging, and the addition of molecular oxygen or peroxide.

2.2.2 Anaerobic Bioremediation

This technique can be employed to remediate a broad range of already oxidized contaminating pollutants including ethenes (PCE, TCE, DCE, VC), chlorinated ethanes (TCA, DCA), chloromethanes (CT, CF), chlorinated cyclic hydrocarbons, various energetics (e.g., perchlorate, RDX, TNT), and nitrate.

Anaerobic bioremediation occurs in two steps:

1. Depletion of background electron acceptors like oxygen (O), nitrate (NO_3^-), ferric ion (Fe^{3+}), etc.
2. Stimulation of biochemical reduction of oxidized contaminating pollutants.

2.2.3 The Fate of Organic Contaminating Pollutants

The knowledge of various catabolic pathways involved in the degradation of contaminating pollutants of both aerobic and anaerobic microorganisms has a major beneficial impact in the development of *in situ* and *ex situ* bioremediation protocols. The intrinsic chemical consideration that limits the biodegradability of aromatic pollutants in both aerobic and anaerobic environments was reviewed by Field et al. 1995.

The aerobic microorganisms make use of oxygenase enzyme to initiate the electrophilic attack on aromatic molecules. The process is greatly suppressed by the presence of electron withdrawing groups such as chloro, azo, and nitro (Dorb and Knackmuss 1978; Knackmuss 1981). The microorganisms involved in the aerobic degradation are *Candida*, *Anabaena*, *Nostoc*, *Chlamydomonas*, *Microcoleus*, *Oscillatoria*, *Saccharomyces*, *Chlorella*, and *Phormidium* (DorotaWolicka et al. 2009). On the other hand, anaerobic microorganisms proceed with the degradation of aromatic pollutants in a completely reciprocal manner, i.e., under anaerobic conditions, the microorganisms make use of enzymes to initiate an electrophilic attack on the aromatic molecules. So here, the presence of electron withdrawing group will enhance the initial reductive attack on aromatic contaminants (Knackmuss 1992, Dolfing and Harrison 1993, Ann-Kathrin Ghattas et al. 2017). Conversely, electron donating groups will hinder the anaerobic transformation of aromatic compounds but will favor the aerobic biotransformation process (Field et al. 1995). However, the complete absence of electron withdrawing as well as electron donating group will enhance the recalcitrance of hydrocarbon in the anaerobic environment (Schink 1985; Schink 1988). It has also been noted that the resulting products of anaerobic biodegradation of complex molecules such as polychlorinated and polynitroaromatic compounds are appropriate products for aerobic mineralization but they resist further anaerobic biodegradation (Zitomer and Speece 1993).

2.3 Microbes Involved in Bioremediation of Soil

The contamination of soil, sediment, and water from industrial and other human inputs is widespread and poses a threat to human and ecological health. Bioremediation is the use of microbes for the beneficial removal of contaminants of concern. The microbial processes involved in bioremediation are normally natural components of respiration or adaptation, often a component of carbon cycling or metal redox cycling. Thus, bioremediation often occurs without direct intervention; however, biostimulation (the addition of nutrients or adjustment of conditions) and bioaugmentation (the addition of microbes capable of bioremediation) are however important for the complete removal of contaminants within an economical timeframe. Various microorganisms involved in bioremediation of various contaminating pollutants are listed in Table 2.1.

Table 2.1 Microbes involved in the bioremediation of contaminants

Contaminants	Microorganisms	References
Monocyclic aromatic hydrocarbons	*Penicillium chrysogenum*	Abdulsalam et al. (2013) and Pedro et al. (2014)
BTEX	*P. chrysogenum*	
Phenols	*Bacillus subtilis*, *Penicillium chrysogenum*, *Corynebacterium propinquum*, *Alcaligenes odorans*, *Pseudomonas aeruginosa*	Singh et al. (2013)
Petrol and diesel oil	*Penicillium alcaligenes*, *P. putida*, *P. veronii*, *P. mendocina*, *Achromobacter*, *Flavobacterium*, *Acinetobacter*	Safiyanu et al. (2015) and Sani et al. (2015)
PAHs	*Pseudomonas putida*, *Pseudomonas* sp., *Coprinellus radians*, *Ralstonia* sp., *Microbacterium* sp.	Sarang et al. (2013), AI-Jawhari (2014) and Safiyanu et al. (2015)
Biphenyls and triphenylmethanes	*Phanerochaete chrysosporium*	Erika et al. (2013)
Hydrocarbons	*Aspergillus niger*, *A. fumigatus*, *F. solani*, *P. funiculosum*, *Tyromyces palustris*, *Gloeophyllum trabeum*, *Trametes*, *Versicolor*	Karigar and Rao (2011), AI-Jawhari (2014) and Xu et al. (2017)
Methylnaphthalene and dibenzofurans	*Coprinellus radians*	Aranda et al. (2010)
Phenanthrene and benzopyrene	*Candida viswanathii*	Hesham et al. (2012)
Oil	*Alcaligenes odorans*, *Bacillus subtilis*, *Fusarium* sp., *Corynebacterium propinquum*, *Penicillium chrysogenum*, *Pseudomonas aeruginosa*	Hidayat and Tachibana (2012) and Singh et al. (2013)
Crude oil	*Aspergillus niger*, *Saccharomyces cerevisiae*, *Candida glabrata*, *Candida krusei*, *B. brevis*, *P. aeruginosa* KH6, *B. licheniformis*, *B. sphaericus*	Aliaa et al. (2016) and Burghal et al. (2016)
Paints (oil based)	*B. subtilis* strain NAP2, NAP1, NAP4	Phulpoto et al. (2016)
Industrial dyes	*Myrothecium roridum* IM 6482, *Pycnoporus sanguineus*, *Phanerochaete chrysosporium*, *Trametes trogii*, *Penicillium ochrochloron*	Shedbalkar and Jadhav (2011) and Hassan et al. (2013)
Textile dyes	*Micrococcus luteus*, *Nocardia atlantica*, *Bacillus* spp. ETL-2012, *Pseudomonas aeruginosa*, *Bacillus pumilus* HKG212, *Listeria denitrificans*	Hassan et al. (2013), Maulin et al. (2013), Das et al. (2015) and Yogesh and Akshaya (2016)
Black liquor	*Bacillus firmus*, *Staphylococcus aureus*, *Bacillus macerans*, *Klebsiella oxytoca*	Adebajo et al. (2017)
Lead, nickel, and mercury	*Saccharomyces cerevisiae*	Chen and Wang (2015), Infante et al. (2014)

(continued)

Table 2.1 (continued)

Contaminants	Microorganisms	References
Fe^{2+}, Zn^{2+}, Pb^{2+}, Mn^{2+}, and Cu^{2+}	*Pseudomonas fluorescence*, *P. aeruginosa*	Paranthaman and Karthikeyan (2015)
Co, Cu, Cr, and Pb	*Lysinibacillus sphaericus* CBAM-5	Peña-Montenegro et al. (2015)
Cadmium	*Aspergillus versicolor*, *Trichoderma* sp., *A. fumigatus*, *Paecilomyces* sp., *Microsporum* sp., *Cladosporium* sp.	Priyalaxmi et al. (2014) and Soleimani et al. (2015)
Endosulfan	*Bacillus*, *Staphylococcus*	Mohamed et al. (2011)
Chlorpyrifos	*Enterobacter*	Niti et al. (2013)
Ridomil MZ 68MG, Fitoraz WP76, Decis 2.5EC, Malation	*Pseudomonas putida*, *Arthrobacter* sp., *Acinetobacter* sp.	Hussaini et al. (2013) and Mónica et al. (2016)
Chlorpyrifos and methyl parathion	*Acinetobacter* sp., *Photobacterium* sp., *Pseudomonas* sp., *Enterobacter* sp.	Ravi et al. (2015)

2.3.1 Bioaugmentation

Bioaugmentation is the process of enhancing/stimulating the rate of bioremediation by addition of single strain or consortia of microorganisms as to mimic the competitiveness among the indigenous microflora and also to remove/decrease adaptation/acclimatization time (Bourier and Zeahnder 1993; Liu and Suflita 1993; Singleton 1994). This technique may involve single strain or consortia of microorganisms but also involve genetically engineered microorganisms (GEMs) within certain strict international rules and regulations. Although GEMs are very efficient in such processes, their accidental release into the environment may pose a serious threat to mankind. Keeping in mind such negative impacts, the use of GEMs has been limited to laboratory-based bioreactor applications.

Bioaugmentation strategy used as per model proposed by Forsyth et al. (1995) for soil is:

1. Where the number of degrading microorganisms is low or sub-detectable.
2. Contaminating pollutants which require a multitude of processes to degrade contaminants.
3. Small-scale contaminated site where non-biological treatment processes are not economical.

2.3.1.1 Factors Affecting Bioaugmentation

Although bioaugmentation has solved a number of issues pertaining to bioremediation of contaminants which are aromatic in nature primarily, still there are a number of ecological constraints which hamper its effectiveness and have kept it to a minimal level. One of the major difficulties that arises during the process is the survival of non-native microbial species which are introduced to the contaminated site. Studies have revealed that the number of exogenous microorganisms has reduced shortly after the inoculation of soil. Hence both abiotic and biotic factors are shown to cause such decrease (Cho et al. 2000; Bento et al. 2005; Wolski et al. 2006). Various abiotic factors include temperature, moisture, pH, and organic content of the soil, and biotic factors include aeration, amount of nutrients, and type of soil.

There are various studies and examples which may prove above mentioned points.

The effect of moisture content in the soil on the survival of *Achromobacter piechaudii* TBPZ and degradation of tribromophenol (TBP) indicating minimum 25% water content was required for rapid degradation, whereas soils with 10% moisture content show limited activity (Ronen et al. 2000). Low moisture content in the soil decreases the efficiency with which microorganisms perform the degradation of contaminants, such effect can be attributed to the fact that the decreased bacterial activity is due to the diffusional limitation of substrate supply and adverse physiological effects associated with cell dehydration (Mashregi and Prosser 2006).

Other most crucial factors influencing the efficiency of bioaugmentation is the organic content of the soil. It plays an important role in the bioavailability of contaminants and hence impairs the survival of inoculated strains and ultimately their availability to degrade contaminants, e.g., the rate of 2,4-D degradation was lower in the soil with high organic content but was considerably higher in soils with lower organic content (Greer and Shelton 1992). Conversely, when the soil was combusted to remove the organic content, microbes completely lost their degradative activity. This indicated that there is presence of some components of insoluble organic matter that is nutritionally beneficial for microorganisms involved in BTEX degradation (Kim et al. 2008).

Other factors including competition primarily between indigenous and exogenous microorganisms for limited C-sources and also antagonistic interaction and predation by protozoa and bacteriophages also play an essential role in the final results of bioaugmentation. All these interactions greatly decrease the number of inoculated cells (England et al. 1993; Sorensen et al. 1999).

2.3.1.2 Microbes in Bioaugmentation

Before performing augmentation in the soil for the purpose of enhanced biodegradation, one should fully know the type and level of contaminants and about the strains of microorganisms and their consortia which play active role in the process. The following features should be kept in mind before augmenting soil:

1. The organism should be easily cultivable.
2. The organism used for the purpose should be able to grow fast under given environmental and nutritional conditions.
3. The organism should be able to withstand a high concentration of contaminants and also should be able to survive in varying environmental conditions.

In case of contaminants such as PAHs, it is especially necessary to use organisms which are capable of producing surfactants, so that these contaminants are more accessible and the process becomes more feasible (Forsyth et al. 1995; van der Gast et al. 2003; Gentry et al. 2004).

Several approaches can be followed to select for the microorganisms useful in bioaugmentation. First being, isolating microorganisms from a contaminated site in question and then growing it under laboratory conditions. Finally, this pre-adapted pure culture is returned to the contaminated site. The process is called reinoculation and involves the use of indigenous microflora. The second approach involves the use of microorganisms from the contaminated site having similar kind of contamination. Various studies revealed that microbial consortia for degradation of aromatic contaminants are effective as compared to selected single strains (Goux et al. 2003; Ghazali et al. 2004).

Both Gram-positive and Gram-negative bacteria play a major role in the bioaugmentation. Experiments pertaining to bioaugmentation were done using both the organisms belonging to genera *Pseudomonas*, *Alcaligenes*, *Flavobacterium*, *Achromobacter*, and *Sphingobium* (Gram-negative bacteria) and *Mycobacterium*, *Bacillus*, and *Rhodococcus* (Gram-positive). Potentially useful fungi in bioaugmentation are represented by genera *Aspergillus*, *Penicillium*, *Absidia*, *Mucor*, *Acremonium*, and *Verticillium.*

2.3.1.3 Delivery of Inoculum

The efficiency of bioaugmentation entirely depends upon the number of microorganisms and total biomass introduced in the soil. The delivery of microbes is also another important factor responsible for efficient bioaugmentation. The conventional delivery mechanisms make use of liquid culture for the introduction of microorganisms into the contaminated site. But nowadays various modifications to such systems have been made. The basic idea of such modifications aimed at maintaining optimum activity of inoculum over an extended period of release which was significantly hampered in case of liquid culture introduction methods. Various modifications include the use of certain carrier material which enhances the activity of microbes and also provides nutrition to the growing microbial population (van Veen et al. 1997). Example of carrier materials includes charcoal-amended soil (Beck 1991), chitin or chitosan (Gentili et al. 2006; Chen et al. 2007), nylon (Heitkamp and Steward 1995), zeolite (Liang et al. 2009), and clay (Omar et al. 1990). A study on activated carbon and zeolite in the treatment of site contaminated with crude oil showed that these materials increased microbial growth and enhanced hydrocarbon degradation (Liang et al. 2009). This revealed that dehydrogenase activity was three times higher in activated carbon than in zeolite. Such an increase in overall activity can be attributed to biocarriers as they improve the diffusion of oxygen, nutrient uptake, and water retention capacity.

Other entirely different approaches primarily used for biodegradation of aromatic compound make use of immobilized cells. This method offers a protective environment to the inoculated microorganisms and provide protection from envi-