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Series Editor: Naveen Kumar Arora

Deepak G. Panpatte
Yogeshvari K. Jhala *Editors*

Microbial Rejuvenation of Polluted Environment

Volume 1

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Microorganisms for Sustainability

Volume 25

Series Editor

Naveen Kumar Arora, Environmental Microbiology, School for Environmental Science, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh, India

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Deepak G. Panpatte • Yogeshvari K. Jhala
Editors

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Deepak G. Panpatte Department of Agricultural Microbiology Anand Agricultural University Anand, Gujarat, India	Yogeshvari K. Jhala Department of Agricultural Microbiology Anand Agricultural University Anand, Gujarat, India

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Preface

Due to rise in global population, agriculture and industrialization increase at an astonishing rate which creates accumulation of pollutants in the environment. Excess loading of pollutants leads to scarcity of clean water and air as well as disturbances of soil, thus limiting agricultural productivity and thereby challenging food security. The key environmental pollutants include agrochemicals, hydrocarbons, heavy metals, dyes, greenhouse gases, and e-waste causing deterioration of environmental health. Microorganisms are wonderful gift of the nature and efficiently explored as the *Solution to Pollution.* Microorganisms have diverse metabolic activities enabling them to break down a wide range of organic pollutants and absorb inorganic substances, which in turn clean up the environment. Eco-restoration of polluted environment by microorganisms includes a variety of approaches such as biostimulation, bioaugmentation, biofilm formation, application of genetically modified microorganisms, and advanced molecular techniques for real-time monitoring of microorganism-mediated bioremediation. Microorganisms can convert the pollutants into nonhazardous and environmentally safe end products and restore the environment to its original state in an eco-friendly manner.

The book entitled *Microbial Rejuvenation of Polluted Environment Volume 1* has a major focus on environmental remediation by exploiting microorganisms for sustainable eco-restoration of polluted environment. Microorganisms are tiny invisible entities which can utilize almost everything. The book focuses on the role of different types of microorganisms including bacteria, algae, fungi, and even archea for mitigation of environmental stress along with a detailed discussion on the mechanisms of action. It also contains reviews and original research of reputed scientists to highlight the latest developments in microbiological research, to cope up with the problem of environmental pollution along with remediation strategies practiced at various stages for improvement of ecological health. This book will be a valuable resource for scientists working to develop mitigation strategies for environmental remediation, will serve as an inspiration and ready reckoner for students who want to pursue studies pertaining to bioremediation of the environment making them ready for future challenges, and also will serve as a single-source reference covering all categories of microorganisms for bioremediation of different pollutants in a well-situated and comprehensive package.

Anand, Gujarat, India	Deepak G. Panpatte
Anand, Gujarat, India	Yogeshvari K. Jhala

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About the Series Editor

Naveen Kumar Arora, PhD in Microbiology Fellow of International Society of Environmental Botanists (FISEB), is Professor and Head, Department of Environmental Science at Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow, Uttar Pradesh, India. He is a renowned researcher in the field of environmental microbiology and biotechnology. His specific area of research is plant-microbe interactions, particularly plant growth promoting rhizobacteria. He has more than 75 research articles published in premium international journals and several articles published in magazines and dailies. He is an editor of 25 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 4 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 advisor to 134 postgraduate and 11 doctoral students. He has been awarded for excellence in research by several societies and national and international bodies/ organizations. 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.

About the Editors

Deepak G. Panpatte has been working as a research scholar for the past 9 years. His main research interest is in agriculturally beneficial microorganisms, such as biofertilizers, biopesticides, and biodegraders. He has also pioneered the development of fortified biocontrol bacterial consortiums with phyto-extracts for the management of phytopathogenic nematodes and fungi. He has received five international conference awards for his research, one award for Best Ph.D., and the Rashtriya Gaurav Award for outstanding contributions to agriculture. He has published 14 research papers, 5 books and 16 book chapters with Springer, 1 practical manual, 33 popular articles, and 2 editorials. He is also an editorial board member for several respected national and international journals.

Yogeshvari K. Jhala is an Assistant Professor with 11 years of teaching and research experience. Her main research interest is in agriculturally beneficial microorganisms such as biofertilizers, biopesticides, and biodegraders. She was the first researcher worldwide to report on five unique strains of methanotrophic bacteria. She has received the All India Best Research Award and Young Faculty Award for her outstanding research on methanotrophic bacteria. Her publications include 17 research papers, 5 books, 12 book chapters, 2 teaching manuals, 24 popular articles, and 2 editorials.

Chapter 1

Rhizosphere: Niche for Microbial Rejuvenation and Biodegradation of Pollutants

M. Gomathy, K. G. Sabarinathan, K. S. Subramanian, K. Ananthi, V. Kalaiyarasi, M. Jeyshri, and Pranab Dutta

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Abstract Several research works have been carried out in the rhizosphere that gives a clear picture and better understanding of the rhizospheric microbes that avid the research interests of many scientists. Rhizosphere was found to be the better environment and hotspot for the microbes for rejuvenation as it is rich in nutrients needed for the microbial growth. Among the nutrients, root exudates influence the

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M. Gomathy (✉) · K. G. Sabarinathan · K. S. Subramanian · K. Ananthi · V. Kalaiyarasi · M. Jeyshri
Tamil Nadu Agricultural University, Agricultural College & Research Institute, Killikulam, Vallanadu, Tamil Nadu, India

P. Dutta
Central Agricultural University, Umiam, Meghalaya, India

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root zone by changing the oxidation reduction potential, enhancing the availability of moisture and nutrients, providing better niche for the growth of plant growth promoting organisms, producing antibiotics, other secondary metabolites and growth regulators, microbial interactions, sheltering the microsymbionts, model system for the study of soil-rhizosphere biology, drought, avoiding soil erosion, etc. Exploring these rhizospheric microorganisms by unravelling their possible relationships with plants has launched a new and fascinating area of investigations in the rhizosphere research. Moreover, the rhizospheric microbes are considered as bioindicators of soil quality that are affected by much of the pollutant that makes the soil infertile. Rhizosphere region that harbours abundant microbes can remediate the polluted soils in a much greener way. Microorganisms and their metabolites like enzymes were involved in bioremediation process. Hence, bioremediation is a much auspicious method to overwhelm the pesticide pollution that can surely solve the problem of pollution in soil.

Keywords Rhizosphere · Microorganisms · Rejuvenation · Bioremediation

1.1 Rhizosphere

Rhizosphere is the nutrient-rich soil region surrounding the root. The term was coined by Hiltner in the year 1904, and he only introduced the concept of rhizosphere first to describe the narrow zone of soil surrounding the root where microbes and their populations are stimulated by root activities. This rhizosphere seems to be a complex environment where the interaction between the plant and the soil microbes are interdependent and highly interacting. However, the original concept has now been extended to include the soil surrounding a root in which physical, chemical and biological properties have been changed by root growth and activity. The discharges of roots will attract many microbes and help the microbes to colonize around the root system is known as rhizosphere effect (Hiltner 1904), and he also observed direct proportional relationship between the population of microorganism around the root system to the yield. Many microbes such as bacteria, fungi and actinomycetes rhizosphere include mycorrhizal fungi, PGPR, biocontrol agents, mycoparasitic organism and antibiotic produces among which many of them play important role as N fixers, P solubilizers, K mobilizers, etc. are onus for the rhizosphere dynamics.

Rhizosphere also known as the warehouse of microbes where the biochemical secretions are influenced by the roots. Rhizospheric bacteria help the plants in various ways through the secretions (Kundan et al. 2015). The composition of the root exudates varies depending on the plant species and physicochemical properties that help the plants to attract many microbes (Kang et al. 2010). Three niches were identified to be the hot spots of root exudates, namely rhizospheric soil, rhizoplane (surface of the root) and the root itself. These regions were profound to have root exudates and can be coined as a fertile zone of the soil. Due to this immense fertility of the soil, microbes are attracted and helpful for the growth and reproduction. Among the microbial population, bacterial population is found to be huge and have the symbiotic or nonsymbiotic relationships, and this microbial community differs based on the texture of soil (Raynaud et al. 2007; Bulgarelli et al. 2013).

Since root exudate composition changes along the root system, according to stages of plant development and plant genotypes, the rhizomicrobiome composition differs accordingly (Bouffaud et al. 2012). In the rhizospheric region, the growth of plants and microorganisms is mutually influenced by the secreted molecules.

The rhizodeposits referred as exudates of plant roots which include amino acids, fatty acids, organic acids, plant growth regulators, carbohydrates, putrescine, nucleotides, sterols, phenolics, polypeptides, polysaccharides, water soluble sugars, sugar phosphate esters and vitamins (Uren 2000). Some of the root exudates act as repellents to microbes and other insects; hence, the nature of exudates depends on the plant species from which it exuded (Kamilova et al. 2008). Several distinct groups of microorganisms are found in the rhizosphere, and these were inducing the growth of plants through the liberation of the above-said chemicals into the rhizosphere (Kundan et al. 2015). Plant growth–promoting rhizobacteria termed as PGPR also dwell in the rhizospheric environment and promote the activity of plants through continuous supply of nutrients to crops (Davison 1988), release of phytohormones to manage or reduce the activity of plant pathogens, to improve soil texture, bioaccumulation, etc. (Ehrlich 1990). As rhizosphere is an exceptionally nutrient dense region compared to nonrhizosphere, it not only is flourished with root exudates but also have dead tissues of plants, animals, proteinaceous mucilage secretions, carbonaceous compounds that obtained from plant roots, etc.

1.2 Niche for Rejuvenation of Soil Microorganisms

As the rhizosphere is the bowl full of essential and important storehouse of organic nutrients, microbes are invigorated and growing under continuous supply of nutrients. They multiply in an exponential rate and releasing primary and secondary metabolite that are an added advantage for the plant growth. In addition to the secreted organic compounds, the organic and inorganic amendments added to the soil for crop growth also influences the growth of microbes in the rhizosphere region. Quite interestingly, microbes that lives in the nonrhizosphere region when it faces deprival of nutrients can sense the availability of nutrients in the rhizosphere and move towards the rhizospheric region through quorum sensing. These signalling processes help the *Rhizobia* to move towards the root tip of leguminous plants (via detection of the flavonoid signal produced by the plant system), to initiate the nodulation process in the soil that has low nitrogen concentration.

Rhizospheric microbes directly or indirectly influence the plant productivity as their richness in the below ground is the indicator of above-ground wellness in various environmental conditions. The production of antibiotic compounds bound to have effect on pathogenic microbes, defence against protozoa, motility, and biofilm formation.

Several interesting metabolites were produced by these rhizospheric microorganisms such as volatile organic compounds (VOCs) that alter and modulate the crop growth and involved in signalling process. They help in the long-distance communication because of its high vapour pressure and small in size that diffused through water and soil pores. These volatiles also proved to arrest the growth of fungal plant pathogens.

Several research works have been carried out in the rhizosphere that gives a clear picture and better understanding of the rhizospheric microbes that avid the research interests of many scientists. The root exudates influence the root zone by changing the oxidation reduction potential, enhancing the availability of moisture and nutrients, providing better niche for the growth of PGPR organisms, producing antibiotics, other secondary metabolites and growth regulators, microbial interactions, sheltering the microsymbionts, model system for the study of soil-rhizosphere biology, drought, avoiding soil erosion, etc.

Science always have two schools of thoughts, crop plants may alter and choose the microbes needed in the rhizosphere for its own benefit and yield while the other researches stated that root exudates are kind of products that overflow out of roots, and further research has to answer postulations.

For growth and reproduction, microbes need water, nutrients, and space that is enormously available in the rhizospheric region and well utilized for doubling its population to a greater extent. The primary and the first speech of signals were exchanged when the seeds start its germination and the seedlings put forth root and shoot. The developing plant interacts with a wide array of microbes present nearby and invites through the release of organic materials that act as a driving force for the development and active growth of microbes. Once after the release of these organic materials by the root the compounds present in the root were subjected to microbial attack and make it difficult to separate from the roots. The efficiency of this exudation is governed by various factors such as nutrient deficiency, temperature, soil type, light intensity, soil pH, microbial existence, plant species and its developmental stage, etc. (Singh et al. 2006). Even though the rhizosphere is abundant with all types of nutrients there, the presence and dominance of the individual organism fluctuate to prove the concept of survival of the fittest. The effect of the root exudates travels to a certain distance as far as the diffusion and the distribution pattern spreads. Studies of molecular fingerprints in different root zones showed that the community composition altered in rhizoplane, emerging roots even in root tips, lateral roots, older roots, etc.

Alterations in the rhizosphere due to depletion of nutrients cause tidal waves in the existing population and death, and lysis of bacterial cells occurs. The exponential growth occurs by the release of nutrients via decay of tissues and cells. Saprophytes flourish in the rhizospheric soil and do the vital processes of decomposition of organic residues and helpful for the nutrient mineralization, turnover processes, and soil dynamics. Carbon flow and its availability are greatly influenced in the rhizospheric region as 12–40% of the total amount of carbohydrates prepared during photosynthesis released into the rhizosphere.

Compared to bulk soil, rhizosphere is the main place where higher amount of conversion of the extracellular compounds such as glucose to gluconic acid and 2 keto gluconic acid occurs. *Pseudomonas* sp. can effectively perform the above conversion to sequester glucose and create a competition over other microbes that need glucose. Competition happens not only for sugars but also for micronutrients such as zinc, manganese, molybdenum, iron, copper, etc. The niche of rhizosphere has phytohormones such as IAA, auxins, cytokinins, and gibberellins secreted by the plants as well as by the microbes that truly enhances the plant growth and the root architecture that further increased the production of exudates.

Rhizospheric microbiome very particularly influences the nutrient status of soil and nutrient uptake of the plants. The best-known miracle doers are known to be the very famous Rhizobium and AM fungi for its nitrogen fixing and phosphorus uptake, respectively. These AM fungi are the important symbionts for translocation of nutrients and minerals, maintaining the soil structure, forming soil aggregates, suppressing soil-borne pathogens, etc. Rhizospheric microbes also influence the uptake of many trace elements such as iron, molybdenum, magnesium, boron, etc. AM fungi has been proven to uptake and enhance the Fe and Zn concentration in chickpea (Pellegrino and Bedini 2014) and maize (Subramanian and Balakrishnan 2013).

Addition of organic amendments into the soil provides abundant carbon and nutrients that are readily available to microorganism for its growth. This was supported by the observation that composting of plant residues with more labile organic matter resulted in higher soil microbial biomass and respiration (Tejada et al. 2009). Besides, Wu et al. (2013) found that compost additions in soil increased the microbial biomass and it may be due to the higher availability of nutrients, labile organic matter, the increased water retention, and aeration (Hu et al. 2011; Duong et al. 2012; Wu et al. 2013).

1.3 Shaping the Rhizospheric Microbiome

Shaping of the rhizospheric microbiome is an important hot topic in the growing ómics' research as they decide many factors of the plant–microbe interaction. During the developmental stages of plant, the microbial communities prevailing in the rhizospheric zone, their functions and pathways in which they are undergoing breakdown of metabolites differ. Barret et al. (2011) have discussed many molecular approaches for the gene expression pattern in the rhizosphere. Studies on in vivo expression technology (IVET) revealed that when the microbes are colonizing in the rhizosphere, different genes and proteins were induced such as the genes for nutrient absorption and stress response. Whereas some proteins which are involved in environmental sensing, metabolic regulation and membrane transport were expressed in *R. leguminosarum*. To analyse the specific processes in the rhizosphere, study of reporter genes were successfully employed by many researchers to find out the production of antimicrobial compounds, response of bacteria to nutrient availability (nitrogen, carbon, phosphorus), availability of water and temperature.

To evaluate the actual effect of root exudates on gene expression of microbes, Mark et al. (2005) studied whole genome transcriptome profiling in *P. aeruginosa* and found significant alterations happened due to the root exudates in sugar beet cultivars. Metaproteogenomic approach was reported by Wang et al. (2011) to reveal the complex interactions of plants and rhizospheric microbiome. MALDI-TOF analysis reported 189 proteins in the rhizosphere of rice that actually originated from plants, bacteria, fungi, and other faunas. Interestingly, by applying stable isotope probing (SIP), scientists found that plant-derived carbon utilized into microbial nucleic acids could be tracked to explore the metabolically active population of rhizobacteria. Exciting information was obtained when the plants like wheat, maize and clover exposed to $^{13}CO^{2-}$ through DNA-SIP technology. Biomarker studies of phospholipid fatty acids revealed that not only bacteria but also rhizospheric fungi metabolized remarkable quantities of root exudates and confirmed through $^{13}CO_2$-exposed plant study. So the provision of simply degradable root exudates in the rhizosphere region also invites diversity of fungi to thrive in.

These fungi not only merely present in the rhizosphere, but it changes the community dynamics by influencing and flourishing especially during flowering and senescence of potato crop. Drigo et al. (2010) clearly indicated that through DNA SIP studies that plant assimilated carbon is quickly transferred to AM fungi that in turn slowly released to the rhizospheric bacterial and fungal communities and pointed that combined approach in the rhizosphere is always found to be a powerful tool to get the ultimate crop response.

Nowadays, the population growth and industrialization totally affected the global ecosystems and 39% of terrestrial biomass (Ellis et al. 2010). Urbanization occupied the cultivated land that lead scarcity of crop production, to produce more food in a shorter period. Hence the farmers tend to use agrochemicals to produce high yields in the small cultivated area. This will happen in developing countries too (Lichtfouse et al. 2010). In the Green Revolution, the inorganic fertilizers and pesticides were applied to produce foster food production in a shorter period of time (Shelton et al. 2012).

The liquid wastes from industries have heavy metal contamination that also affects the soil fertility, water quality, plant growth and overall environmental degradation and finally causes serious threat to human health (Oves et al. 2012). In worldwide 22 million hectares of soil are highly affected by chemical pollution especially in Europe and Asia (Bai et al. 2008). Pollution of ecosystem is the major and emerging problem in the twenty-first century. A number of methods are available to meet food requirements without affecting environment, for this purpose microbes play a vital role to ensure the food security during climatic change (Timmusk et al. 2017).

Recently, the agriculture and industries have released lot of chemical wastes as xenobiotics, which is very harmful to human growth, crops, livestock and wild life. Various methods like bioremediation offer to destroy the harmful things by using the natural materials (Fulekar 2014). Bioremediation and phytoremediation are widely emerging technologies used to eliminate the contaminants from soil and water (Raskin and Ensley 2000). The microbial products also help to destroy the pollutants from soil (Vidali 2001). Microbial metabolites like proteins and enzymes are used to breakdown the contaminants from soil through the mutualistic relationship with the plants (Fulekar 2014).

1.4 Plant Physiological Effects on Rhizosphere Enzyme Activity

Root is a major vegetative organ that supply water, minerals and substances essential for plant growth and development. Roots are believed to be the primary source of the growth regulators gibberellins and cytokinins, which influence the overall plant growth and development. The rhizosphere is a unique hotspot in soil from the viewpoint of microbial ecology, as soil microorganisms are considerably stimulated by the activity of the roots.

Increased soil temperatures, elevated atmospheric carbon dioxide and more frequent wetting and drying cycles (water stress) will change microbial community composition and possibly increase biomass and enzyme activities either directly or stimulation of plant growth and increases in litter deposition and root exudation. The climate is changing as the concentrations of CO_2 and other greenhouse gases in the atmosphere increase, resulting in global warming and altered precipitation patterns. Because the activities of enzymes in natural environments are controlled by both abiotic factors (e.g. temperature, water potential and pH) and biotic processes (e.g. enzyme synthesis and secretion), they are likely to be responsive to atmospheric warming and more frequent and extreme variations in precipitation patterns. These changes will have important consequences for ecosystem functions such as decomposition, nutrient cycling and plant microbe interactions, which will ultimately affect plant growth and productivity.

The study of different hydrolase enzyme activities in the rhizosphere soil and their changes is important in plant growth and development. Since they indicate the potential of a soil to carry out specific biochemical reactions, and these hydrolytic enzymes are important in maintaining soil fertility and plant productivity. Because plant nutrient uptake occurs through the rhizosphere, the activity of rhizosphere microbial community is of great importance for plant growth.

Soil enzymes are involved in the catalysis of a large number of reactions necessary for life processes of microorganisms in soils, decomposition of organic residues, cycling of nutrients, formation of organic matter and soil structure. These enzymes include amylase, arylsulphatases, beta glucosidase, cellulase, chitinase, dehydrogenase, phosphatase, protease, urease and others, derived from plant, animal or microbial origins (Gupta et al. 2016). These enzymes can be accumulated, stabilized and or decomposed in the soil.

Lignin degradation is principally an oxidative process catalysed by enzymes broadly categorized as phenol oxidases, peroxidases and dehydrogenases. Phenol oxidases are enzymes that oxidize phenolic compounds using oxygen as an electron acceptor. Peroxidases have heme prosthetic groups that use H_2O_2 as an electron acceptor. With redox potentials up to 1490 mV, they can oxidize lignin linkages either directly or through redox intermediates such as Mn^{3+}. The third group of ligninases, the dehydrogenases, are primarily intracellular oxidative enzymes that transfer hydride groups from a substrate to an acceptor such as NAD^+. They are generally considered substrate-specific but play a key role in the decomposition process, particularly for bacteria. However, at least a few bacteria, e.g. Sphingomonas, depolymerize lignin extracellularly using dehydrogenases. The extracellular oxidative enzymes associated with the degradation of recalcitrant plant and microbial components include saccharide-oxidizing enzymes such as glyoxal oxidase, galactose oxidase and glucose oxidase that reduce oxygen to H_2O_2 in support of peroxidase activity; and cellobiose dehydrogenase, which reduces phenoxy radicals, quinones and metal cations, contributing to the supply of redox mediators.

1.5 Role of Enzymes and Plant Growth Regulators

Plant growth–promoting substances, regulators such as auxins and gibberellins, are present in root exudates and thus enter the rhizosphere. Auxin is the generic term for growth substances that typically stimulate cell elongation, while IAA (indoleacetic acid) is recognized as the principal auxin in plants. The level of auxin is usually higher in the rhizosphere than in the free bulk soil, a consequence of an increased microbial population or of accelerated metabolism owing to the presence of root exudates. A large number of gibberellins have been isolated from bacteria, fungi and ferns and identified as GA-like substances. The best known GA response is the stimulation of internode growth.

Microorganisms present in the rhizosphere of various crops appear to have a greater potential to synthesize and release plant growth substances as secondary metabolites because of the rich supply of substances, and it is an important factor in soil fertility. According to several reports, 86% of the bacterial isolates from the rhizosphere of various plants produced phytohormones such auxins, gibberelins and kinetin-like substances, but also different hydrolytic enzymes such protease, lipase, pectinase and amylase.

Acid and alkaline phosphatase activities in wheat rhizosphere were strongly correlated with the depletion of organic P. Protease activity is involved in the hydrolysis of N compounds to NH_4, using low-molecular-weight protein substrates, and microorganisms are responsible for breaking down urea into ammonium. Urease enzyme is responsible for the hydrolysis of urea fertiliser applied to the soil into NH_3 and CO_2 with the concomitant rise in soil pH.

In earlier studies on plant growth regulators, the activities of rhizosphere bacteria including nitrogen fixation, production of cytokinin, auxin or hydrolytic enzymes such protease, lipase, pectinase and amylase increased the N, P and K uptake of plant components.

1.6 Common Source of Pollutants in Soil

Soil pollution is one of the worldwide problems, which is caused due to the assimilation of toxic compounds through the discharge of industrial waste into the soil, salts due the application of pesticides, herbicides and fertilizers, seepage from landfills, solid waste and radioactive materials affecting plant and animal growth.

The agriculture mainly depends on main factors, namely organic inputs and inorganic fertilizers and pesticides. Vehicles also release some major sources like petroleum hydrocarbons, dioxins and polycyclic aromatic hydrocarbons that affect soil health. Industrial wastes are disposed through the pits and affect the groundwater supply.

1.7 Biological Degradation of Pollutants

Microorganisms are omnipresent which are distributed widely because they can easily grow and multiplied, for their nutritional requirements, they can degrade the pollutants and wastes for their energy. Biodegradation of pollutants are called as bioremediation. Some microbes convert, modify and finally utilize the toxics for their survival (Tang et al. 2007).

Instead of collecting pollutants, bioremediation is a process applied to break down and transform the heavy molecules in to simple things like less toxic or nontoxic compounds (Strong and Burgess 2008). Different types of biological processes include bio-attenuation, biostimulation, bioaugmentation, Bioventing, Biosparging and biopiles.

1.7.1 Principles of Bioremediation

Bioremediation is defined as the process whereby biological organisms are used to break down hazardous substances into less toxic or nontoxic substances. Microbes in the environment are much suited for this purpose to destruct the contaminants by the secretion of several enzymes, namely oxidoreductases, hydrolases, lyases, transferases, isomerases and ligases that convert the pollutants into harmless unpolluted products. The microbes involved in bioremediation processes are encouraged, and the purpose has been improved by the supply of continuous nutrients and other chemicals which triggered the reaction at optimum conditions (Kumar et al. 2011). This is a naturally occurring process which encourages the waste products into carbon dioxide, water and other inorganic compounds that are safe for animals, plants, human and aquatic living things (Jain and Bajpai 2012). The degradation of pesticides through bioremediation is an important process to remove dangerous toxic chemicals and reduce the environmental pollution. Bioremediation is more effective, eco-friendly and versatile to remove the pesticide from the fertile lands (Finley et al. 2010).

Biostimulation

Biostimulation is the technique of enhanced bioremediation along with bioaugmentation where specific native of non-native microorganisms are introduced with an aim to enhance the biodegradation of target compound or serving as donors of the catabolic genes. In enhanced bioremediation process, the microbial population in the environment will be stimulated and modified by the addition of various nutrients such as carbon, phosphorus and nitrogen in the form of organic substrates (Nikolopoulou and Kalogerakis 2008).

Bio-Augmentation

Bio-augmentation is the process to degrade contamination by adding excess amount of bacterial inoculum. The soil sediment contains lot of microbes which is adapted to pollutants. Two percent bioremediated soil is used to facilitate biodegradation of polyaromatic hydrocarbon compounds (Lamberts et al. 2008).

Bioventing

It is one of the first in situ treatments applied to degrade the oil spills and petroleum products. The gases and nutrients are applied through the small wells at very low level air flow rates to minimize the volatilization of petroleum products and hydrocarbons. Bioventing method induces the aerobic biodegradation in the subsurface bacteria leads to improve the subsurface bioremediation. Bioventing is the cost-effective method to clear subsurface contaminants, and also highly effective in colder and dried areas (Robinson et al. 2011).

Biosparging

In this method, the air is supplied under pressure to the groundwater table to increase the groundwater oxygen concentrations and to improve the rate of biodegradation of pollutants using bacteria (Adams and Ready 2003). Biosparging helps to increase the mixing of saturated zone and thus increase the interaction between the groundwater and soil. This method is very easy and low cost, requires small diameter injection point to supply the air to the pollutant areas to reduce the petroleum components mixed with the groundwater.

It is more effective to reduce petroleum products to the underground storage tank sites (USEPA 2004). It is very similar to bioventing for the remediation of soils from heavy metals.

Biopiles

Biopiles is one of the cleanup techniques where the excavated soil materials are mixed with hydrocarbons which is treated with biodegradation by oxidation process by the injection of oxygen. The oxidation process increases the availability of microbes in soils. The contaminated materials are excavated which is further mixed with sawdust, sand, compost, nutrients, wood chips, etc. These things improve the moisture retention, allow the permeability of microbes and stimulate the biological reactions very fast and to oxidize the hydrocarbons. Biopiles are also called as compost piles, biomounds, biocells, and bioheaps (Delille et al. 2008).

1.8 Microorganisms and Pollutants

The microorganisms present in the rhizosphere soil can transform the pollutants from one oxidation state to another. Microbes protect its own structure from metal toxicity through various mechanisms, namely oxidation, reduction, methylation, adsorption, etc. Methylation is an important method that play important role in bioremediation. For example, mercury, bioethylated by a number of bacterial species, namely *Alcaligenes faecalis*, *Bacillus pumilus*, *Bacillus* sp., *P. aeruginosa* and *Brevibacterium iodinium* to gaseous methyl mercury. Environmental factors also play an important role for the growth and activity of microbes to enhance bioremediation (Vidali 2001). The long-term application of pesticides can also promote biodegrading enzymes in the indigenous microflora, as they serve as a source of carbon and energy, making the remediation of pesticide contaminated sites easier (Qiu et al. 2009).

1.8.1 Degradation of Pesticides by Rhizospheric Microbes

Rhizospheric microorganisms are universal scavengers for decaying or recycling the waste materials into harmless things. It includes bacteria, fungi and actinomycetes which are able to eliminate the pesticides from the environment (Parte et al. 2017). Many researchers reported that soil microorganisms such as *Burkholderia*, *Arthobacter*, *Aztobacter* and *Flavobacterium* degraded the pesticides (Shi and Bending 2007). Single microbe can degrade more than one herbicide and pesticide and also involved in plant growth promotion and zinc and phosphorus solubilization.

Staphylococcus sp. and *Bacillus circulans* isolated from the surrounding area of pesticide production industry degraded 72–76% of endosulfan under aerobic and facultative anaerobic conditions (Kumar and Philip 2007). In the chemical industry, various chlorinated compounds are used as the industrial solvents and degraded by *Aminobacter* and *Mesorhizobium* sp. by the secretion of enzymes (Osborn et al. 2010). Organophosphorus pesticides were degraded extensively (Singh 2008).

Acinetobacter sp., *Serratia* sp., *Proteus vulgaris* and *Vibrio* sp. are able to degrade dichlorvos by the excretion of several enzymes (Agarry et al. 2013).

Pseudomonas species are efficient to degrade profenofos (Malghani et al. 2009), and *Xanthomonas* sp. and *Pseudomonas* sp. were obtained from its source of carbon and nitrogen from chlorpyriphos and 3,5,6-trichloro-2-pyridinol under in vitro conditions (Rayu et al. 2017). Similarly, *B. thuringiensis* degrades cyhalothrin and pyrethroids (Chen et al. 2015).

Pseudomonas putida and *Acinetobacter rhizosphaerae* degraded organophosphate fenamiphos (FEN) and hydrolysed fenamiphosphenol, and both the strains are obtained C and N from FEN (Chanika et al. 2011). Rhizospheric microbes exposed to agrochemical environment for quite a longer time become resistant to that particular environment. Hence, these kinds of microbes are used as bioremediation of pesticides (Khan et al. 2009). The resistant microbes utilize the pesticides as their energy source (Reddy et al. 2016).

P. aeruginosa G1, *Stenotrophomonas maltophilia* G2, *B. atrophaeus* G3, *Citrobacter amolonaticus* G4 and *Acinetobacter lowffii* G5 are able to degrade the organochlorine, endosulfan (Ozdal et al. 2016).

Biopesticide activity of *Penicillium raistrickii*, *Trichoderma* sp., *Aspergillus sydowii*, *Penicillium miczynskii*, *Bionectria* sp. and *Aspergillus sydowii* was studied using solid and liquid medium at the concentration of 5, 10 and 15 mg of dichloro diphenyl dichloroethane (DDD). Among the organisms tested, *Trichoderma* degraded the pollutant efficiently (Ortega-Gonzalez et al. 2015). In vitro condition results stated that among the sugar sources tested, glucose was found to be the preferred source that speeds up the biodegradation process of *Sphingobacterium* sp. (Fang et al. 2014).

Fungi are also involved in the degradation of organochlorine pesticides. Siddique et al. (2003) identified that along with bacteria, fungi also isolated from soil that degraded 84–91% of isomers of endosulfan. Okeke et al. (2002) isolated *Pandoraea* sp. from soil slurry of biodegradation of hydrocarbons. The following fungi such as *P. acanthocystis* (90%), *P. brevispora* (74%), and *P. tremellosa* (71%) removed the heptachlor from soil by the hydrolysis and hydroxylation processes (Xiao et al. 2010). Rousidou et al. (2016) identified four oxamyl-degrading bacteria by multilocus sequence analysis (MLSA) and found they belong to genus *Pseudomonas*. They can also reutilize methylamine as C and N sources that possess methylamine dehydrogenase enzyme which is similar to carbamate hydrolase gene. He et al. (2006) isolated *Penicillium* sp. from herbicide production unit soil sample which degraded metsulfuron methyl in soil and water.

Several studies showed that several organisms degrade pesticides, herbicides, organophosphates and carbamates (Dinamarca et al. 2007). Yang et al. (2006) isolated *Stenotrophomonas* sp. from solid waste water of organophosphorus pesticide manufacturing unit that degraded chlorpyrifos contaminated soil. Yuanfan et al. (2010) suggested that genetically modified organism persist the gene *mpd*, able to bioremediate multiple pesticides at once. Genetic engineering studies introduced methyl parathion (MP) degrading gene into *Pseudomonas putida* X3 which strongly degraded the soil contaminated with MP and Cd (Zhang et al. 2016). Diuron widely used herbicide in sugarcane fields, which is degraded by DP8-1 strain to the level of 99% diuron within 3 days under optimal condition. This strain also degrades the monuron, isoproturon, linuron, fenuron, metobromuron, chlorbromuron and chlortoluron (Wang et al. 2018).

Organic Pollutants

Various industries such as textile and dye industries release effluent waste called persistent organic pollutants (POPs) that affect the environment and human health, and these complex chemical compounds are named as xenobiotics which is removed from the environment through microbial degradation (Ahmad et al. 2018).

1.8.2 Dyes

Rhizospheric microbes, especially bacteria can effectively degrade all the xenobiotics and industrial wastes (Khalid et al. 2008a, b). Along with bacteria, fungi are also involved in the degradation of industrial effluents. Bacteria can degrade the dyes by the process called biosorption through the release of enzymes to digest the organic pollutants. Researchers identified that industrial effluent containing the dye called azodyes is degraded by the bacteria via enzymatic degradation or biosorption or combination of both (Wu et al. 2012). Bacteria are able to degrade the azodyes with an enzyme azoreductase enzyme, which possess strong bonding properties (Chen 2006). Several microbes are involved to digests the xenobiotic compounds, and during these reactions, bacteria can produce hydroxylase and oxygenase enzymes that act on the intermediate products, released during decolourization of xenobiotics (Khalid et al. 2009). Many researchers studied that fungi, yeasts and algae also involved in the digestion of industrial effluents (Olguín 2003). During the biodegradation, several factors (pH, temperature and salts) interfered in the degradation process (Prasad and Rao 2011).

The species of fungi, lignolytic mushroom *Lenzites elegans* WDP2 can decolorize the dyes viz. Brilliant green 93%, malachite green 21%, and Congo red 99% reported by Pandey et al. (2018). Paper mill water wastes are biodegraded by actinomycetes, bacteria and fungi (Hossain and Ismail 2015). *Bacillus cereus* and *Pseudomonas aeruginosa* are identified for degradation and decolourization of the papermill wastes (Tiku et al. 2010). *Pseudomonas putida* and *Acinetobacter calcoaceticus* are able to decolourize around 80% in the black liquor derived from the kraft pulp and papermills (Abd El-Rahim and Zaki 2005). *Microcystis* spp. removed 70% colour from the papermill effluents within 2 months (Sharma et al. 2014).

1.8.3 Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are originated from anthropogenic activities, highly organic pollutants and more carcinogens and mutagens. These compounds have influence on the microbial population. PAH assists as energy for microorganisms, and also it converts ineffective and not as much toxic compounds by the highly effective and expensive process of biodegradation (Anwar et al. 2016). PAH contaminations are difficult to degrade and persist in water for longer periods; hence, it effects the microbial population (Gałązka et al. 2018).

Among the microbes, bacteria is more effective in the degradation of PAHs in aquatic environments (Johnsen et al. 2005). The bacterial activity is less in soil- or sediment-based PAHs (Yuan et al. 2001), and in that particular cases, sludge-based degradation bacteria has to be introduced (Hwang et al. 2003). Dissolution and vaporization process make the degradable bacteria live than sorption process (Kim et al. 2007).

Uyttebroek et al. (2007) revealed that PAH degradation is mainly based on the soil age and its nutrient concentration. Wang et al. (2009) identified that nonspecific enzymes are not able to degrade the PAHs and remain in the soil for long time. Teng et al. (2010) studied that dihydriol is an oxygenated intermediate compound that helps for the degradation of anthracene by the presence of *Nocardia*, *Beijerinckia*, *Sphingomonas*, *Rhodococcus* and *Paracoccus*. Other than bacteria, many aerobic and anaerobic fungi species are also involved in the degradation of PAHs (Aydin et al. 2017).

Kadri et al. (2016) observed that many fungal species like *Phanerochaete chrysosporium* and *Pleurotus ostreatus* are able to produce lignolytic enzymes, namely laccase, Mn peroxidase and lignin peroxidase that degraded the PAH compounds. Jin et al. (2016) reported that plant growth–promoting rhizobacteria have the ability to degrade pyrene and other aromatic contaminants.

1.8.4 Microbial Detoxification of Heavy Metals

The entire ecosystem has been contaminated by heavy metals. They are toxic not only to humans but also to the microorganisms in the soil. Among the microorganisms, mycorrhizal fungi are the only ones which provide a direct link between soil and root of the crops (Gomathy et al. 2018a, b). Pollutants including heavy metals are detoxified by microbes in the presence or absence of plant system. Heavy metals are either beneficial or harmful to microbes (Ayangbenro and Babalola 2017). Some of the heavy metals like manganese, Fe, nickel, Mg, copper, chromium, cobalt and Zn are beneficial to microbes during the enzymatic reactions, redox reactions and stabilization of biomolecules (Bruins et al. 2000). Certain heavy metals like mercury, lead, antimony, gold, cadmium and silver are not involved in any biological functions and toxic to microbes at high concentrations (Bruins et al. 2000).

1.8.5 Phytoremediation of Heavy Metals

Phytoremediation is the process where the plants are involved in cleaning up the contaminants from soil (Ojuederie and Babalola 2017). To speed up the reaction, scientists discovered that rhizospheric bacteria helped the plants to uptake the heavy metals in a faster rate (Kuffner et al. 2008). *Flavobacterium*, *Pseudomonas*, *Streptomyces*, *Agromyces* and *Serratia* were observed in rhizosphere regions and reported to absorb Zn and Cd (Ghasemi et al. 2018). ACC deaminase activity in bacteria can induce heavy metal stress tolerance in crop plants, and also it enhanced the phytoextraction and phytoremediation in plants. Rodriguez et al. (2008) isolated four bacterial strains from Ni-contaminated soil based on ACC deaminase activity.

Endophytic bacteria is also involved in the process of metal stress tolerance in crop plants. Sheng et al. (2008) identified two heavy metal–resistant bacteria, namely *Pseudomonas fluorescens G10* and *Microbacterium* sp. *G16*, from the root of canola plants which grow in Pb-contaminated areas. The microbes mentioned above were resistant to heavy metals and improved the growth of canola in pot experiment. Bioremediation is essential for the detoxification of heavy metal–polluted environments and to prevent the toxic effects from the environment and organisms (Emenike et al. 2018a, b).

1.9 Mycoremediation

Mycoremediation is the term coined by Stamets. It is a kind of bioremediation using fungi to digests and eliminate the contaminants from the environments followed by repair or retain the nutrients in the environments. Mycofilteration is the process to filter the toxic waste and microbes using fungal mycelia by the secretion of enzymes. Fungal mycelium secrets several enzymes and acids which break the lignin and cellulose (Stamets 2005). The Mycorrhizal fungi can secrete a protein called glomalin that stabilized the aluminium occurred in the soil, when planted with Gmelina plants (Dudhane et al. 2012). Say et al. (2003) revealed that the following fungi species are identified as they are involved in the mycoremediation process to recover the plants from pollution. *Aspergillus niger, Aureobasidium pullulans, Cladosporium resinae, Funalia trogii, Ganoderma lucidum, Penicillium* spp. (Loukidou et al. 2003). *Aspergillus fumigates* is the suitable strain used to remove Pb(II) ions from the aquatic solution. AM fungi have the wider mycelia network, and they release glomalin protein that has the ability to sequester all types of heavy metals and renders a metal-free environment to the root zone (Gomathy et al. 2018a, b). Glomalin protein released by the AM fungi has the ability to sequester the metals in their cell wall.

1.10 Cyanoremediation

It is the process to remediate metals in the environment using cyanobacterial or blue green algae (BGA). This controls the heavy metals using either wild or genetically engineered cyanobacteria (Yin et al. 2012). This blue green algae help to remediate the arsenic from the aquatic environments. BGA prefer to remediate the heavy metals from aquatic and wetland ecosystem (Fiset et al. 2008) especially agricultural rice cultivated areas (Tripathi et al. 2012).

Deng et al. (2007) studied that green algae *Cladophora fascicularis* used to eliminate Pb(II) from waste water. Lee and Chang (2011) estimated the biosorption capacity of Cyanobacteria species and found *Spirogyra* and Cladophora removed the Pb and copper from the aquatic environment. Mane and Bhosle (2012) observed that *Spirogyra* showed the maximum biodegradation of metals from the environment Cu (89.6%), Cr (98.23%), Mn (99.6%), Fe (99.73%), Se (98.16%) and Zn (81.53) and in case of *Spirulina* sp. Cr (98.3%), Fe (98.93%), Se (98.83%), Cu (81.2), Se (98.83) and Zn (79%).

1.11 Factors Affecting Bioremediation

Rhizospheric microbes react on the pollutants through the secretion of various catalysts based on the wastes. Bioremediation reactions depend on various factors that include nature of pollutants, chemical concentration of pollutants, physicochemical properties of wastes, environmental characters and availability of microbial numbers. In the environment, the wastes and biodegradable microbes are not equally present; hence, for this purpose, controlling and optimizing of bioremediation is the complex process due to many factors including pollutions, microbial contents and environmental factors, viz. temperature, pH, soil, electron acceptors, presence and absence of oxygen and nutrients.

1.12 Conclusion

Studying the rhizospheric microbial diversity in a wide array of plant root system is a major struggle for research involving plant microbe interactions as it is quite difficult to answer specific community structures, how the particular community interacting with other microbes, influence of biotic and abiotic stress conditions and their alteration towards the rhizospheric microbes, etc. While considering the beneficial microbes in the root system, it conveys that the root exudates and other nutrients discussed in this chapter certainly influence the presence of beneficial microbes in the rhizospheric region and their interaction with the plant system. Rhizospheric microbes are highly beneficial in nutrient solubilization, mobilization, providing plant growth hormones, remediating the soil, improving the soil health, etc. Rhizospheric microbes are vital in bioremediation process. Further studies have to explore the molecular mechanisms behind the metal tolerance of many microbes in the root region. So the role of rhizospheric microbes are inevitable, and they will rejuvenate themselves by the help of elixir given by the roots, and they will continue their job of doing wonders and challenge the researchers all over the world to explore the rhizospheric region.

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Chapter 2
Bioremediation of Pesticides: An Eco-Friendly Approach for Environment Sustainability

Anju Sehrawat, Manisha Phour, Rakesh Kumar, and Satyavir S. Sindhu

Contents

A. Sehrawat · M. Phour · R. Kumar · S. S. Sindhu (✉)
Department of Microbiology, CCS Haryana Agricultural University, Hisar, Haryana, India
e-mail: sindhuss@hau.ac.in

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Abstract Various pesticides including organochlorines, organophosphates, carbamate, pyrethroids, chloronicotinyl etc., are used in agriculture for protection against plant diseases and insects. Only a fraction of the applied pesticides is utilized in killing of target pests and the leftover residual pesticides either remains associated with cereal grains, vegetables, and fruits or may cause environmental pollution. In addition to the traditional physical and chemical degradation methods, the microbial degradation method is commonly more efficient and low-cost method used for pesticide degradation. Microorganisms have been characterized which have the capability to degrade residual pesticides. The microbes that demolish these pesticides use the pesticides as nutrients and break them down into tiny nontoxic molecules. Pesticide degrading microbes belong to different microbial groups, i.e., bacteria, fungi, actinomycetes, and algae. Bacteria possessing pesticide degradation capability include *Pseudomonas* spp., *Bacillus* spp., *Burkholderia*, *Klebsiella* spp., *Streptomyces*, etc. and the fungi include *Trichoderma* spp., *Aspergillus* spp., *Phanerochaete chrysosporium*, white rot fungi, etc., whereas algae include *Chlamydomonas* and marine *Chlorella*. Major reactions in pesticide destruction include mineralization and co-metabolism. Pesticide degradation is influenced by many factors such as type of pesticide, type of microorganism, temperature, humidity, and acidity in the environment. Plasmid-located genes usually encode many enzymes and degrade a large number of pesticides. Microorganisms may acquire pesticide-degradation capabilities in soil through horizontal gene transfer from degradative plasmids, by modification of substrate specificity, or through altered regulation of preexisting enzymes. With the progress of molecular biology, the genetically engineered rhizobacteria may be built to enhance the bioremediation of pollutants and pesticides. Such recombinant microbial populations may be of immense value in bioremediation of diverse pesticides from the surroundings.

Keywords Pesticides · Microbial degradation · Mineralization · Co-metabolism · Genetically engineered rhizobacteria

2.1 Introduction

Currently, various pesticides are extensively applied in agriculture to target pests, weeds, and pathogens to protect crops in order to obtain high biomass and yield productivity (Cycoń et al. 2017; Sindhu et al. 2018). In developing countries, synthetic pesticides are widely used to control plant pathogens, weeds, and insect pests. Most widely used pesticides include phorate, simazine pendimethalin, malathion, glyphosate, carbofuran, chlorpyrifos, endosulfan, diazinon, methyl parathion, mancozeb, and carbendazim (Moneke et al. 2010). Fifty-seven thousand metric tons of chemical pesticides was used in India, while only 6340 metric tons of bio-pesticides was consumed during 2016–2017 (www.ppgs.gov.in/divisions/pesticides-monitoring-documentation). Usually, very low fraction (only 10–15%) of the applied pesticides are utilized in killing of target pests, and the leftover residual pesticides either leach down in soil or remain associated with grains, vegetables, and fruits (Sogorb et al. 2004; Jiang et al. 2019), which became a global pollution problem (Wang et al. 2016a; Rayu et al. 2017). Insecticides, especially organochlorine and organophosphates, enter any fresh water bodies through agricultural run-off (Karunya and Saranraj 2014). Many recalcitrant pesticides accumulate in the soil and migrate through the soil, into various environmental components such as air and surface water, directly or indirectly endangering human health and the environment (Bisht et al. 2019).

Chlorinated pesticides, especially chloroaromatics, contribute to pollution problems because of their recalcitrant nature. Therefore, the use of organochlorine pesticides such as 1,1,1-trichloro-2,2-bis-*p*-chlorophenylethane (DDT) and lindane has been banned or drastically reduced in developed countries due to prolonged persistence, prone to bioaccumulation and toxic to nontarget organisms. Similarly, endosulfan binds to soil particles and has a relatively long shelf life of 60–800 days. Recently, these recalcitrant compounds have been replaced by less persistent and more effective pesticide compounds belonging to chemical classes such as the organophosphates, carbamates, and synthetic pyrethroids, which are easily biodegradable and pose less environmental hazards.

Pesticide use in modern agriculture increases the quantity of pesticide residues in vegetables, grains, and cereals and the development of pest resistance, which has led to many problems (Fig. 2.1). Irregular and indiscriminate use of chemical pesticides in the crop system can contaminate soil, water, and air, as well as reduce soil microflora and fauna (Mwangi et al. 2010; Martin et al. 2011; Chauhan and Singh 2015). Excess bio-pollution and pesticide residues in the food chain and water have been found to cause carcinogenesis, neurotoxicity, and reproductive disorders (Burrows et al. 2002; Prüss-Ustün et al. 2011; Myers et al. 2016). Additionally, the accumulation of these contaminants in the soil not only adversely affects microorganisms and populations but also has hazardous effects on human health (Prashar et al. 2014; Wang et al. 2016a; Walia et al. 2018).

Therapeutic technologies in the remediation of pesticides have been developed with adaptation, oxidation, catalytic degradation, membrane filtration, and bioremediation treatment as well as a number of physical, chemical, and biological methods (Smith et al. 2004; Li et al. 2010b; Rani et al. 2017). But microbial-mediated pesticide diminution is the primary mechanism for remediation and detoxification of contaminants (Sindhu et al. 2014; Akbar and Sultan 2016; Javaid et al. 2016). Therefore, soil microbial communities are of great importance due to their multiple attenuation capabilities (Das and Chandran 2011; Dechesne et al. 2014) (Fig. 2.2).

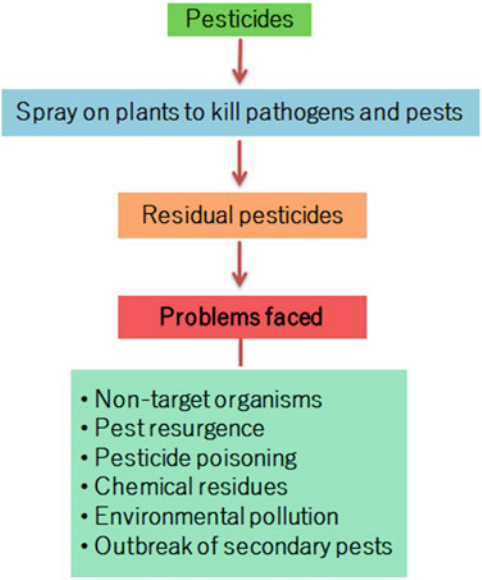

Fig. 2.1 Adverse outcomes of excessive pesticide application on surroundings and human being's health

Beneficial microorganisms isolated from crop rhizosphere could be exploited to provide sustainable solutions to agricultural crop production by reducing pesticide use (Philippot et al. 2013; Sindhu et al. 2017; Sehrawat and Sindhu 2019) or by degradation of residual pesticides in soil (Sindhu et al. 2014; Huang et al. 2018). Therefore, the microbial biodegradation or biological catalytic process of organic contaminants in the soil or crop rhizosphere is of major importance for environmental restoration (Karigar and Rao 2011; Joutey et al. 2013; Kehinde and Isaac 2016; Bharagava and Mishra 2018).

In bioremediation process, microorganisms and plants are used as biological intermediates to eliminate toxic/hazardous organic and inorganic chemicals into less hazardous compounds (Chandra et al. 2015; Saxena and Bharagava 2016). It is an environment-friendly and efficient method that can be used as an alternative to chemical and physical methods (Gilani et al. 2016). Antimicrobial control is an effective tool for cleaning pesticide-contaminated areas. Toxic chemicals/substances are converted to low-level toxic substances by the microbial control process (Saez et al. 2014; Kurade et al. 2016; Pan et al. 2017). The main benefits of microbial remediation of pesticides are easy multiplication and rapid growth leading to high microbial population. Under suitable growth conditions (sufficient humidity, moderate or warm temperature, adequate pH, and air circulation), microbial decay can be improved, leading to complete deprivation of pesticides.

Microbial degradation of pesticides, xenobiotic compounds, and biochemicals has been broadly reported (McGuinness and Dowling 2009; Porto et al. 2011; Ladino-Orjuela et al. 2016) to reduce pesticide residues in food and feed (Kadam and Gangawane 2005; Castillo et al. 2011). Microbes like fungal and bacterial species break down a variety of pesticide compounds counting phenols, substituted

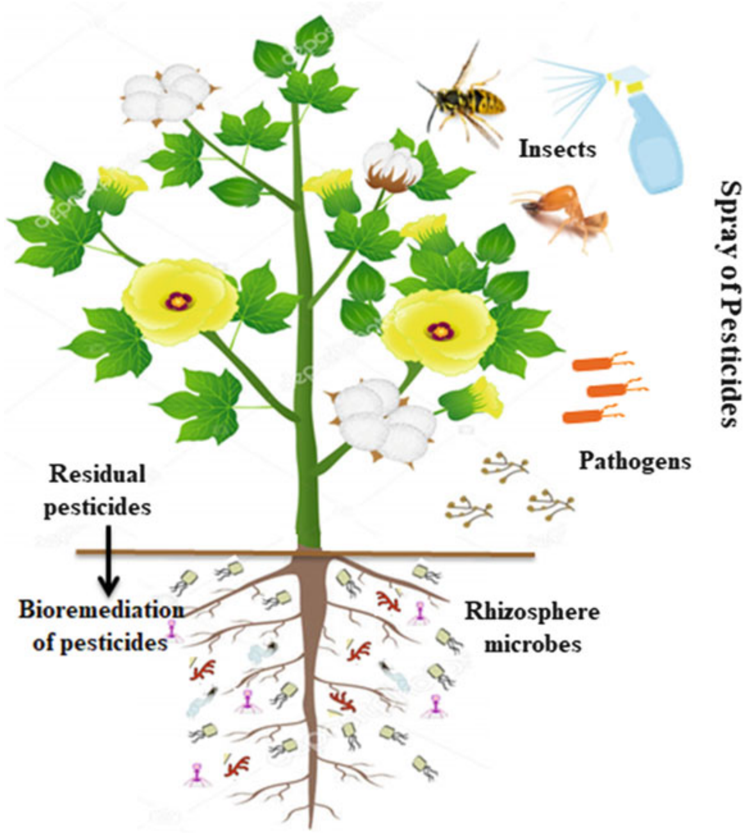

Fig. 2.2 Various kinds of pesticides are sprayed for the control of pathogens and pests. Residual pesticides are degraded by various microorganisms in the rhizosphere

phenolics, and noxious compounds (Moneke et al. 2010; Castillo et al. 2011). The commonly used microorganisms for the pesticide bioremediation belong to the species of *Agrobacterium*, *Azospirillum*, *Bacillus, Burkholderia*, *Flavobacterium, Klebsiella*, *Mycobacterium, Methylococcus*, *Pandoraea*, *Pseudomonas*, and *Streptomyces* (Nakkeeran et al. 2005; Glazer and Nikaido 2007; Rani and Dhania 2014; Parte et al. 2017; Kumar et al. 2018b). Some fungi that degrade pesticides include *Aspergillus*, *Candida*, *Lecanicillum*, *Penicillium, Rhizopus*, *Trichoderma*, and *Phanerochaete chrysosporium* (Mateen et al. 1994; Pimentel 2002; Martins et al. 2017). Bacteria that degrade certain pesticides have not only survived in stressful conditions of pesticides but have also shown biocontrol activity (Castillo et al. 2011; Chennappa et al. 2014). For example, the pesticide (methomyl)-degrading bacterial strains, i.e., Disha A and Disha B isolated from pesticide-infested rhizospheric soil, were recognized as *Bacillus cereus* and *Bacillus safensis*, respectively (Roy and Das 2017). Recent awareness about human health and concern about food safety has necessitated the characterization of efficient bioremediation agents from soil/

rhizosphere. Further, understanding of the involved degradation mechanisms and enhancing degradation of pesticides by genetic engineering of microbes are essential for improving soil health and ecosystem performance (Pellegrino and Bedini 2014) by improving crop productivity in the sustainable agriculture sector.

2.2 Categorization of Pesticides

Pesticides are classified into different categories based on their activity, including herbicides, insecticides, fungicides, nematicides, rodenticides, and molluscicides (Rani et al. 2017). Based on the chemical composition, pesticides can be classified as: (1) organochlorines, (2) organophosphates, (3) carbamates, and (4) substituted urea. Organophosphates and organochlorines are highly hazardous and persistent organic pollutants, which are even more dangerous to the environment. Organophosphorus pesticides (OPs), referring to chemical structures and functional groups, are phosphates, phosphoramides, or phosphorothioates, usually contain P-O, P-N, or P-S bonds, respectively (Liang et al. 2004; Shaker and Elsharkawy 2015). Twelve organophosphorus pesticides (OPs) have been listed in the US EPA's latest Candidate Contaminant List (CCL4) (Parker et al. 2017). OPs comprising high mammalian toxicity (Singh et al. 2004) can inhibit the activity of acetylcholine esters (AChE) (Pino and Peñuela 2011), and prolonged exposure to OPs poses a serious threat to human health (Wang et al. 2010a, b; Granella et al. 2013). Excessive consumption of OPs can lead to severe environmental pollution and hazards. The use of pesticides refers to the practical method by which pesticides are delivered to their biological targets (e.g. pests, crops or other plants). Introduction of other synthetic pesticides, organophosphate pesticides in the 1960s, carbamates in the 1970s, pyrethroids in the 1980s, and herbicides and fungicides introduced from the 1970s to the 1980s has been instrumental in pest control and agricultural production (Table 2.1).

2.3 Pesticides and Their Toxic Effects

To control pests and diseases, farmers are using higher doses of pesticides compared to the recommended. Most pesticides such as glyphosate, malathion, phorate, monocrotophos, chlorpyrifos, simazine, pendimethalin, carbofuran, phosphamidon, diazinon, mancozeb, methyl parathion, and carbendazim (Moneke et al. 2010; Chennappa et al. 2016) are often applied for the cultivation of agricultural crops. Organochlorine pesticides explored for pest management consist of dichlorodiphenyltrichloro-ethane (DDT), aldrin, dieldrin, endosulfan, endrin, hexachlorocyclohexane (HCH), heptachlor, sodium pentachlorophenate, and toxaphene. Different genera of bacteria and fungi can degrade these pesticides. Although endosulfan and HCH are banned in developed countries, these pesticides are still

Table 2.1 Types of various pesticides commonly used in crop protection

Type of pesticides		Name of pesticides
Insecticide	Organic nitrogen	Benzoylphenyl ureas, chlordimeform
	Organic phosphorus	Acephate, azinphos-methyl, bromophos, chlorpyrifos, coumaphos, diazinon, dimethoate, dioxathion, disulfoto, diazinon, ectophos, fenitrothion, fenitrooxon, fonofos, glyphosate, leptophos, malathion, mathamidophos, parathion, phenthoate, profenofos, phorate, phosmet, phosphothion, trichloffon, trichlorfon
	Organic chlorine	Aldrin, chlordane, DDT, dieldrin, dicofol, endosulfan, endrin, fipronil, heptachlor, lindane, γ-hexachlorocyclohexane
	Carbamate	Aldicarb, carbaryl, carbofuran, carbosulfan, artap
	Pyrethroid	Cypermethrin, chlorfenvinphos, deltamethrin, fenvalerate, flumethrin, permethrin, ivermectin
	Insect growth regulators	Azadirachtin, benzoylphenyl urea, diflubenzuron, methoxyfenozide, pyriproxyfen, spinosad, tebufenozide
Acaricides		Amitraz, coumaphos, dimethoatet, fenpyroximate, formic acid, menthol, tau-fluvalinate
Herbicide		Acetanilides, alachlor, barban, chlorbromuron, hlorophenoxy, dalapon, diuron, glyphosate, linuron, monuron, neburon, pendimethalin, pentachlorophenol, propham, salted iron phosphorus, swep, 2,4-D, 2,4,5-T
Bactericide		Bayleton, blue copper, chlorothalonil, copper hydrochloride, copper oxychloride, copper sulfate, dithane, dithiocarbamates, mancozeb, metalaxyl, methyl phosphorus, polytrin, ridomil, rice blast net, triazoles, thiocarbamates, thiovit

Adapted and modified from Huang et al. (2018)

used in developing countries (Niewiadomska 2004; Kadam and Gangawane 2005; Moneke et al. 2010; Castillo et al. 2011). Extreme use of these chemical substances directs to microbial unevenness, health risks, and environmental pollution by upsetting soil and aquatic habitats. Ultimately, they may cross the human and animal food chain causing neurotoxicological diseases.

2.3.1 *Impact of Pesticides on Environment*

When pesticide residues are suspended in the air and spread through the air to other areas that pose a threat to the surrounding environment, the pesticide effect is caused by the flow of pesticides. Physical parameters such as weather, temperature, wind speed, and relative humidity of the area during pesticide use contribute to its spread. Large amounts of pesticides evaporate as a result of low relative humidity and high temperature of the location. Some pesticides applied for soil fumigation can synthesize volatile organic compounds, which react with other chemicals to form a contaminant that affects tropospheric ozone. Drops of liquid pesticide sprayed on the fields will stick to the dust and spread as dust particles.

2.3.2 Impact on Soil and Water

Soil is an important and primary source of pesticide pollution. Extensive application of pesticides alters the normal metabolism of microorganisms and has detrimental effects on soil microorganisms and other natural microflora of soil ecosystems (Chennappa et al. 2019). The occurrence of pesticides in water resources such as lakes, canals, and rivers has been reported to pose a threat to water bodies. The causes of pesticide infiltration into water are pesticide flow, percolation through soil, water flow, accidental spraying or soil erosion when sprayed (Karunya and Saranraj 2014). All of these factors lead to suffocation due to the toxicity of the aquatic biota and zooplankton.

2.3.3 Impact of Pesticides on Human Beings

Pesticides applied in agricultural areas enter the human body through inhalation of dust aerosols and vapors or through oral exposure by ingesting pesticide-contaminated foods and water. The severity of the pesticide depends on the toxicity and chemical nature and prolonged exposure to the pesticide. The severity may be severe with long-term consequences. Severe effects include headache, nausea, abdominal pain, vomiting, dizziness, respiratory infections, sore throat, allergies, skin, and eye problems. Long-term outcomes include neurological disorders, reproductive effects, birth defects, fetal death, and other reproductive problems. Cancer-related complications have also been reported in lymphoma, brain, prostate, liver, blood, and skin. Pesticides are also known as endocrine disruptors (Aleem et al. 2003; Naik et al. 2007; Martin et al. 2011) because the use of these chemicals can lead to hormonal imbalances in the body. Furthermore, some of these pesticides are easily transmitted from nursing mothers to children through breast feeding (Muñoz-de-Toro et al. 2006). Organophosphates are an important group of neurotoxic pesticides that act by inhibiting acetylcholine esterase in the central and peripheral nervous system, resulting in the formation of choline and acetate (Eleršek and Filipič 2011). In addition, the nerves are significantly inhibited, and this suppression can lead to heart attack, strokes, and eventually death in insects and mammals (Singh and Walker 2006).

Chlorpyrifos is moderately toxic to humans because it acts on the nervous system by inhibiting acetylcholine esterase activity (Schuh et al. 2002; Reiss et al. 2012). There have been reports of genetic and mutagenic effects of chlorpyrifos in humans (Sobti et al. 1982; Sandal and Yilmaz 2011) and rat (Ojha et al. 2013). Nasr et al. (2016) reported that chlorpyrifos tends to cause significant oxidative damage in the brain and kidney of rat. Recently, Jegede et al. (2017) reported that changes in temperature affect the toxicity of chlorpyrifos to soil microarthropods. Humans exposed to methyl parathion have reported headaches, nausea, insomnia, diarrhea, dizziness, shortness of breath, dizziness, abdominal cramps, excessive sweating, and mental confusion (Rubin et al. 2002). Toxicity of methyl parathion is associated with disruption of acetylcholine esterase in mammals, especially in humans and pests leading to serious health problems (Liu et al. 2016b). Abhijith et al. (2016) reported that an acute and mild dose of methyl parathion induces significant variations in the enzymatic profiles of *Catla*.

Quinalphos is another pesticide that affects acetylcholine esterase resistance and is also present on the stomach and respiratory system (Yashwanth et al. 2016). Debnath and Mandal (2000) reported that quinalphos is an environmental xenoestrogenic insecticide that obstructs with the expression of sex hormones and lead to abnormalities in mammals. Furthermore, quinalphos is toxic in female reproduction in certain concentrations (Khera et al. 2016). The presence of profenofos residues in the soil causes a high environmental risk as it adversely affects the ecosystem (He et al. 2010; Fosu-Mensah et al. 2016). The presence of profenofos and its intermediate (4-bromo-2-chlorophenol) in human plasma and urine has been reported (Gotoh et al. 2001). Profenosios is highly toxic to fish and invertebrates (Talwar and Ninnekar 2015). Furthermore, samples of metaphase plates treated with dosages of profenofos showed satellite links, chromatid interruptions, and gaps, and the effect of profenofos on chromosomes was demonstrated (Kushwaha et al. 2016).

2.3.4 Effect of Pesticides on Natural Biodiversity

Depending on the type of pesticide and the dosage recommended for field application, pesticides may have a temporary effect on microbial and enzyme activity. The changes in number, function, and diversity of soil microorganisms serve as indicators of soil fertility and reflect soil quality (Sharma et al. 2018; Dahiya et al. 2019a). Ataikiru et al. (2019) investigated the effect of pesticides on soil biochemical properties on some soils and observed variations in the different enzyme activities of soils treated with carbofuran and paraquat. Increased dehydrogenase activity in pesticide-treated soils was recorded. Urease activity was lower than other enzyme activities. Differences in the organic carbon values of the soil were also observed. The number of microorganisms gradually increased with the temporary mineralization of pesticides and their ability to utilize carbon as energy sources. The population of *Azotobacter* was affected by many factors in the soil, and these factors consisted high consumption of pesticides and chemical fertilizers that are usually used to control pests and diseases in agricultural crops.

2.3.5 Effect of Insecticides on Plant Growth Promoting Attributes

Indole acetic acid (IAA) is produced by different rhizobacterial strains belonging to *Serratia*, *Bacillus*, and *Pseudomonas* even under exposure stress of residual insecticide but decreased consistently with increasing insecticide concentration among all bacterial strains (Wani et al. 2005). Ahemad and Khan (2011) reported that substantial IAA was produced by the *Klebsiella* sp. strain PS19 even when exposed to three times the recommended dose of insecticides. In addition, *Azotobacter* species was found to fix nitrogen, produced hormones IAA, gibberellic acid (GA) and solubilized phosphate in the media containing a variety of pesticides (Chennappa et al. 2014; Gurikar et al. 2016). Castillo et al. (2011) found that endosulfan did not affect IAA production in *Azotobacter chroococcum* and very few differences were found. On the other hand, Asma et al. (2012) reported the effect of endosulfan on IAA production in *Azotobacter* and found that even 50 ppm of endosulfan inhibited IAA production. The effect of pesticides (chlorpyrifos and phorate) on IAA production by *Azotobacter* species was observed at different concentrations compared to control (Chennappa 2016). The highest IAA-producing *Azotobacter salinestris* supplemented with 1 mg tryptophan at 1% chlorpyrifos indicated that 1% chlorpyrifos did not affect bacterial growth and function. Significant differences were recorded in the different isolates at 3% and 5% phorate, and *A. salinestris* produced the maximum IAA at 5% phorate (Chennappa 2016). Similarly, *Azotobacter* species that are resistant to pesticides isolated from paddy soils produce IAA in media supplemented with 5% pesticides (Chennappa et al. 2013).

Gibberellic acid (GA) is one more important plant growth substance produced by plant growth–promoting rhizobacteria (PGPR) of various species, including *Azotobacter* species. Asma et al. (2012) reported the effect of endosulfan on GA production in *Azotobacter*, and 50 ppm concentration of endosulfan was found to inhibit GA production. *Azotobacter salinestris* isolate produced a maximum of GA at 1% chlorpyrifos (Chennappa 2016). Higher than 1% concentration, chlorpyrifos reduced the GA production capacity of *Azotobacter* and also reduced bacterial growth by 20–25%.

Castillo et al. (2011) reported that endosulfan at 2–10 mg L^{-1} inhibited 94% and 96% of the nitrogenase activity of the *Azotobacter chroococcum* but *A. chroococcum* completely degraded endosulfan. Of the total five isolates, the highest nitrogen fixation was observed with *A. salinestris* isolate at 1% phorate concentration (Chennappa 2016). Moneke et al. (2010) reported that *Azotobacter* and other bacterial species, such as *Pseudomonas*, *Escherichia*, and *Acetobacter*, were tolerant and degraded glyphosate herbicides, and all the isolates were resistant to 1%, 3%, and 5% pesticides, although the bacterial activity was inhibited compared to control. Wani et al. (2005) assessed the toxic effects of different types of pesticides on the solubility of phosphate of 12 bacteria on phosphate, isolated from various rhizospheric soils such as *Serratia*, *Pseudomonas*, and *Bacillus*. Among various bacterial cultures, *Serratia* exhibited the highest phosphate solubilization. *Klebsiella* spp. significantly solubilized inorganic phosphate even in the presence of recommended and high levels of pesticides (Ahemad and Khan 2011). *Azotobacter* phosphate solubility was detected at a maximum of 1% chlorpyrifos concentration and reduced to 35–40% at high concentration of chlorpyrifos (Chennappa 2016).

2.4 Microorganisms Involved in Degradation of Pesticides

Pesticides are usually toxic and have a xenobiotic nature. When constantly exposed to high concentrations of toxic and persistent pesticides, a wide range of soil-dwelling microorganisms, including bacteria and fungi, may develop the ability to use pesticides as a source of energy and nutrients. Partial or complete mineralization/conversion of such pesticides in the soil make them more or less non-toxic than the parent molecule, leading to bioremediation of such contaminated areas (Alexander 1999). In most cases, high levels of pesticides increase bacterial and fungal populations, where soil microorganisms utilize pesticides as a source of carbon, energy, and other nutrients. For example, the pesticide diazinon and herbicide linuron significantly increased the number of heterotrophic bacteria and fungi in the soil after 28 days, when the concentration from 15 mg kg^{-1} soil to 1500 mg kg^{-1} soil was gradually increased (Cycon and Piotrowska-Seget 2007).

Due to environmental issues such as accumulation of pesticides in food and water supply, biodegradation has been recognized as a safe, convenient, and economically viable tool for the cleaning of pesticide-contaminated soils due to low cost, ease of use, high efficiency, and no secondary pollution (Sindhu 2007; Ning et al. 2012; Ramu and Seetharaman 2014; Ozdal et al. 2017). Most recalcitrant pesticides are captivated into the soil and, therefore, are not properly accessible to bacteria due to intracellular degradation processes. Among microbial species, bacteria, fungi, and actinomycetes are main pesticide degraders (Table 2.2) have been isolated from soils either by direct serial dilution method (Fig. 2.3) or by enrichment culture technique using particular pesticides as substrate.

Several microorganisms that can mineralize organophosphates (OPs) have been isolated, including bacteria such as *Pseudomonas aeruginosa* F10B (Das and Singh 2003), *Ochrobactrum anthropi* B2 (Qiu et al. 2006), *Hyphomicrobium* spp. MAP-1 (Wang et al. 2010a, b), *Agrobacterium* sp. Yw12 (Wang et al. 2012), and belonging to *Bacillus*, *Flavobacterium*, *Micrococcus*, and *Pseudomonas* (Singh and Walker 2006), as well as fungi *Penicillium oxalicum* ZHJ6 (Zhao et al. 2010), *Fusarium* spp. F1 (Zhao et al. 2009), *Aspergillus sydowii* PA F-2 (Tian et al. 2016), and *Saccharomyces* (Gao et al. 2011). Another pesticide monocrotophos (MCP) was degraded by *Pseudomonas aeruginosa* F10B, and *Clavibacter michiganense* subsp *insidiosum* SBL 11, which used MCP as a source of phosphorus (Das and Singh 2003; Singh and Singh 2003). MCP can also be degraded by *Bacillus megaterium* (Bhadbhade et al. 2002). *Aspergillus sydowii* PAF-2 has been reported to metabolize 75.31% OP trichlorofon (100 mg L^{-1}) in 7 days (Tian et al. 2016). Salt-resistant actinomycete

Table 2.2 Microorganisms involved in pesticide degradation

Types of microbes	Microbial genera and species	Example of pesticide degradation	References
Bacteria	*Pseudomonas*	Aldrin, chlorpyrifos, coumaphos, DDT, diazinon, endosulfan, parathion, hexachlorocyclohexane, methyl parathion, monocrotophos	Verma et al. (2014), Parte et al. (2017), Kumar et al. (2018a)
	Bacillus	Chlorpyrifos, coumaphos, DDT, diazinon, dieldrin, endosulfan, endrin, glyphosate, methyl parathion, monocrotophos, parathion, polycyclic aromatic hydrocarbons	Verma et al. (2014), Upadhyay and Dutt (2017), Rani et al. (2019)
	Alcaligenes, *Flavobacterium*, *Klebsiella*	Chlorpyrifos, endosulfan, diazinon, glyphosate, methyl parathion, parathion	Verma et al. (2014); Kafilzadeh et al. (2015), Upadhyay and Dutt (2017), Osadebe et al. (2018), John et al. (2018)
Actinomycetes	*Micromonospora*, *Actinomyces*, *Nocardia*, *Streptomyces*	Aldrin, carbofuran, chlorpyrifos, diazinon	Jayabarath et al. (2010), Verma et al. (2014), Briceno et al. (2018)
Fungus	*Rhizopus*, *Cladosporium*, *Aspergillus fumigatus*, *Penicillium*, *Fusarium*, *Mucor*, *Trichoderma*, *Mortierella* sp.	Alachlor, aldicarb, atrazine, carbofuran, chlordane, chlorpyrifos, DDT, diuron, endosulfan, esfenvalerate, fenitrothion, fenitrooxon, fipronil, heptachlor epoxide, lindane, malathion, metalaxyl, pentachlorophenol, terbuthylazine, 2,4-D	Bending et al. (2002), Kataoka et al. (2010), Xiao et al. (2012), Romero-Aguilar et al. (2014), Birolli et al. (2016), Martins et al. (2017), Parte et al. (2017), Osadebe et al. (2018), Spina et al. (2018)
Algae	*Chlamydomonas*, diatoms	Phorate, parathion, atrazine, fenvalerate, DDT, patoran	Shehata et al. (1997), Kabra et al. (2014), Tang (2018)

S. alanosinicus was found to be highly effective in carbofuran degradation and led to 95% decomposition. It utilized carbofuran as a source of carbon in saline soils (Chougale and Deshmukh 2007).

Furthermore, some metabolic mediators formed from OPs were highly toxic compared to their parents (Li et al. 2010a). For example, parathion can be oxidized to paraoxon, which is more toxic than parathion (Zhang et al. 2000). The biodegradable products of dimethoate are highly water soluble, can easily migrate to other

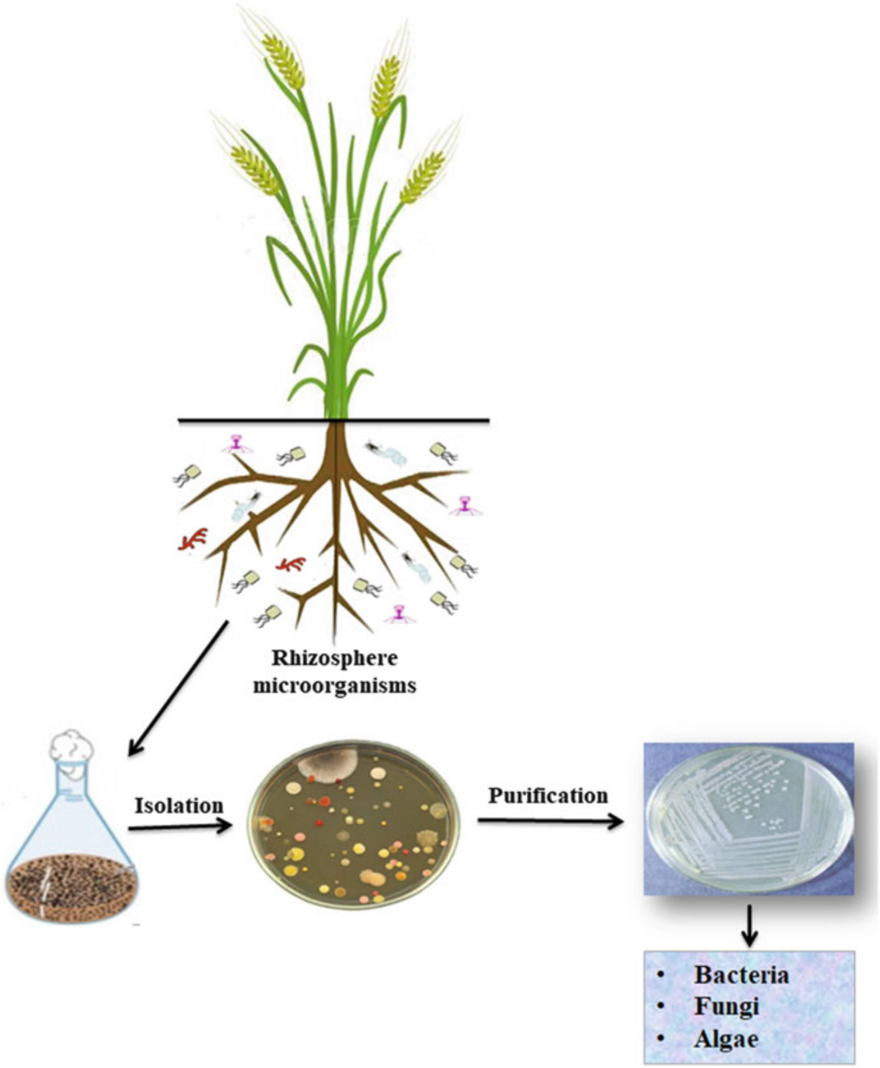

Fig. 2.3 Characterization of various kinds of microorganisms viz. bacteria, fungi, and algae from the rhizosphere soil for bioremediation of pesticides

systems such as groundwater and soils causing greater ecotoxicological risks (Li et al. 2010b). The complete breakdown of pesticides into inorganic components, water, CO_2, and other elements by microorganisms is termed as biomineralization (Odukkathil and Vasudevan 2013). Most of the pesticides that reach the soil are biodegradable, but some pesticides are completely resistant to biodegradation and are called recalcitrant pesticides (Mulchandani et al. 1999).

In the rhizosphere, microbial communities accelerate biodegradation processes and improve co-metabolism to degrade organic pollutants and pesticides by (1) facilitating selective enrichment of biodegrading microorganisms for xenobiotics degradation in root-free soils (Nichols et al. 1997), (2) enhancing metabolism of microbial growth by secreting natural substrates which depends on the quantity of xenobiotics (Haby and Crowley 1996), or (3) enriching natural compounds that provoke the co-metabolism of xenobiotics in specific microorganisms that exhibit genes or plasmids with degradation functions (Gupta et al. 2016). Thus, the rhizosphere microorganisms inhibit or tolerate the level of organic contaminants, mainly with the help of microorganisms linked to metabolic degradation, partially or completely detoxifying, leading to a decrease in the quality and quantity of contaminants in the soils (Furukawa et al. 2004; Balcom et al. 2016).

2.4.1 Pesticide Degradation by Bacteria

The biodegradability of microorganisms depends on the physical, chemical, and microbiological properties of the soil and the chemical properties of the pollutant (Banat et al. 2000; Van Hamme et al. 2003). Pesticide degradability decreases as molecular weight and degree of branching increases in pesticide structure. During degradation processes, bacteria and fungi produce intra- or extracellular enzymes such as hydrolases, peroxidases, oxygenases, and other enzymes for the degradation of toxic pesticide molecules (Li et al. 2007; Ortiz-Hernández et al. 2011). Profenophos, a well-known organophosphate pesticide, is widely used to control lepidopteron pests of cotton, tobacco, and vegetable crops and is degraded through hydrolysis by *Pseudomonas aeruginosa* (Malghani et al. 2009a). Similarly, *P. putida* utilized and degraded another organophosphate pesticide, cadusafos, which is used to control nematode and insect pests (Abo-Amer 2012). Organophosphate pesticide, chlorpyrifos was utilized by soil bacterium *Providencia stuartii* under in vitro conditions up to a concentration of more than 700 mg L^{-1} (Rani et al. 2008). Malathion is a broad-spectrum organophosphate used in agricultural soils. *Acinetobacter johnsonii* MA19 was isolated from malathion-contaminated soil samples using enrichment culture method. The degradation rates were significantly improved by the use of sodium succinate and sodium acetate as additional carbon sources for the degradation of malathion (Shan et al. 2009).

Kafilzadeh et al. (2015) isolated *Klebsiella*, *Acinetobacter*, *Alcaligenes*, *Flavobacterium*, and *Bacillus* form sediments and water samples, which could degrade endosulfan effectively. Jayabarath et al. (2010) selected 319 actinomycetes from saline soils in Sangli district (Maharashtra) for carbofuran tolerance, while *Streptomyces alanosinicus*, *S. atratus*, *Streptoverticillium album*, *Nocardia farcinia*, *N. amarae*, and *Micromonospora chalcea* could degrade carbofuran pesticide. Elgueta et al. (2016) used white rot fungi for degradation of atrazine and reported that growth and consumption of atrazine by fungi reduced the half-life of atrazine to 6 days. Kabra et al. (2014) reported the degradation ability of green microalga

Chlamydomonas mexicana to atrazine by accumulating atrazine in cells and subsequently degrading it, reaching a degradation rate of 14–36%. There are many other microorganisms such as *Streptomyces* spp., *Arthrobacter fluorescens*, and *A. giacomelloi*, *Chlorella vulgaris*, *Chlamydomonas reinhardtii*, *Clostridium sphenoides*, and *S. japonicum* UT26, which possessed the potential of degrading organochlorine insecticides (Boudh et al. 2017; Boudh and Singh 2019).

Endosulfan is another toxic, persistent, and widely used broad-spectrum cyclodine organochlorine pesticide. *Achromobacter xylosoxidans* strain C8B was isolated from the soil, using endosulfan as the only sulfur source, through the selective enrichment technique (Singh and Singh 2011). This bacterial strain degraded 94.12% α-endosulfan, 84.52% β-endosulfan, and 80.10% endosulfan sulfates, respectively, possibly by the formation of dichloro-diphenyl-trichloroethane (DDT), an organochlorine compound still used in agriculture and mosquito control in many developing countries. A *p,p′*-DDT degrading bacterial strain *Staphylococcus haemolyticus* was isolated from soil containing DDT residues in the range of 0.17–9.84 ng g^{-1} soil and reduced by 37.4% of *p,p′*-DDT in 10 days (Sonkong et al. 2008). A *Micrococcus* strain degraded pyrethroid pesticide cypermethrin, which yields 3-phenoxybenzoate yield by hydrolysis of ester linkage, resulting in loss of its insecticidal activity (Tallur et al. 2008). The degradation product 3-phenoxybenzoate was further metabolized by diphenyl ether cleavage to give protocatechuate and phenol. Both of which on oxidation by the ortho-cleavage pathway led to the complete mineralization of cypermethrin. Similarly, Naphade et al. (2012) isolated five different strains of soil bacteria, namely *Pseudomonas psychrophila*, *P. aeruginosa*, *Devosia yakushimensis*, *Paracoccus chinensis*, and *Planococcus rifietoensis*. Simazine, the active ingredient of 2-chloro-*S*-triazene herbicides, was biodegraded to almost 100% efficiency in 4 days by the *Arthrobacter ureafaciens* strain NC isolated from the rhizosphere soil (Błaszak et al. 2011).

Bromoxinil octanoate (BOO) is a toxic and common herbicide used for control of annual broad-leaved weeds applied in the maize crop (Cai et al. 2011). Cai et al. (2011) reported the degradation of these herbicides by the bacterial strain *Acinetobacter* spp. XB2 isolated from contaminated soil. The strain XB2 reduced 100 mg L^{-1} BOO to undetectable levels within 72 h under optimal conditions. Similarly, broad-spectrum herbicide glyphosate is widely used to control perennial and annual post-emergent weeds (Duke 2018). Fan et al. (2012) isolated *Bacillus cereus* strain from herbicide-contaminated soil, which exhibited potent glyphosate degradation potential. This strain utilized 94.47% of glyphosate and reduced it to AMPA, glycosylate, sarcosine, glycine, and formaldehyde through C-P lyase and glyphosate oxidoreductase activity.

Liu et al. (2010) isolated an *Arthrobacter* strain T3AB1, which used atrazine as the only carbon and nitrogen source, from maize field treated with atrazine in Heilongjiang province. The bacterium degraded more than 99% at 500 mg L^{-1} atrazine (pH 8.0) within 72 h under optimal conditions. Furthermore, this strain was found to use other herbicides such as imazamox, imazethapyr, trifluralin, clomazone, and fomesafen as a single carbon and nitrogen source at a degradation rate of

12.66–40.54% after 168 h. Besides, Kabra et al. (2014) studied the ability of green microalgae *Chlamydomonas mexicana* to degrade atrazine and found that microalgae accumulate atrazine in cells and then degrading it effectively, reaching a degradation rate of 14–36%. Another popular herbicide 2,4-dichlorophenoxyacetic acid (2,4-D) is used in many crops around the world in various crops such as wheat, rice, corn, sorghum, and sugarcane. World Health Organization has classified this herbicide as a carcinogen agent of level II toxicity. However, some microbial species, such as *Acinetobacter* spp., *Serratia marcescens*, *Stenotrophomonas maltophilia*, *Flavobacterium* spp., and *Penicillium* spp., have been reported to be rapidly consistent with the presence of 2,4-D, with subsequent degradation under in vitro conditions (Silva et al. 2011).

Polycyclic aromatic hydrocarbons (PAH), a class of hazardous chemicals containing two or more fused benzene rings in various structural configurations, are listed as priority pollutants by the U.S. Environmental Protection Agency due to their carcinogenic, mutagenic, and toxic effects (Poonthrigpun et al. 2006). Ahmad et al. (1997) characterized *Rhizobium meliloti* strains in soils contaminated with aromatic/chloroaromatic hydrocarbons. The rhizobial population was composed of many phenotypic and genetically diverse strains, and all rhizobial cells are effective in symbiotic N_2 fixation. Another group of ubiquitous PAHs in the environment includes acenaphthylene and phenanthrene. Acenaphthylene can be completely degraded by *Rhizobium* spp. strain CU-A1 in 3 days by the metabolic pathway of naphthalene-1,8-dicarboxylic acid (Poonthrigpun et al. 2006). On the other hand, *Sinorhizobium* spp. C4 was found to use phenanthrene as a single carbon source, and 16 intermediate metabolites involved in this degradation pathway were identified (Keum et al. 2006). Some toxic aromatic acids as well as hydrodynamic biosynthetic intermediates (i.e., quinate and shikimate) commonly found in plants and in the rhizosphere contribute to the growth of different rhizobial species (Parke et al. 1985). Many free-living rhizobial strains of the genus *Agrobacterium*, *Bradyrizobium*, *Rhizobium*, and *Sinorhizobium* have demonstrated the utilization of PAHs, PCBs, aromatic heterocycles (i.e., pyridine), or other toxic organic compounds (Poonthrigpun et al. 2006; Tu et al. 2011).

Polychlorinated biphenyls (PCBs) are another class of POPs that differ in the number of chlorine atoms attached to their biphenyl rings (Passatore et al. 2014). Tu et al. (2011) demonstrated that *Sinorhizobium meliloti* strain ACCC17519 degraded more than 70% of 2,4,4′-TCB (PCB28) compared to other rhizobial strains. Aromatic toxin produced by the sources of mimosine, *Leucaena* sp. is toxic to both bacteria and eukaryotic cells (Awaya et al. 2005). Some *Leucaena*-nodulating *Rhizobium* strains have been reported to utilize mimosine as a source of carbon and nitrogen (Soedarjo et al. 1995; Soedarjo and Borthakur 1998), indicating the catalytic ability of rhizobia to use aromatic compounds. Strains of *R. meliloti* could utilize 2,4,4′-TCB (PCB28) as a sole carbon and energy source under aerobic conditions, and HOPDA has been identified as a major intermediate during the biotransformation of 2,4,4-TCB by *S. meliloti* (Xu et al. 2010; Tu et al. 2011).

Chlorpyrifos is one of the most widely used insecticides to control mosquitoes (larvae and adults), flies, and various soil, leaf crop, and household pests. *Klebsiella* spp. degraded toxic chlorpyrifos into non-toxic products and increased the microbial growth along with the improved dehydrogenase activity (John et al. 2018). Diverse species of *Pseudomonas* including *P. putida*, *P. aeruginosa*, *P. stutzeri*, *P. nitroreducens*, and *P. fluorescens* isolated from agricultural soils significantly degraded the chlorpyrifos (Bhagobaty and Malik 2008; Maya et al. 2011; Sasikala et al. 2012). Similarly, *Bacillus aryabhattai* effectively degraded parathion as well as chlorpyrifos at optimal concentrations of 200 mg mL^{-1} (Pailan et al. 2015). Abraham and Silambarasan (2016) studied the biodegradation of chlorpyrifos and its by-product TCP by a novel bacterium *Ochrobactrum* spp. JAS2 isolated from the rice rhizosphere soil. The *mpd* gene responsible for the production of organophosphorus hydrolase was identified in *Ochrobactrum* spp. JAS2 (Abraham and Silambarasan 2016). The engineered *Pseudomonas putida* MB285 was capable of completely mineralizing chlorpyrifos by direct biodegradation, and two intermediates, namely TCP and diethyl phosphate, appeared in the culture medium (Liu et al. 2016a). Rayu et al. (2017) isolated species of *Xanthomonas*, *Pseudomonas*, and *Rhizobium* from sugarcane farm soils, which showed complete mineralization of chlorpyrifos (10 mg L^{-1}).

Nair et al. (2015) isolated 12 different bacterial species capable of growing on quinalphos and three isolates, namely *Pseudomonas* spp., *Serratia* spp., and *Pseudomonas aeruginosa*, efficiently degraded quinalphos. In *Pseudomonas aeruginosa*, 2-hydroxyquinoxaline and phosphorothioic acid were accumulated during quinalphos degradation (Nair et al. 2015). Gangireddygari et al. (2017) studied the effect of environmental factors on quinalphos depletion in *Bacillus thuringiensis*. The highest quinalphos degradation was achieved by using an inoculum of 1.0 optical density (OD) with an optimum pH (6.5–7.5) and an incubation temperature of 35–37 °C. Furthermore, the addition of yeast extracts improved quinalphos degradation rate to some extent. Archana et al. (2018) isolated *Bacillus cereus* and *Asaccharospora irregularis* isolates from contaminated soil from pesticides that effectively degraded pendimethalin contaminated environment. Meng et al. (2019) found that an alkaline phosphatase from *Bacillus amyloliquefaciens* strain YP6 may cause biodegradation of five broad-spectrum organophosphorus pesticides.

Profenofos was degraded by bacterial strains including *Pseudomonas aeruginosa*, *P. putida*, *Burkholderia gladioli* (Malghani et al. 2009a, b), *Bacillus subtilis* (Salunkhe et al. 2013), and *Stenotrophomonas* spp. G1 (Deng et al. 2015). 4-Bromo-2-chlorophenol was identified as a major intermediate during profenofos catabolism, providing a sensitive and accurate biomarker of profenofos degradation (Dadson et al. 2013). Talwar and Ninnekar (2015) studied profenofos degradation by free- and immobilized cells of *Pseudoxanthomonas suwonensis* strain HNM isolated from pesticide-contaminated soil samples by enrichment technique in sodium alginate-polyvinyl alcohol and sodium alginate-bentonite clay matrices. Sodium alginate-bentonite clay immobilized cells showed enhanced degradation rate of profenofos than freely suspended cells and other matrices (Talwar and Ninnekar 2015). Abdullah et al. (2016) reported that *Pseudomonas putida* isolate DB17 showed maximum potential for profenofos degradation.

2.4.2 Algae and Cyanobacterial Degradation

Thengodkar and Sivakami (2010) reported that hydrolysis of the chlorpyrifos pesticide by the secretion of the enzyme alkaline phosphatase by *Spirulina platensis* led to production of its non-toxic primary metabolite 3,5,6-trichloro-2-pyridinol. Kabra et al. (2014) studied degradation of atrazine by the microalgal species *Chlamydomonas mexicana*. The carbohydrate content in algae increases, which proved that *C. mexicana* can evacuate the pesticides at polluted streams. Pesticide remediation rate was found to vary depending on algal strain used, nature of pollutants, and environmental factors such as nutrients, water, pH, salinity, oxygen tension, temperature, and light intensity. Furthermore, physical and chemical parameters such as molecular chemistry, weight concentration, and toxicity have been shown to have an effect on atrazine degradation (Priyadarshani et al. 2011; Varsha et al. 2011).

Megharaj et al. (1987) reported the degradation of monocrotophos and quinalphos (organophosphorus insecticides) over a period of 30 days by *Chlorella vulgaris*, *Scenedesmus bijugatus*, *Synechococcus elongatus*, *Phormidium tenue*, and *Nostoc linckia*. *Anabaena* spp. and *Aulosira fertilissima* were found to metabolize DDT to DDD and DDE, respectively, by the process of bioaccumulation and transformation (Lal and Lal 1987). Microalgae degraded the organophosphorus insecticide methyl parathion and used it as a source of phosphate through a reductive process (Barton et al. 2004). *Chlamydomonas reinhardtii* has been shown to be useful in the bioremediation of prometryne (herbicide)-contaminated aquatic systems because it can rapidly uptake and catabolize prometryne (Jin et al. 2012). *C. vulgaris* accumulated the triazine group of herbicides, while *I. galbana* and *Dunaliella tertiolecta* accumulated atrazine (Weiner et al. 2004).

The endocrine disrupting insecticide, α-endosulfan was converted to endosulfan sulfate, endosulfadiol, β-endosulfan, endosulfan aldehyde, and endosulfan ether by *Scenedesmus* spp. and *Chlorococcum* spp. at cell densities of 1550×10^6 and 600×10^6 mg L^{-1} in a defined liquid medium (Sethunathan et al. 2004). Zhang et al. (2012) reported that *Anabaena azotica* strain 118 isolated from Chinese rice soils degraded lindane at a concentration of 0.2 mg L^{-1}. However, exposure to microalgae to multiple toxic compounds could lead to the development of resistant species, which may contribute to the degradation of more pesticide contaminants. Therefore, microalgae species are highly recommended for the ecosystems contaminated with lindane pesticide.

2.4.3 Degradation by Fungi

The filamentous nature of fungal growth provides a major advantage over bacteria, as it helps fungi to effectively propagate in the soil environment. In addition, during hyphae colonization in the soil, the fungi produce substrate-specific extracellular enzymes that are somewhat more tolerant to high concentrations of contaminants and lead to improved bioremediation (Fragoeiro 2005). Fungi can degrade a wide variety of pesticides by introducing small structural changes in the molecule. Fungal bioremediation of pesticides is caused by the release of a mixture of extracellular enzymes such as laccases, polyphenol oxidases, and lignin peroxidases. Intracellular enzymes such as reductases, methyltransferases, and cytochrome oxygenase were also involved in the remediation of organic pollutants and reduced these pollutants to a lesser or nontoxic form. The biotransformed pesticide was released into the soil, where it was further degraded by bacteria (Gianfreda and Rao 2004; Slaoui et al. 2007; Bisht et al. 2015; Bisht and Harsh 2017).

Various fungi such as *Penicillium* (Peng et al. 2012), *Aspergillus* (Mohamed et al. 2011), and *Phanerochaete* spp. (Chirnside et al. 2011) showed an effective remediation of pesticides. *Fusarium verticillioides* showed the potential to use lindane as a source of carbon and energy under aerobic conditions (Pinto et al. 2012). Other fungal strains, viz. *Fusarium oxysporum*, *Lentinula edodes*, *Penicillium brevicompactum*, and *Lecanicillium saksenae*, caused the biodegradation of the pesticides terbuthylazine, pendimethalin, and difenoconazole (Hai et al. 2012). Ellegaard-Jensen et al. (2014) mineralized the phenyl urea herbicide diuron using a consortium of fungi and bacteria. Clothianidin was biotransformed by a white rot fungus *Phanerochaete sordida*, which converted clothionidin into the non-toxic metabolite *N*-(2-chlorothiazol-5-methyl)-*N*-methyl urea (TZMU) (Mori et al. 2017).

Endosulfan-decomposing aerobic fungal strains were found useful in soil contaminated with organochlorine pesticides. For example, *Mortierella* spp. strains W8 and Cm1–45 caused 50–70% degradation of endosulfan lactone (Kataoka et al. 2010). During endosulfan degradation, diol was initially formed, which was later converted to endosulfan lactone. Mixed fungal species have more likely to degrade mixed pesticides such as chlorpyrifos and DDT. Decomposition efficiency was found to be higher using low mixed insecticide concentrations (Kulshrestha and Kumari 2010). The efficacy was observed in degradation of DDT and chlorpyrifos at 26.94% and 24.94%, respectively. Under severe conditions, the genus *Sphingomonas yanoikuyae* can decompose carbamate and pyrethrin (OPs) in enrichment culture with high efficiency (Ouyang et al. 2008). *Gliocladium* showed maximum potential for degradation of carbofuran (Seo et al. 2005). *Trichoderma harzianum* and *T. viride* showed a high efficiency in the degradation of pyrimicarb and increased degradation potential when activated charcoal was added (Romeh 2001).

2.5 Factors Affecting Microbial Degradation of Pesticides

The use of pesticides is essential for agricultural production, and hence, many problems of environmental pollution and health hazards have become increasingly prominent. Various microorganisms play an important role in the bioremediation of pesticides. However, the microbial degradation of pesticide residues is limited by a number of intrinsic and extrinsic environmental factors. The effect of intrinsic factors has derived from the structure of the pesticide and the microorganisms. The physical and chemical parameters of the soil, i.e., organic matter, nutrients, temperature, pH, humidity, redox conditions, amount, and nature of clay were found to have a direct impact on the success of bioremediation. Schroll et al. (2006) investigated the potential of soil moisture in the aerobic microbial mineralization of certain pesticides, i.e., glyphosate and benzoin ethyl in different soils. They observed a linear relationship between increasing soil moisture and pesticide degradation.

2.5.1 Effect of Microbial Species, Metabolic Activity, and Adaptability

Different species of microorganisms perform different reactions to the same organic substrate and pesticide degradation products were found to be different, and the microorganisms showed strong potential for adaptation in pesticide-contaminated soils (Hugo et al. 2014). Through the adapted process, new intermediate compounds were discovered to stimulate microorganisms to produce the corresponding enzyme system or to establish a new enzyme system to degrade the pesticide. Changes in the functional properties and degradation of the pesticide were the most important factors (Hussain et al. 2009; Tsai et al. 2011; Zhang et al. 2015).

2.5.2 Effect of Pesticide Structure

The molecular weight, spatial structure, number and type of substituents, substituted properties, and location were identified to affect the rate and efficiency of microbial degradation of pesticides (Mahro et al. 2012; Chaw and Stoklas 2013). In general, the polymer and composite pesticides were more resistant to biodegradation, and the simpler structure was more easily degradable (Luan et al. 2006). The main route of phytoremediation on soil contaminated by polycyclic aromatic hydrocarbons (PAHs) was microbial degradation in the rhizosphere. The number of benzene rings of PAHs had a great effect on the microbial degradation of PAHs. Two-rings and tricyclic compounds such as naphthalene, phenanthrene, anthracene, and fluorene existed in the atmosphere for a short time and microorganisms easily mineralized these compounds with using PAHs as a sole carbon source. However, high-molecular-weight four-ring and other multi-ring PAHs were stable in the atmosphere. However, white rot fungi could degrade these compounds through metabolism (Acevedo et al. 2011). In general, as the number of benzene rings of PAHs increased, the octanol/water partition coefficient increased, and the rate of degradation was decreased.

Most of the current contaminants or pesticides were synthesized as biologically diverse organic substances that are not present in nature. They often showed strong resistance to degradation by microbes. It may be explained that the time it takes for these compounds to come into nature was so short that not a single microbe has developed metabolic mechanisms regarding the degradation of such compounds. Compared to the currently widely used synthetic heterologous substances, the natural evolutionary process of microorganism was not able to meet the requirements of microbial pesticide degradation, because the speed of this process was far from reaching what the environment and human needed. Therefore, the balance of the entire ecosystem would be disturbed having a long-term impact (Ye et al. 2018).

2.5.3 Soil Organic Matter

Degradation of herbicides in modified soils with paddy straw, compost and NPK chemical fertilizer under upland, oxidative-flooded (aerobic-flooded), and reductive-flooded (anaerobic-flooded) conditions was studied (Kumar et al. 2018b). The crop residues acted as a source of organic matter and provided nutrients. Paddy straw, compost, and NPK amendments accelerated the degradation of herbicides under upland and oxidative-flooded conditions. But in reductive-flooded conditions, herbicide degradation was very slow. The degradation of benthiocarb resulted in the formation of 4-chlorobenzoic acid, desethyl benthiocarb, benthiocarb-sulfoxide, and 4-chlorobenzyl methyl sulfone. Paddy straw amendments increased the amount of benthiocarb sulfoxide. Under the upland conditions the amount of desethyl benthiocarb was reduced by paddy straw and compost. The major degradation product of MCPA was 4-chloro-2-methylphenol, resulting in large amounts of paddy straw amendments in oxidative-flooded and NPK amendments under upland conditions (Duah-Yentumi and Kuwatsuka 1980). Boivin et al. (2005) studied the interaction of pesticides, viz. isoproturon, trifluralin, and atrazine, in relation to the organic matter of the soil. Singh et al. (2006) studied fenomiphos and chlorpyrifos for its biodegradation, but could not observe the potential of soil organic matter in pesticide biodegradation. Fenlon et al. (2007) found that diazinon mineralized in two types of the organic soils. Gupta et al. (2015) observed that the effect of the organic substrate content on pesticide's degradation in composting was greater than that of the bacterial population when compost was mixed with soil contaminated by PAHs.

2.5.4 Environmental Factors

Temperature, humidity, salinity, pH, nutrition, carbon dioxide, oxygen, substrate concentration, surfactant, etc. were found to affect pesticide depletion (Martin et al. 2009; Sartoros et al. 2015; Bhattacharya et al. 2006; Munawar 2010). Bacteria or their enzymes require adequate temperature, pH, and substrate concentration for growth and enzymatic function (Nakajima and Shigeno 2014). Furthermore, biochemical reactions depend on the temperature of microbial activities, which having a direct effect on cell physiology by altering proteins and permeability of the cell membrane (Alberty 2006). Temperature and humidity were found to affect the growth, biochemical activity, and reproduction of bacteria (Arbeli and Fuentes 2007; Parmar and Sindhu 2013). Bacteria usually degrade chlorpyrifos and fenamiphos at temperature of 15–35 °C, but its degradation potential was severely reduced at low or high temperatures, i.e., 5 or 50 °C (Singh et al. 2006). Siddique et al. (2002) observed similar results during biodegradation of HCH isomers of soil slurry. For α- and γ-HCH isomers, the incubation temperature of 30 °C was found optimum for degradation.

The surfactant can alter the solubility, absorption, and dehydration balance of PAHs in soils and the interaction between PAHs and soil microorganisms, thereby altering the bioavailability of PAHs. For example, Yuan et al. (2003) used a way to reduce the interfacial tension between soil and water to increase the solubility of PAHs, facilitated the transport of PAHs, and increased the bioavailability of PAHs. However, due to the toxic effects of surfactants on microbes or the use of non-toxic surfactants as a microbial growth matrix, the bioavailability of PAHs might be inhibited. In addition, the effect of surfactants on the bioavailability of different forms of PAHs in soils was found different, so that surfactant could be added to increase the solubility of PAHs in the aqueous phase, to promote and improve the solid phase transfer to the water phase and reduce the bioavailability and surface and interfacial tension of the matrix (Yuan et al. 2003). Zhu et al. (2015) observed the degradation and mineralization of biaryl compounds in soil and compost by bacteria called *Ralstonia* and *Pickettii* and found that the nonionic surfactant Tween 80 increases bacterial utilization of biaryl compounds under appropriate soil moisture conditions, such as biphenyl, 4-chlorobiphenyl.

2.6 Removal of Pesticides Through Phytoremediation

Phytoremediation is a comprehensive strategy to isolate or detoxify environmental pollutants and pesticides using plants and their associated microorganisms (Bhat and Bhat 2016; Mitton et al. 2016). Plants are capable of degrading or removing metals, pesticides, explosives, solvent, crude oil, and many industrial contaminants. Phytoremediation is a clean, cost-effective, environmental-friendly technology, particularly for the treatment of large contaminated areas. It has been engaged in the environmental cleaning industry (Macek et al. 2000; Suresh and Ravishankar 2004).

Various mechanisms involved in the phytoremediation process include: (1) phytotransformation, which reduces toxicity, inactivates, or neutralizes contaminants caused by plant metabolism; (2) rhizodegradation which enhances the activity of soil microorganisms to degrade contaminants by rhizosphere bacteria; (3) phytoextraction, which absorbs contaminants from the polluted solids and stores the substances in the plant biomass, with a potential to recover and reuse valuable metals, and (4) phytostabilization, which reduces mobility of toxic substances in the soils, as in the case of mine tailings. Plants that are relatively tolerant of environmental pollutants often remain small in the presence of contaminants and remove only small amounts per plant. In order to obtain a more efficient degradation of organic compounds and pollutants, plants must rely on their associated microorganisms (Pilon-Smits and Freeman 2006). Therefore, inoculation with plant growth–promoting bacteria (PGPB), which have the property of remediation, has been found to stimulate plant growth, especially under stressful conditions. Growing plant biomass to microbial inoculants makes phytoremediation a faster and more efficient process (Glick 2003).

Phytoremediation technique involves the cultivation of pesticide/metal-tolerant plants having pesticide/metal accumulating ability to remediate the contaminated area. These plants can accumulate, absorb, and detoxify chemicals from the site through their metabolic processes. Suresh et al. (2005) reported that *Cichorium intybus* and *Brassica juncea* plants are effective in degradation of DDT and triazophos (Cheng et al. 2007), chlorpyrifos (Prasertsup and Ariyakanon 2011; Romeh and Hendawi 2013), methyl parathion (Khan et al. 2011), and atrazine (Wang et al. 2012). Aquatic plants such as *Eichhornia crassipes*, *Lemna minor*, and *Elodea canadensis* have been used in water treatment due to high photosynthesis, high growth rate, easy harvesting, and high pollutant absorption rates (Syuhaida et al. 2014). Pesticide uptake and phytodegradation of pesticides by *Eichhornia crassipes* in water resources can be used as a potential, economical, and alternative biological method (Xia and Ma 2006). However, the removal efficiency of *E. crassipes* and *P. strateotes* for pyrethroids has been observed significantly higher as compared to organochlorine (Riaz et al. 2017).

Lemna minor and *Spirodela polyrhiza* were found to remove dimethomorph until its concentration is highly toxic and inhibit depuration mechanisms (Dosnon-Olette et al. 2010). *Lemna minor* has also been reported to decontaminate organic metal such as heavy metal and pesticides by rhizofiltration (Sasmaz et al. 2017). *Acorus gramineus* showed the ability to absorb many OP and OC pesticides (diazinon, fenitrothion, malathion, parathion, dieldrin, HCB) and remove them from aquatic ecosystems (Chuluun et al. 2009). *Plantago major* was found to absorb cyanophos (Romeh 2014). *Acorus calamus* has been reported to exhibit great phytoremediation potential in terms of biomass growth and atrazine removal (Roman et al. 2012). *Azolla caroliniana* and *Lemna gibba* have also been reported to remove atrazine from the water (Guimarães et al. 2011). Five macrophyte species, namely *L. minor*, *S. polyrhiza*, *C. aquatica*, *C. palustris*, and *E. canadensis*, removed two fungicides dimethomorph and pyrimethanil from water, and two species *L. minor* and *S. polyrhiza* showed the highest efficiency in removal of fungicides (Dosnon-Olette et al. 2009).

2.7 Integrated Remediation Technologies

Plant microbial–associated bioremediation has been used for agricultural soil remediation. Synergistic interactions between plants and microbial population in the rhizosphere are effective for the degradation of recalcitrant organochlorines (OCs) (Vergani et al. 2017). Root exudates (amino acids, flavonones, sugars, enzymes, phenolic compounds, and other organic substances) could increase the bioavailability of OCs and microbial activities in the immediate vicinity of the roots (Javorska et al. 2009). Microbial strains capable of breaking down OCs were widespread in the rhizosphere soils (Chaudhry et al. 2005). Root exudates were found to increase the degradation of PAHs with increasing ring numbers (Sun et al. 2010).

2.7.1 Surfactant-Enhanced Bioremediation

Bioremediation alone has not been able to quickly remove persistent and highly toxic pollutants from farm soil in general (Huang et al. 2017). The use of bioremediation is a secondary step after chemical remediation and was found more effective in PAH removal than the single approach (Kulik et al. 2006). Surfactant-enhanced bioremediation (SEBR) is a hopeful technology to improve the bioavailability and removal efficiency of OCs in agricultural soil (Chirakkara et al. 2016; Wang et al. 2016b). Surfactant increased the partition of OCs to microbial cells and also facilitated the transmembrane transportation of OCs into the cells and thus accelerated intracellular biodegradation (Zhang and Zhu 2012; Li and Zhu 2014; Li et al. 2014). Different surfactants exerted various effects on the biodegradation of PAHs through different approaches, such as disrupting bacterial membranes and modifying cell surface hydrophobicity (Zhang et al. 2013; Ni et al. 2014). Recently, the ring-hydroxylating dioxygenase (RHDase) and 1-hydroxyl-2-naphthoate dioxygenase genes (1H2Nase) were found to be induced in the presence of surfactants, which played a key role in the decomposition of hydrophobic aromatic compounds (Li et al. 2015). Surfactants were also found to enhance the degradation of DDT by microorganisms in agricultural soil (Wang et al. 2016c). Therefore, surfactant-enhanced bioremediation could be a promising technology for addressing combined organic pollution in agricultural soil.

2.7.2 Enhanced Phytodegradation by Plant Growth Promoting Bacteria

The rhizosphere has a population of microorganisms that can degrade xenobiotic substances (Donnelly et al. 1994; Macková et al. 2007; Mendez and Maier 2008; Sindhu and Sharma 2020). Kuiper et al. (2001) reported that inoculation of effective root-colonizing pollutant-degrading bacteria on a suitable crop plant resulted in improved bioremediation of the pesticide. The niche combination of plant and microorganisms led to the effective degradation of naphthalene and protected the grass seeds against the toxic concentrations of naphthalene. In another soil, contaminated with creosote, on inoculation of tall fescue (*Festuca arundinacea*) with polycyclic aromatic hydrocarbons (PAH) degrading bacteria and PGPB (*Pseudomonas putida*, *A. brasilense*, and *Enterobacter cloacae*) substantially increased the removal rate of PAH (Guo et al. 2018). Large-sized PAH were eliminated in the presence of these PGPB because these specific bacterial species reduced stress in plants through ACC-deaminase activity (Huang et al. 2004). *Pseudomonas* spp. have been reported to increase the growth of the canola plant and common weed *Phragmites australis* in the presence of copper or PAH (Reed and Glick 2005; Reed et al. 2005). PGPB degraded 2-chlorobenzoic acid and oil-contaminated soils for growing *Vicia faba* and many forage grasses, but no clear relationship between contaminant disappearance of pollutants and enhanced plant biomass was observed (Siciliano and Germida 1997; Radwan et al. 2005). The bioremediation potential of legumes *Galega orientalis* and its symbiont, *Rhizobium galegae*, has been assessed in soils contaminated with benzene, toluene, and/or xylene (BTX). The *Galega* plants showed good growth, nodulation, and nitrogen fixation in soils contaminated with oil or spiked with m-toluate, a model compound representing BTX (Suominen et al. 2000).

Several endophytic bacteria were also found to help host plants overcome contaminant-induced stress, and resulted in improved plant growth (Correa-Galeote et al. 2018). During phytoremediation of organic contaminants in soils, plants benefit more from their endophytes, which have degenerative pathways and metabolic abilities that are not inherent in the plant. This strategy leads to a more effective degradation and reduction of phytotoxicity and evaporation of volatile contaminants (Weyens et al. 2009). For example, tall fescue *Festuca arundinacea* grass selects the prevalence of endophytes containing pollutant catabolic genes in an environment contaminated with different pollutants (hydrocarbons and nitro-aromatics) (Siciliano et al. 2001). Barac et al. (2009) reported that when remediation reduced BTX below a detectable level, the ability of the endophytic community in poplar plants to degrade BTX disappeared. Similarly, inoculation of the *Pisum sativum* plant with an endophyte (isolated from poplar), having the capability to degrade the herbicide 2,4-D, increased the removal of 2,4-D from the soil (Germaine et al. 2006).

The excretion of root exudates may also stimulate growth of specific, pollutant-degrading bacteria in the rhizosphere by secreting phospholipid surfactants (Sindhu et al. 2017) that make organic pollutants more bioavailable or by releasing secondary metabolites that induce the expression of genes with organic pollutant-degradation potential (Pilon-Smits 2005). For example, *Rhodococcus* species are the most common group in the rhizosphere of trees, which naturally colonized and improved a PCB-contaminated site in the Czech Republic (Van der Geize and Dijkhuizen 2004). Barley growing in PAH-contaminated soils has contributed to the growth of *Mycobacterium* species capable of mineralizing the PAH (Child et al. 2007). Soils contaminated with petroleum derivatives generally have high concentrations of m-toluate. The rhizosphere of oriental goat's rue *Galega orientalis* grown on these polluted sites showed predominant population of m-toluate degraders *Pseudomonas* spp., *Rhodococcus*, *Arthrobacter*, *Bacillus*, and *Nocardia* (Jussila et al. 2006).

2.8 Mechanisms and Enzymes Involved in Pesticide Degradation

The remarkable variety and complexity of different pesticide structures and diversity of pesticide degrading microorganisms belonging to all physiological types indicated that a wide variety of transformation reactions are catalyzed by various microorganisms in the pesticide degradation. Pesticides and other organic pollutants in soil and water can be degraded by photolytic, chemical, and biological mechanism. Photolytic degradation can occur when a pesticide molecule is irradiated by sunlight. Chemical degradation occurs when the molecule is chemically unstable in the conditions of its environment, whereas biodegradation refers to the transformation of pesticides by living microorganisms. In nature, biological and non-biological processes work together to degrade pesticide compounds.

Soil pesticides can be degraded in a variety of ways; traditional methods include physical, chemical, and physico-chemical degradation, which primarily causes secondary pollution (Qu et al. 2015; Kaur et al. 2016; Zhang et al. 2017). Recently, microbial degradation was regularly used as microbes decomposed pesticides into some smaller molecules, such as CO_2 and H_2O (Chen et al. 2011; Tang 2018).

Pesticide degradation follows different metabolic pathways depending on the structure of the pesticide, environmental conditions, and the nature of the microorganisms (Fig. 2.4). The mechanism consists of (1) oxidative transformation mediated by oxidative enzymes (cytochrome P450, peroxidases, and polyphenol

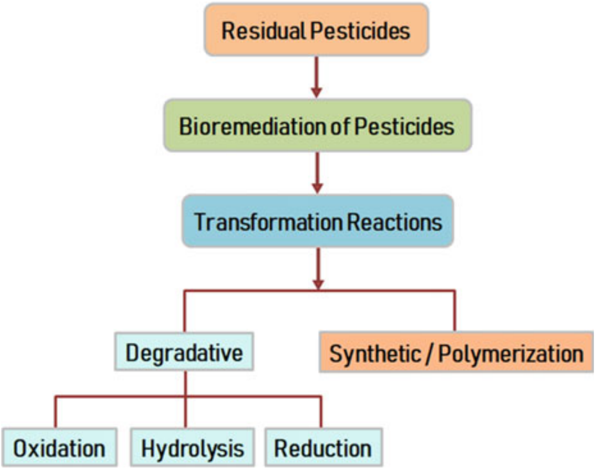

Fig. 2.4 Predominant transformation reactions involved in degradation of various pesticides

oxidases), (2) hydrolytic transition mediated by hydrolytic enzymes (hydrolases) that cleaves bonds of substrate by adding hydrogen or hydroxyl group from water molecules, (3) reductive transformation mediated by reductive enzymes (nitroreductase) by which removal of anion occurs by reduction, and reductive dehalogenation is mediated by reductive dehalogenase enzyme (Commandeur and Parsons 1994; Odukkathil and Vasudevan 2013), and (4) synthetic/conjugation reactions by which an exogenous or endogenous natural compound is added to the pesticide to facilitate mineralization.

Three enzymes were involved in the first few stages of the degradation of atrazine by *Pseudomonas* spp. strain ADP, which used atrazine as the sole carbon source (De Souza et al. 1996; Wackett et al. 2002). Most of the catabolic genes encoding these degradative enzymes were located on the plasmid (Nour et al. 2017; Nayak et al. 2018). Likewise, biodegradation of 2,4-D is regulated by genes carried on the plasmid (Don and Pemberton 1985). Studies have shown that mineralization and co-metabolism were the major mechanisms for further degradation of pesticides and their by-products (Boivin et al. 2005; Arora et al. 2012; Ye et al. 2018). On ingestion, inhalation, or absorption dermally, chlorpyrifos may be metabolized by the enzymes of cytochrome P450 that cause derylation (oxidative ester cleavage) of the chlorpyrifos and formed 3,5,6-trichloro-2 pyridinol (TCP) and diethylthiophophate (Komori et al. 1990). Chlorpyrifos degradation mainly leads to TCP, which is then degraded by bacterial enzymatic oxidation and hydrolytic reactions (Li et al. 2010b). TCP is broken down via the release of three chlorine molecules during its sequential dechlorination, in which one oxidation and two hydrolytic steps 3,6-dihydroxypyridine-2,5-dione (Li et al. 2010b; Ramakrishnan et al. 2011) were formed.

The degradation of 2,4-dichlorophenoxy acetic acid (2,4-D) was shown to have two different pathways (Amy et al. 1985). These two degradation pathways were mediated by *Pseudomonas* spp. and *Alcaligenes* spp. isolates, respectively (Amy et al. 1985). In one way, the sixth carbon is oxidized by the addition of the OH group, yielding 6-OH-2, 4-D, and followed by removal of acetate, resulting in the formation of 3,5-dichlorocatechol. In the second path, two carbon side chains are removed, resulting in glyoxylate and 2,4-DCP. The oxygenases synthesized by *Pseudomonas* spp. caused degradation of tetrachlorobenzene to trichlorocatechol by removing HCl from the compound (Sander et al. 1991). Mono- and dioxygenases were actively involved in the dehalogenation-mediated degradation of halogen-based pesticides (Braus-Stromeyer et al. 1993). Peroxidases synthesized by fungi and bacteria were reported to biodegrade pesticides and their derivatives. For example, the compound 3,4-dichloroaniline was converted to 4,4-tetrachloroazobenzene by peroxidases produced by soil microorganisms (Bordeleau et al. 1972). The peroxidases secreted by *P. chrysosporium* added on chlorine to 2,4-di-, trichlorophenol, 2,4,6-trichlorophenol and pentachlorophenol at their para positions and formed *p*-benzoquinone (Hammel and Tardone 1988). These peroxidases mineralized 2,4,5-TCP rapidly. Different reactions and enzymes involved in pesticides degradation are illustrated in Fig. 2.4.

2.8.1 Oxidoreductases

Oxidoreductases are a broad group of enzymes that catalyze the transfer of electrons from one molecule (redundant or electron donor) to another (oxidant or electron acceptor). Most of these enzymes require additional cofactors to function as electron donors, electron acceptors, or both. These enzymes have applications in bioremediation, during which they catalyze the oxidation/reduction reaction by electronically incorporating molecular oxygen (O_2). In these reactions, oxygen is reduced to water (H_2O) or hydrogen peroxide (H_2O_2).

A fungus *Cladosporium cladosporioides* was isolated from organophosphate contaminated soil, which showed the potential to use chlorpyrifos as the sole carbon source (Gao et al. 2012). The parent chlorpyrifos was first produced by hydrolysis of 3,5,6-trichloro-2 pyridinol (TCP) and diethylthiophosphoric acid (DETP). The hydrolysis product is further transformed by the breakage of the TCP ring, resulting in its complete detoxification (Chen et al. 2012). Likewise, Lu et al. (2013) isolated a bacterial strain called *Cupriavidus* spp. DT-1 responsible for the degradation of chlorpyrifos. In the degradation path, chlorpyrifos was first hydrolyzed to TCP, dechlorinated to 2-pyridinol, respectively, and then to the cleavage of the pyridine ring and further degradation. The *mpd* gene, which encodes the enzyme responsible for chlorpyrifos hydrolysis to TCP, was cloned and expressed in *Escherichia coli* BL21. Inoculation of chlorpyrifos-contaminated soil with strain DT-1 reduced chlorpyrifos and TCP at 100% and 94.3%, compared to 28.2% and 19.9% in uninoculated soil, respectively.

Oxidases constitute a subclass of oxidoreductase enzymes (Scott et al. 2008). The products of oxidation reactions often contain anionic hydroxyl or carboxyl substituents and are more polar and water soluble than parent pesticides. Glyphosate oxidase (GOX) is the best characterized oxidase involved in pesticide bioremediation (Scott et al. 2008). Most of the chloroaromatics molecules are converted by bacteria to chlorocatechol or chloroprotocatechuate, which become the starting substrate for subsequent reactions involving oxidative cleavage. Monooxygenases metabolize the xenobiotics by often enhancing their reactivity and/or the water solubility through the addition of oxygen atom. A two-component flavin diffusible monooxygenase family (TC-FDM) (Galan et al. 2000) is a monooxygenase that plays a role in the degradation of environmental pesticide residues. The cytochrome P450 family is another large group of monooxygenase enzymes that have a wide substrate range and have been reported to catalyze biochemically recalcitrant reactions, such as oxidation or hydroxylation of non-activated carbon atoms (Werck-Reichhart et al. 2000). An example of the use of cytochrome P450 in the bioremediation of herbicides is cytochrome CYP1A1 (also known as aryl hydrocarbon hydroxylase) from mammalian liver, which has been found to degrade atrazine, norflurazon and chlortoluron (Kawahigashi et al. 2005).

2.8.2 Hydrolases

Another group of enzymes commonly used in pesticide bioremediation is hydrolases (Zhongli et al. 2001). The presence of hydrolysable groups in a pesticide or xenobiotic molecule is an important factor in determining its anaerobic biodegradability. Hydrolases catalyze the hydrolysis of many major biochemical classes of pesticides (esters, peptide bonds, carbon–halide bonds, ureas, thiosters, etc.) and generally function in the absence of redox cofactors (Scott et al. 2008). Esterases are enzymes that catalyze the hydrolysis of carboxylic esters (carboxyestrases), amides (amidases), phosphate esters (phosphatases), etc. (Bansal 2012). Many insecticides (organophosphates, carbamates, and pyrethroids) contain the carboxylic ester component, and the enzymes that can hydrolyze this type of ester bond are called carboxyl-esterases. In this group, phosphotriesterases (PTEs) are one of the most important classes (Chino-Flores et al. 2012). The first phosphotriestrase was isolated from *Pseudomonas aeruginosa* strain MG, and this enzyme showed high catalytic action against organophosphate pesticides. PTEs are encoded by genes called *opd* (organophosphate-degrading), and the *opd* genes were first characterized in *Flavobacterium* strain ATCC 27551 (Latifi et al. 2012). These enzymes distinctively hydrolyzed phosphorus bonds such as P-O, P-F, P-NC, and P-S, and the hydrolysis mechanism involved a water molecule at the phosphorus center. This enzyme showed its potential to eliminate organophosphorus pesticide-contaminated environments (Ortiz-Hernandez et al. 2003).

Microbial degradation of organophosphorus compounds by hydrolysis of P-O-alkyl and P-O-aryl bonds is considered to be the most important step in detoxification (Sogorb and Vilanova 2002). Analogous phosphor-monoesterase and diesterase, which degraded methyl and dimethyl phosphate, respectively, have been reported in *Klebsiella aerogenes* (Wolfenden and Spence 1967). Organophosphorus hydrolase (OPH) and organophosphorus acid anhydrolase (OPAA) are one of the most widely studied organophosphorus degrading enzymes (Mulbry and Karns 1989; Singh et al. 1999). In bacterial enzymes, OPH from *P. diminuta* has a wide range of substrate specificity (Manavathi et al. 2005). The highly active OPAA molecule from *Alteromonas undina* is composed of a single polypeptide with a molecular weight of 53 kDa (Cheng et al. 1993). However, another OPAA was isolated from *Alteromonas* spp. JD6.5 is composed of 517 amino acids with a molecular weight of 60 kDa and has been reported to play an important role in cellular dipeptide metabolism (DeFrank and White 2002).

Other structurally and functionally distinct organophosphorus degradation enzymes were three unique parathion hydrolases, which were characterized from Gram-negative bacterial isolates. An exclusive phosphotriesterase has been characterized from *Nocardioides simplex* NRRL B-24074. Another novel phosphotriesterase HOCA (Hydrolysis of Caroxone) was isolated from *P. monteilii* (Horne et al. 2002a, b). This enzyme is required by the host for phosphate metabolism and was suggested to originate from phosphodi- or monoesterase. The enzyme phosphonatase was found capable to degrade phosphonates was isolated from *B. cereus* (La Nauze et al. 1970). One more interesting enzyme involved in the degradation of phosphonates is C-P lyase refined from *Pseudomonas* spp. GLC 11 (Selvapandiyan and Bhatnagar 1994).

Numerous examples of hydrolases with applications in the bioremediation of pesticide residues include carboxylesterases, phosphotriesterases (Oph and OpdA), and haloalkane dehalogenases (LinB, AtzA, and TrzN) (Mohn and Tiedje 1992). Halidohydrolases use water to replace halogens with hydroxyl groups, and this is affected by the number and types of halogen substituents and by the presence of unsaturated carbon-carbon bonds. Use of either oxygenases or hydrolases to dehalogenate pentachlorophenol (PCP) illustrates the potential for microbes to develop diverse mechanisms for metabolizing such chemicals. A carbofuran degrading methylotroph strain ER2 initiated the attack on carbofuran by hydrolyzing the carbamate linkage, producing 7-phenol carbofuran, CO_2, and methylamine (Chaudhary and Ali 1988).

2.8.3 Lyases

In the absence of redox cofactors or water, the enzyme lyase catalyzes the cleavage of bonds, including carbon-carbon bonds such as pyruvate formate-lyase (PFL) (Sawers 1998) and carbon bonds with phosphorus, oxygen, nitrogen, halides, and sulfur. The haloelimination reaction catalyzed by lindane hydrochlorinase is active against the insecticide hexachlorocyclohexane (Nagata et al. 1993) have been linked to the aminomethyl phosphonic acid (MPA) is susceptible to lyse-producing bacteria (Zhang et al. 1999), and the use of MPA as a source of phosphorus by *Pseudomonas putida* has been observed (Cook et al. 1978). *Arthrobacter* sp. GLP-1 and *Pseudomonas* sp. PG2982 degraded glyphosate and produced sarcosine (*N*-methylglycine) by C-P lyse activity (Dick and Quinn 1995). *Rhizobium meliloti* has also been reported to degrade glyphosate by lyase activity (Park and Hausinger 1995). A similar pathway has been observed in *Arthrobacter atrocyaneus* (Pipke and Amrhein 1988) and *Flavobacterium* sp. (Pipke et al. 1987). *Enterobacter cloacae* strain K7 possessed C-P lyase activity and degraded glyphosate to sarcosine, which was subsequently oxidized to glycine (Kryuchkova et al. 2014).

2.8.4 Synthetic Reactions and the Formation of Immobilized Residues

Synthetic reactions covalently attach pesticide or pesticide transformation products to other organic molecules. For example, molecules which contain reactive nucleophilic groups, amino ($-NH_2$), hydroxyl (-OH), or carboxyl (-COOH) can participate in these reactions. Usually, all products of synthetic reactions are larger than the parent compounds but the mobility and bioavailability of the reaction products are variable, depending on the size of the molecule to which the residue is attached. For example, the methylation of the hydroxyl group in PCP by fungi produces a volatile methoxy derivative, tetrachloroanisole (Cserjsei and Johnson 1972). This is a rare example of reaction products being much more volatile, and therefore, more mobile in the environment than the parent compound. Activated transformation products can react together to form polymers. For example, the hydrolysis of the phenylurea herbicides produced chlorinated anilines that readily dimerized to form azobenzene and other condensation products (Bartha and Pramer 1970).

The covalent attachment of pesticide residues to soil humus also effectively immobilized the pesticide residues to the soil matrix and greatly reduced their bioavailability and movement through the soil profile. Bound pesticide residues perhaps may be slowly released during turnover of organic matter. Microbial population and abiotic mechanisms in soil often transform parent pesticide residues in humus to intermediate compounds that are subsequently incorporated into soil organic components, and this phenomenon is often noticed in the case of polyaromatic hydrocarbons, polychlorinated biphenyls, pentachlorophenol, etc. (Bossert et al. 1984; Chauhan et al. 2008). Polymerization of various phenolic compounds was found less toxic after their copolymerization with natural soil components such as syringic acid (Bollag et al. 1988). Some pesticide residues could be immobilized to soil organic material via “oxidative coupling.” In this process, the parent compound is enzymatically transformed by oxidation to a reactive intermediate, which rapidly reacts with soil organic matter. For example, laccase and peroxidase enzymes can catalyze the oxidative coupling of oxidized 2,4-dichlorophenol with fulvic acid and humic acids (Nannipieri and Bollag 1991). It has been suggested that the covalently attached residues are not bioavailable or mobile and, therefore, are effectively detoxified.

2.9 Genetic Engineering of Microbes to Enhance Degradation of Pesticides

The production of extracellular enzymes by soil microorganisms can be enhanced by genetic modification to degrade residual pesticides in the soil (Scott et al. 2008; Sindhu et al. 2010a; Bass and Field 2011; Riya and Jagapati 2012). Various measures can be taken to reduce the stress of bacteria that are constantly exposed/stressed by pesticides available under soil conditions. Various mechanisms involved in pesticide detoxification include: (1) increasing the copy number of genes that allow the organism to produce more protective enzymes such as esterases, glutathione transferases, and other oxidases; (2) reducing the number of receptors that bind to pesticides; and (3) mutating single genes that lead to pesticide resistance. When different pesticides are used sequentially in the field, bacteria may adapt to or develop resistance to other pesticides, leading to the development of strains with multiple pesticide resistance properties. When adaptation occurs through genetic mutations, the pesticide-resistant organism may also tolerate other xenobiotic compounds that have mechanisms of action similar to those already exposed to pesticides; such resistance is called cross-resistance.

Genetic engineering of endophytic and rhizospheric bacteria for the degradation of toxic compounds in the soil is considered to be one of the most promising new technologies for the remediation of contaminated environmental sites (Divya and Kumar 2011). To select the appropriate strain for genetic recombination and its subsequent inoculation into the rhizosphere, three criteria have been recommended: first, the strain should be stable after cloning, and the target gene should have high expression; second, the species must be tolerant or insensitive to the contaminated/ toxic compound; and third, these strains may establish and live in specific plant rhizosphere (Sindhu and Dadarwal 2000; Huang et al. 2004). In general, most bacteria in the rhizosphere show only limited ability to reduce organic pollutants. With the development of molecular biology, the genetically engineered rhizobacteria with the contaminate-degenerating genes are constructed to enhance the rhizoremediation (Glick 2010).

The microbial PCB-degradation system consists of two main metabolic stages: (1) anaerobic reduction dechlorination, where PCBs are converted to low chlorinated congeners; and (2) aerobic breakdown of the biphenyl structure in low-halogenated congeners (less than five chlorines), resulting in chloro-HOPDA (2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoate), chlorobenzoic acid, ring opening, and complete mineralization (Passatore et al. 2014). The aerobic rhizobial degradation of PCBs is usually carried out by the oxidative biphenyl pathway encoded by the *bph* genes, which include the multicomponent dioxygenase (*bph*A, E, F, and G), dehydrogenase (*bph*B), secondary dioxygenase (*bph*C), and a hydrolase (*bph*D) in other bacteria. Genomic DNAs from *Rhizobium* and *Bradyrhizobium* have been found to be strongly hybridized with the *Comamonas testosteroni*-derived *bph*ABC gene probe, suggesting the presence of a similar oxidative degradation system in rhizobia (Damaj and Ahmad 1996; Ahmad et al. 1997). Molecular mechanisms involved in the degradation of certain pollutants, such as trichloroethylene (TCE) and PCBs, have also been studied.

Gong et al. (2016) reported the metabolic engineering of *Pseudomonas putida* KT2440 for complete mineralization of methyl parathion. The strain was found genetically stable, and its growth was not inhibited. Furthermore, engineering of the strain showed a high degradation of methyl parathion (50 mg kg^{-1} soil) in soil samples. In another study, genetically engineered *Pseudomonas putida* X3 strain was reported to utilize methyl parathion as the sole source of carbon for growth. Engineered X3 strain hydrolyzed methyl parathion to *p*-nitrophenol. However, no further degradation was observed, which may be due to the absence of *p*-nitrophenol degrading genes in the X3 strain (Zhang et al. 2016).

In general, the combination of multiple OP degrading genes causes pesticides to be converted into intermediate metabolites and eventually into small-molecule and non-toxic substances (Barman et al. 2014; Acharya et al. 2015). OP-degrading genes involved in the biodegradation and detoxification of OPs include *opd*, *opd*E, *mpd* and *opd*A (Somara et al. 2002). During microbial degradation of atrazine, the degrading microbe *Citrichoccus* spp. strain TT3 possessed the genes *trz*N, *atz*B, and *atz*C, all of which were involved in the biodegradation process of atrazine (Yang et al. 2018).

Similarly, genes responsible for the degradation of chlorobenzene acids, other halogenated pesticides and toxic wastes have been identified. Friello et al. (2001) successfully produced *Pseudomonas*, a multiplasmid containing oxidizers of aliphatic, aromatic, terpenic, and polyromatic hydrocarbons. *Pseudomonas putida* that contained XYL and NAH plasmid, as well as hybrid plasmid derived by the recombinating components of CAM and OCT developed by conjugation could degrade camphor, octane, salicylate, and naphthalene (Sayler and Ripp 2000). Degradation of environmental pollutants by genetically engineered microorganisms is primarily focused on genetically engineered bacteria using various genetically engineered technologies, such as modification and substrate specificity by *Comamonas testosteroni* strain VP44 (Hrywna et al. 1999). For the degradation of polychlorinated biphenyls, chromosomally located PCB catabolic genes of *R. eutropha* A5, *Acromobacter* spp. LBS1C1, and *A. denitrificans* JB1 were transferred into the heavy metal–resistant strain *R. eutropha* CH34 by natural conjugation (Menn et al. 2008).

For heavy metals, Sriprang et al. (2003) introduced *Arabidopsis thaliana* gene for phytochelatin synthase (PCS; PCSATt) into the *Mesorhizobium huakuii* subspp. *rengei* strain B3, which established a symbiosis between the *M. huakuii* subs. *rengei* strain B3 and *Astragalus sinicus*. The gene was expressed to produce phytochelatins, and it accumulated CD^{2+}, under the control of the bacteroid-specific promoter of the *nif*H gene (encoding Fe protein, dinitrogenase reductase) (Sussman et al. 1988). Finally, the use of genetically engineered microorganism (GEM) strains as an inoculum during seeding avoids problems related with competition between strains in mixed culture. However, there is much controversy about the release of such genetically engineered microbial strains into the environment, so field testing of these organisms must be delayed until safety and environmental damage issues are resolved (Wackett 2004).

2.9.1 Adaption and Development of New Degradation Capabilities

Microorganisms can occupy an infinite variety of niches in the environment because of their rapid growth rate, large numbers, and small size. The breadth of selective pressures experienced by these microbes provided them the opportunity to develop tremendous biochemical diversity. There is an important selective advantage in the ability to utilize a new substrate in otherwise carbon-limited soils. A number of microorganisms possess the enzymes required to degrade xenobiotic molecules, whose structures are apparently foreign to anything previously seen in nature. The degree of "foreignness" is actually variable from pesticide to pesticide. There appear to be no natural counterparts for many pesticide structures, for example, the chlorinated hydrocarbons DDT, mirex, and dialdrin. However, some seemingly unusual structures found in pesticide molecules are also found in nature. For example, soil fungi can produce large amounts of various halogenated aromatic compounds (De Jong et al. 1994).

Microorganisms growing at the expense of a xenobiotic (i.e., foreign to nature) pesticide can frequently be isolated from soil only a few years after the introduction of the chemical at particular field. Some soils degrade pesticide much more rapidly after repeated applications, suggesting that some kind of adaption and change in the properties of the degrading microflora may have taken place. This observation has prompted speculation that the biodegradation of newly introduced pesticides becomes possible because of the rapid evolution and selection of catabolic phenotypes (van der Meer et al. 1992). Enzyme involved in the metabolism of natural chemicals may have sufficiently low substrate specificity, and they may also attack xenobiotic analogs. Therefore, pesticides can also be degraded co-metabolically by enzymes with low substrate specificities. For example, the oxidative lignin-degrading system of *Phanerochaete chrysosporium* is remarkably nonspecific and can also degrade a very wide variety of pollutants (Yadav and Reddy 1993). Under suitable environmental conditions, all-natural compounds can be catabolized, because the evolution of biosynthesis of natural chemicals was sufficiently slow to permit the parallel evaluation of new catabolic functions required for their degradation.

A number of genetic mechanisms may be involved in the evolution of new pesticide-degrading capabilities in soil. Microorganisms can in principle acquire new catabolic capabilities: (1) through the recruitment of genes encoding pesticide-degrading enzymes; (2) by modification of substrate specificity; and (3) regulation of preexisting enzymes which have other functions (van der Meer et al. 1992). Genes encoding various enzymes, which are involved in the degradation of organic pollutants, are frequently located on the plasmids and sometimes in transposons (DNA elements able to replicate and insert new copies in the genome). These mobile genetics elements can be exchanged between microorganisms in soil and water (Fulthorpe and Wyndham 1992).

2.9.2 Mobilization of Genes to Enhance Catabolic Steps in Pesticide Degradation Pathway

Genes encoding various enzymes, involved in the degradation of a large number of pesticides, are located on the plasmids (Table 2.3). For example, the plasmids contain the genes for degradation of phenoxyalkanoic and thiocarbamate herbicides, methyl carbamate, and organophosphorus insecticides. Degradative plasmids (DP) may encode a complete degradative pathway such as those for toluene or

Table 2.3 Plasmids involved in degradation of chlorinated hydrocarbons

Plasmids	Compound degraded	Size of the plasmid
181 PKFI	4-Chlorobiphenyl	82 kb
P44204	2-Monochloropropionic acid	53 kb
185pAC21	1,4-Dichloro biphenyl	65 MDa
pAC27	3-Chloro-benzoic acid	110 kb
189	2,4-D	50–150 MDa
190	PCP	80–100 kb
194	Chlorotoluene	72 MDa
196 p401	Fluoracetate	44 MDa
Tol	Toluene	–

Adapted from Chaudhry and Chapalamadugu 1991

xylene Tol (pWWO) catabolic plasmid or partial degradative steps such as those for naphthalene (NAH) to salicylate (SAL) and camphor (CAM). In addition, genes involved in a catabolic pathway are frequently clustered together, facilitating correlation and transfer among microorganisms. Moreover, genes involved in the degradation of pesticides may evolve in different microorganisms followed by their assembly on the same plasmid in a single organism through horizontal gene transfer. There are now evidences indicating that transposable elements cause rearrangement of genetic material and may be transferred between unrelated strains and ultimately resulting in the construction of new degradative plasmids (Tan 1999).

The movement and rearrangement of sections of DNA through genetic recombination or transposition of DNA can modify the regulation and expression of catabolic genes. Success of a catabolic pathway depends upon its catabolic components and regulatory elements particularly the promoters. Understanding of the behaviors of such regulatory promoters in and off the field is the prerequisite for engineering of the catabolic pathways for pesticide bioremediation. Genetic engineering techniques have been used to construct plasmids that code for the catabolism of halo-aromatic compounds (Rojo et al. 1987; Ramos et al. 1987). The transmissible nature of genes specifying dissimilation of xenobiotic compounds may lead to a rapid spread of degradative capabilities in microbial population, once a degradative plasmid has evolved.

The transfer of these catabolic plasmids may be involved in the adaption of the soil microflora and development of enhanced degradation capability. The 2,4-D degradation encoding plasmid pJP4 was transferred into a wide variety of bacteria including *E. coli*, *Rhodopseudomonas sphaeroides*, *A. tumefaciens*, *Rhizobium* spp., *P. fluorescens*, *P. putida*, and *Acinetobacter calcoaceticus* (Don and Pemberton 1981). Haugland et al. (1990) reported that mixtures of the herbicides 2,4-D and 2,4,5-T were toxic to *P. cepacia* strain AC 1100 (2,4,5-T degrader) and *Alcaligenes eutrophus* strain JMP134 (2,4-D degrader) due to production of inhibitory metabolites. A derivative of strain *P. cepacia* AC 1100 was constructed by the transfer of 2,4-D degradative plasmids pJP4 from *Alcaligenes eutrophus* strain JMP134. The new strain RHJ1 efficiently degraded mixture of 2,4-D and 2,4,5-T. Such microbial populations can be of immense value in bioremediation of persistent chlorinated compounds especially the PHAs and PCBs.

Gottschalk and Knackmuss (1993) designed a mixed culture of *Pseudomonas* spp. N31 and B13 that oxidized 4-chloro-2-nitro phenol and its toxic metabolite 4-chlorophenol involving oxygenase of *Pseudomonas* spp. N31 and 4-chlorocatechol dioxygenase of *Pseudomonas* spp. B-13, respectively. However, use of bacterial consortia resulted in the formation of undesirable amount of dark colored toxic metabolites. The presence of chlro- and methyl-arenes at that site-induced meta- and ortho-pathways caused misrouting of methyl- and chloro-arenes. This leads to substrate incompatibilities and production of metabolic dead-end products, which are toxic to the bacterial cells culminating in the cessation of mineralization activity.

2.9.3 Modification of Substrate Specificity by Manipulations of Enzymes

Small modifications to a catabolic gene sequence may alter the properties of the encoded enzymes. The enzyme substrate specificity or gene transfer specificity can be altered by substitutions of single base pairs or point mutations. Deoxygenases and dehalogenases are the two enzymes that have been modified by enzyme bioengineering. By comparison of amino acid sequences and models of tertiary structures of haloalkane dehalogenases, their active centers were identified and selected as possible targets for site directed mutagenesis. Erickson and mondello (1993) reported that biphenyl deoxygenases of *Pseudomonas* spp. LB 400 possess broad substrate specificity, whereas the high efficiency of enzyme was reported in *P. pseudoalcaligenes* strain KF707. The enzyme showed 95.6% amino acid sequence similarity in the large subunit, but had different substrate specificities. A site-directed mutagenesis of four nucleotides that cause a change in these four amino acid sequences of biphenyls *bph*A gene (encoding dioxygenase reductase component) was performed. It resulted in a novel dioxygenase that combined the broad substrate specificity of *Pseudomonas* spp. LB 400 and efficiency of homologous enzyme from *P. pseudoalcaligenes* KF 707 to degrade a range of di-, tri- and tetra-para-substituted polychlorinated biphenyls. Bosma et al. (2002) reported heterologous expression of haloalkane dehalogenase gene *dha*A of *Rhodococcus* spp. M15-3 by mutating the enzyme at two amino acid levels by Cys 176→Tyr and Tyr 273→Phe (phenylalanine) substitutions. The mutated enzyme was placed under the control of a constitutive promoter in 2,3-dichloro-1-propanol, employing *Agrobacterium radiobacter* AD1. The engineered pathway could completely degrade TCP (1,2,3-trichlorpropane), a waste product from epichlorhydrin manufacture.

2.9.4 Rapid Evolution Through Duplicated Genes

One gene copy can accumulate mutations and yield enzymes with altered properties, while the other copy of the gene may continue its normal function. A number of bacterial genes have been used to modify plants genetically and make them resistant to specific herbicides. For example, the herbicide bromoxynil inhibits photosynthesis and uncouples oxidative phosphorylation. A gene originating from *Klebsiella pneumoniae* subspp. *ozaenae* encoding a bromoxynil-modifying nitrilase was used to generate bromoxynil-resistant transgenic plants (Stalker et al. 1988). Moreover, in situ pesticide degradation rates can be manipulated by modifying the soil environment. For example, the plant rhizosphere can accelerate pesticide degradation presumably through enhancement of microbial activity via the provision of carbon in the form of root exudate or modification of O_2 concentrations. The organophosphorus insecticides diazinon and parathion were mineralized about twice as fast in soil containing a bush bean (*Phaseolus vulgaris*) plant as in soil without a plant (Hsu and Bartha 1979).

Focht and Reineke (2002) studied application of hybrid bacterium containing sequences for complete degradation of polychlorinated biphenyls, Aroclor1221 in a soil microcosm and found that both introduced bacterium and native microbes remained unaffected. Although these studies demonstrated the potential of genetically engineered microorganisms in bioremediation of environmentally hazardous compounds, there is also an example of GEM adversely affecting the indigenous microbe during degradation of 2,4-D. Short et al. (1992) reported that genetically engineered *P. putida* PP301 (pR0103) accumulated 2,4-dichlorophenol in arid soils affecting an indigenous fungus.

2.9.5 Development of Transgenic Plants with Enhanced Pesticide Degradation

To minimize the application of pesticides, transgenic plants have been developed, which express the Bt (*Bacillus thuringiensis*) toxin. Such transgenic plants have been released for cultivation in cotton, corn, brinjal, etc. Due to the cultivation of these transgenic crops, lower amounts of pesticides are applied for control of pathogens and insects. There is also possibility to develop transgenic plants with enhanced ability to detoxify persistent organic compounds. To increase the natural abilities of plants in the removal/detoxification of organic compounds, different cytochromes have been introduced into plants, which are considered to be responsible for the first phase in plant detoxification. Doty et al. (2000) showed the enhanced metabolism of halogenated hydrocarbons in transgenic plants containing mammalian cytochrome P450 2EI. Similarly, overexpression of a basic peroxidase in tomato (Wevar Oller et al. 2005) resulted in increased phenol phytoremediation, thereby supporting the hypothesis that apart from P450 cytochromes, peroxidases are also involved in the first phase of detoxification.

The development of GM tobacco, which overexpressed glutathione-*S*-transferase for the phytoremediation of chloroacetanilide herbicide (Karavangeli et al. 2005), addresses the second phase in plant detoxification, namely the conjugation of the activated compound. Similarly, the biodegradation of explosives by transgenic plants expressing pentaerythritol tetranitrate reductase (French et al. 1999) is the classic example of the exploitation of a bacterial gene for phytoremediation. In addition, plants have been constructed that express bacterial enzymes capable of TNT (trinitrotoluene) transformation and RDX degradation (hexahydro-1,3,5-trinitro-1,3,5-triazine), an explosive nitroamine widely used in military and industrial applications (Bruce 2007).

The vital missing step in the efficient degradation of hydroxylated PCBs by plant cells is the opening of the biphenyl ring by the bacterial enzyme encoded by *bph*C, which is responsible for the cleavage of hydroxylated PCB derivatives, even those formed by plants. Francova et al. (2003) reported the generation of tobacco plants carrying the *bph*C gene. Subsequent testing of seeds for their ability to germinate in high concentration of PCB showed significant germination. Besides this, improved substrate specificity has been achieved by the expression of bacterial biphenyl-chlorophenyl dioxygenase gene in tobacco (Mohammadi et al. 2007).

2.10 Future Perspectives

Insect pests, pathogenic fungi, and weeds have always responded to the chemical pesticides sprayed on them by developing resistance (Sindhu et al. 2010b, 2016). Indeed, repeated use of the same agrochemicals is considered to be the main cause behind the development of resistance (Miyata and Saito 1984; Heap 2014). On the contrary, resistance has become the incentive for innovation on their fight against the enemies of the crops (Jeschke 2016). For instance, widespread pest resistance to the old organochlorine insecticides was the main reason that led to ban DDT and cyclodienes. Their replacement with cholinesterase inhibitors was envisaged well before the sublethal effects on birds of prey were noticed. The search for molecules with different mode of actions in subsequent years was a necessity to confront resistance mechanisms among insect pests. Despite this, pest resistance has developed within a few years of the introduction of the novel neonicotinoids and diamide insecticides (Uchiyama and Ozawa 2014; Bass et al. 2015). Even more dramatic change has been the development of resistance against glyphosate by many weeds due to overuse of this herbicide in genetically modified crop varieties of cotton, soybean, and maize (Shaner 2000; Beckie and Hall 2014; Dahiya et al. 2019b).

To address the resistance problem, the pesticide chemical industry is looking for the production of novel chemicals to control the crop pests (Jeschke 2016). In recent years, huge growth has been noticed in the marketing of neonicotinoids, phenylpyrazoles and diamide insecticides, strobilurin fungicides, and 4-HPPD herbicides

(Jeschke 2016). There are evidences to demonstrate that the routine application of insecticides and fungicides have little or no significant increase in today's crop yields (Lechenet et al. 2017). The solution to this problem, therefore, is not to add new chemicals to the already saturated pesticide market but to find different ways of combating this war against weeds and pests (Owen et al. 2015; Chauhan et al. 2017; Phour and Sindhu 2019), without affecting the ecosystem resources and services provided by soil biota and pollinators, which are essential for agricultural productivity (van Hoesel et al. 2017).

Two types of GM crops have been developed, one for pest control (i.e., Bt-crops) and the other for herbicide resistance (glyphosate-GM crops). Bt-crops produce the toxin of *Bacillus thuringiensis*, which are very effective against caterpillars and grubs (Hutchison 1999) without damaging natural enemies of the pests (Thomazoni et al. 2010), thus avoiding the use of insecticide sprays against such pests (Wadhwa and Gill 2007); however, this has created secondary pests that require the use of other insecticides. By contrast, glyphosate-GM crops are resistant against this herbicide, so the farmers can apply glyphosate products without harming the crop plants. Unfortunately, this has led to an overuse of the herbicides that fostered rapid development of resistance among various weeds plus contamination of the environment (Powles 2008; Beckie and Hall 2014; Sindhu and Sehrawat 2017).

2.11 Conclusion

Bioremediation has tremendous potential for the remediation of contaminated soils infested with pesticides. Rhizosphere microorganisms play an important role in the degradation of various pesticide residues. A consortium of microorganisms thrives, which degrades pesticide contaminants into a simple chemical compound that may be used by the crop plant and reduce the use of chemical pesticides in agriculture. The continuous degradation of chemicals by enzymatic reactions represents the most important strategy with high bioremediation efficiency. These biocatalysts may be formed in large numbers by genetic engineering technology, expression of enzymes, or indigenous organisms that are used in agriculture to remove pesticides from contaminated sites. Further research on the biodegradation or biotransformation mechanisms in plants, bacteria, fungi, or algae is essential to improve bioremediation strategies. An in-depth study of the microorganisms is needed to excavate the pesticide degradation process and the mechanisms by which their enzymes are used.

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Chapter 3
Microbial Indicators of Bioremediation: Potential and Success

Sarita K. Yadav

Contents

Abstract The human race has been involved in lot many activities on energy reservoirs, seeking the commercialization of agriculture and swift in industrial growth apart from mining activities, which has led to environmental pollution by many folds. There are a number of reasons for this environmental pollution; ingress of heavy metals into ecosystem, nuclear wastes as part of residue created due to nuclear energy power stations or atomic research activities, uncontrolled utilization of pesticides in our farmers, greenhouse gases and hydrocarbons generated due to various human activities are to name a few of them. If bioremediation activities are to be carried out successfully, they require a lot of time, but time and again have proved to be successful. In both ex situ and in situ ways, it is possible to carry out bioremediation.

S. K. Yadav (✉)
Regional Centre of Organic Farming, Department of Agriculture and Cooperation, Ministry of Agriculture and Farmers Welfare, Government of India, Jabalpur, Madhya Pradesh, India

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Keywords Environment · Pollution · Bioremediation · Eco-friendly

3.1 Introduction

Sustainability on this planet is dependent on the resources which Mother Nature provides us. However, human has failed in the utilization of the available resources in a justified and environment friendly manner. Human activities led to the release of enormous quantities of toxic compounds which include both organic and inorganic. Deliberated and well-regulated industrial emissions may occur through industrial emissions or chemical or oil spills which may occur accidentally. These toxic compounds create irreversible contamination in various ecosystems.

In the recent times, it is well understood that the contaminated sites act as threat not only for life on the planet but also it has adverse effects on the environment. Hence, efforts have been made in this direction all over the world in an effort to make a world better place to live in (Baker and Herson 1994; Kensa 2011; Vidali 2001).

3.2 Bioremediation: A Better Approach

Removal of the contaminated soil and taking it out to a landfill is the conventional method which is still being used to control the contaminants of an area. Although, this technique may provide an initial depiction that the problem of contamination is being solved. But it has the potential of creating even greater risks in the process of digging the contaminated soil, dealing with the contaminated soil and carrying out the material to the destination i.e. a selected landfill. The transportation of the contaminated material may also be quite tedious and may cost hugely financially. Nowadays, various methods like introduction of contaminants to ignition at extreme temperature along with several chemical methods are being used effectively to reduce the contamination levels, but they find a much low acceptability on the public front due to technical complexity and exposure to contaminants. In this scenario, bioremediation emerged as an option in terms of destroying or converting the pollutants into much less harmful ingredients using natural biological activity (Evanko and Dzombak 1997; Gómez Orea 2004; Kensa 2011; Prasad 2004; Vidali 2001).

The process of bioremediation is an environment and expenditure friendly approach to retrieve the ecosystems which have been polluted due to human activities. Bioremediation is a combination of various techniques and methods which helps in achieving successful results (Abatenh et al. 2017; Azubuike et al. 2016; Verma and Jaiswal 2016).

George Robinson (US Microbics 2003) is the pioneer for initiating the microbes' usage for the process of bioremediation. During 1960s, during the incident of oil spill along the coast of Santa Barbara, California, microbes were used by him. In the 1980s, the usage of bioremediation techniques has considerably increased in the cases of oil spills as well as hazardous wastes (Shannon and Unterman 1993). Each ecosystem has a set of native microorganisms which are well acclimatized in the respective system. The same stands true in the case of soil microbes. These well-established indigenous soil microbes carry out an extreme important role in which they perform as the agents of biochemical reactions and enable the transformation of complex (organic) into smaller (inorganic) compounds. This whole process of transformation is defined as mineralization. The property of ionic exchange facilitates the microbes to get adsorbed to soil particles, as soil particles possess a negative charge, thus soil and bacteria are held together by ionic bond (Killham 1994). Microorganisms assist in the process of bioremediation by either destroying or immobilizing waste materials (Shanahan 2004). The processes of mineralization, transformation and alteration of the hazardous chemicals are carried out for the detoxification (Shannon and Unterman 1993). Natural bioremediation was also being used by several civilizations, but now it is dealt with a scientific, systematic approach of the same. The reactions which occur in the process of bioremediation involve the release of energy in the form of redox reaction inside the microbial cell. Various water bodies, viz. underground water, soil, lagoons etc. can be sanitized by using various bioremediation methods. The oil spill in the water bodies occurs quite frequently due to various activities like discharge of crude oil from tankers, platforms near the shore, assembly for drilling and wells, spills of refined petroleum products (such as gasoline, diesel), bunker fuel spillage and haphazard discharge of waste oil in the sea (Adams et al. 2015). Most extensive and successful application of bioremediation was Alaska oil spill cleanup after Exxon oil spill (Boopathy 2000; Katyayan 2019). Oil contamination creates havoc in a tremendous manner to the environment. The penetration of oil into sea creatures reduces the ability of insulating themselves to a great extent, thus making them more susceptible to the temperature fluctuations. Also their ability of keeping themselves buoyant in water reduces significantly. These alterations make the survival of sea organisms very tough. The strong smell of oil makes it difficult for babies and mothers to locate each other. Eventually, babies are left on their own leading to their deaths (Hogan 2008). It even disables a bird from flying, preventing it from foraging, resulting in dehydration and metabolic imbalance or escaping from predators. The ingestion of oil leads to disabled liver and kidney function. It is difficult to protect the birds from dying. It is suggested that only 1% of birds affected by oil spill are able to survive (Dunnet et al. 1982). The oil spill is equally harmful for humans too. In 2013, alone, in such two incidences, water supply for 3000 people was contaminated in Miri, Malaysia.

In 2000, around 80,000 people in Coca, Ecuador, similarly, Springs were contaminated in Clark County, Kentucky in 2010. Tourism is also affected adversely due to contamination, which creates an economic impact (Yang et al. 2009).

The adverse effects of the spilled oil can be easily judged from the fact that once the oil seeps into soil, the ability to support the growth of the plants is significantly reduced. This leads to an increase in the accumulation of the heavy metals causing adverse effects. Once the heavy metals enter the food chain, they have extreme toxic effects. It may also damage nerves, liver and bones along with blocking functional groups of vital enzymes (Moore 1990; Ewan and Pamphlett 1996).

The soils in which contaminants are associated with soil particles and their presence can also be seen in soil liquids and in the soil atmosphere, i.e. multiphasic environments, then an interdisciplinary approach has to be considered (Boopathy 2000).

It is well known that the microorganisms are cosmopolitan due to their amazing metabolism. Thus, they act as significant solution givers to a wide range of polluted habitats by carrying out the biodegradation and bioremediation activities, provided that environmental conditions are suitable for their growth and metabolism (Abatenh et al. 2017; Azubuike et al. 2016; Verma and Jaiswal 2016). Microorganisms stand out over other biological tools for the removal of pollutants in various ecosystems, due to their fast growth and metabolic activities (Demnerovà et al. 2005).

A group of biological mechanisms, which degrades, detoxifies and mineralizes concentrated pollutants into harmless or significantly less harmful substances, can be easily utilized by other organisms.

3.3 Criteria for the Selection of Bioremediation Techniques

Pollutants are of various types: agrochemicals, dyes, heavy metals, greenhouse gases, hydrocarbons, chlorinated compounds, nuclear waste, plastics and sewage. Depending on the nature of pollutant, ex situ or in situ type of remedial may be considered (Frutos et al. 2012; Smith et al. 2015).

Before starting off any remediation project, it is extremely essential to check upon the method or technique based upon the selection and performance criteria. Nutrient and O_2 concentrations, pH and other abiotic factors are included in the performance criteria, which will ultimately lead the project towards success. There are a vast variety of bioremediation techniques available, but most of them are concentrated on remediation on hydrocarbons pollution, since it is the most common type of pollution (Frutos et al. 2010; Sui and Li 2011; Kim et al. 2014; Firmino et al. 2015; Abatenh et al. 2017).

Basically, bioremediation is done by the utilization of living organisms. Microorganisms, including both bacteria and fungi, are considered most suitable to degrade the environmental contaminants into less toxic or toxic-free forms. Even plants with some basic features too can be very useful for carrying out bioremediation successfully. The selected living organisms may be native to a contaminated area or they may be introduced to the contaminated area from somewhere else. These organisms have the ability to transform the contaminant compounds through the metabolic processes, which enables to convert the pollutants into harmless products. Bioaugmentation takes place when a set of suitable microbes are brought to the contaminated site, so as to boost the process of degradation. It is very much essential to provide favourable environmental conditions for the proper growth of the microorganisms, so that the degradation can be achieved at a faster pace. Ordinarily, bioremediation systems work under aerobic conditions, so as to permit microbial organisms to degrade even recalcitrant molecules (Colberg and Young 1995; Strong and Burgess 2008).

Advantages of Bioremediation

- Bioremediation is generally carried out using normal biological activities (Vidali 2001).
- This technique is low cost and requires low technical assistance (Vidali 2001).
- It also has acquired high public acceptance due to its environment friendly nature (Vidali 2001).
- When compared with the traditional methods of incineration, bioremediation methods are much more economical (Colberg and Young 1995).

Disadvantages

- The variety of contaminants on which bioremediation works is quite narrow, viz. chlorinated organic or high aromatic hydrocarbons are tough contaminants and are found to be resistant microbial attack. Hence, the process may not be able to see any degradation reaction, or if it takes place, it may be very slow (Colberg and Young 1995).
- The process is relatively long, sometimes may even require decades.
- Designing and implementation of a successful bioremediation programme require experience and expertise.
- The process and factors of bioremediation is not fully understood (Vidali 2001).

3.4 Types of Bioremediation

The location of contaminants plays a very important role in the feasibility of the bioremediation process. If the implementation of the process is to be done on site, then the process is called in situ.

Feasibility of bioremediation depends on the location of contaminants. Approaches for the implementation of bioremediation depend on whether the impacted soil to be treated is intact in the environment or it is to be excavated for treatment in an offsite facility. If on site, the term in situ remediation suffices and if offsite then ex situ terms are used (Kumar et al. 2011; Orji et al. 2012; Hamzah et al. 2013) (Fig. 3.1).

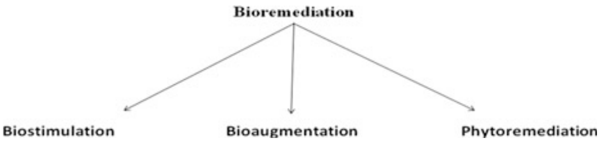

Fig. 3.1 Types of bioremediation

3.4.1 Biostimulation

The process of biodegradation in soil is dependent on various factors, viz. pH, temperature, moisture content, oxygen availability, soil properties and quantity in which contaminant is present (Atagana 2008; Al-Sulaimani et al. 2011; Bundy et al. 2002). In this process, limiting nutrients are added in various forms. Phosphorus, nitrogen, oxygen and carbon (electron acceptors) are added in the form of molasses or by optimizing conditions by aeration, etc., which stimulates the growth and activities of microbes (Elektorowicz 1994; Perfumo et al. 2007; Piehler et al. 1999; Margesin and Schinner 2001; Rhykerd et al. 1999).

This method was considered most suitable or appropriate for remediation of petroleum pollutants present in soil (Margesin and Schinner 2001). In the process of biostimulation, bioremediation process would be carried out by the microbes which are already present in the environment, along with well-established and distributed within the subsurface environment. The local geology of the subsurface plays an important role in proper distribution, growth and availability of the additives. If the subsurface lithology is tight and impermeable due to the presence of tight clays, etc., then it becomes a hurdle for the additives to spread thoroughly in the desired area. On the other hand, preferential pathways may be created by subsurface with fractures which may create advantages for the additives. Nutrients added to the subsurface may enable the growth of heterotrophic microbes which may not participate in the process of degradation; hence, rivalry or antagonism may be created between the resident micro flora (Adams et al. 2014).

3.4.2 Bioaugmentation

Bioaugmentation is the addition of microbes to supplement the indigenous populations of microbes. The approach of this process is that if the indigenous microbial populations present are incapable of degrading the complex mixtures, then the desired and capable microbes can be added (Leahy and Colwell 1990). Speed of decontamination is the primary factor for deciding any process, and if it is slow, then bioaugmentation can be followed (Forsyth et al. 1995). For the required process of degradation to be carried out successfully, it is essential for the seed microbes to have the features, viz. conduct the degradation process, genetic stability and viability should not be altered, should acclimatize in foreign habitats effectively (Atlas 1984; Goldstein et al. 1985).

Depending on the enzymatic capability of the microorganisms, they may be able to degrade contaminants of various properties (linear, branched or cyclic alkanes, mono-or polynuclear aromatics). With the assistance of biotechnological techniques, various studies are being carried out to select the microorganisms with the potential to degrade the compounds with toxic nature.

Microbial strains or consortia, which are acclimatized to the contamination site, lead to the successful bioaugmentation. Without the ability to compete with the indigenous microbes, predators and various abiotic factors of a particular microbe cannot succeed in carrying out the process of degradation. For the screening of microbe, it is also important to study the chemical structure and concentration of pollutants along with the availability of the contaminant to the microorganisms, the size and nature of microbial population and physical environment (Adams et al. 2015).

There is also a section of degradation which occurs only when oxygen is absent. Conversion of organic parts of degradable organic solid waste and refuse into biogas comprises of methane and carbon dioxide and a humus-like material by anaerobic bacteria like methanogens (methane-producing archeobacteria). This process is called Dranco process, which has been followed by countries like Brecht, Belgium and Salzburg, Austria (Katyayan 2019).

Nitrate is removed from water by the introduction of methylotrophic bacteria like *Methylophilus methylotrophus* to carry out the process of denitrification (Katyayan 2019). Eutrophication can be prevented by the removal of nitrate from waste water (Sharma 2016).

With the addition of methanol to bioreactor, growth of methylotrophs can be enhanced. To biodegrade chlorinated hydrocarbons present in effluents of pesticide industries, which manufacture DDT, heptachlor, chlordane, etc., bacteria like *Pseudomonas cepacia* are utilized. In Hong Kong, *Acetobacter liquefaciens* S-1 is used to treat waste water in textile and dye industries.

Multiple microbial communities are grown in bioscrubbers and biotrickling filters, to produce multilayered complexes called biofilms. Organic pollutants, along with gas streams, are passed through biofilms/biofilters, and the pollutants can be easily degraded. Due to great network area of fungal mycelia, greater surface area is created, thereby elimination of pollutants is done to a greater capacity. Thus fungus like *Candida tropicalis* is used to treat volatile organic compounds in air. Biofilters are used to eliminate ethanol and isopropyl alcohol, which are released into air while drying ceramics. The injection of air to stimulate and aerobic degradation and volatization is called air sparging. The contaminated water is pumped to the surface and then is reinjected which is called bioventing. Microbes like *Thiobacillus ferrooxidans* conduct metal solubilization or leaching to recover Cu, Pb, Zn and Ur through metal solubilization (Table 3.1) (Katyayan 2019).

Table 3.1 List of bacteria used to bioaugmentation

S. No.	Contaminant/industry	Microorganism
1	Organic solid waste	Methanogens
2	Nitrate	*Methylophilus methylotrophus*
3	Chlorinated hydrocarbons	*Pseudomonas cepacia*
4	Waste water from textiles and dye industries	*Acetobacter liquefaciens* S-1
5	Volatile organic compounds in air	*Candida tropicalis*
6	Ethanol and isopropyl alcohol	Biofilters
7	Oil spills	*Pseudomonas* using recombinant technology
8	Cu, Pb, Zn and Ur	*Thiobacillus ferrooxidans*
9	Cd, Pb	*Pseudomonas aeruginosa*
10	Heavy metals	*Pseudomonas putida*, *Arthrobacter viscous*, *Citrobacter* spp.
11	Radioactive metals (uranium and thorium)	*Rhizopus arrhizus*, *Penicillium chrysogenum*
12	Bioleaching of Zn, Co and Ni from sulphide rocks	*Thiobacillus thiooxidans*

3.4.3 Phytoremediation

The usage of vegetation to eliminate, accumulate, degrade or contain harmful pollutants from soil or water is called phytoremediation. The origin of 'phytoremediation' is from Greek word, 'phyton' which means 'plant' and Latin word 'remedium', which means 'to remedy' or 'to correct'. Some aquatic vegetations, viz. *Salvinia* sp., *Lemna* sp., *Azolla* sp. and *Eichhnoria* sp., sedges, for instance, *Typha latifolia* and a few herbaceous and woody flowering plants have the potential to absorb, abide and accumulate heavy metals and other toxic substances from soil and water along with concentrating them into roots, stems and leaves (Adams et al. 2015; Chaney et al. 1997; Dickinson et al. 2009; Ensley 2000; Mendez and Maier 2008; Prasad 2004; Prasad and Freitas 2003). *Thlaspi caerulescens*, the alpine pennycress, if grown on zinc-contaminated soil yields up to 30–40% zinc. Thus this plant is bio-ore of Zn. *Sebertia acuminate* (Sapotaceae), which is native to Caledonia, accumulate 20–25% Ni of its dry weight (Katyayan 2019). Phytoremediation can be divided into six categories:

Enhanced rhizosphere degradation, phytodegration, phytoextraction (phytoaccumulation), rhizofiltration, phytovolatilization and phytostabilisation.

- *Phytodegradation* (*phytotransformation*): Certain enzymes, viz. nitroreductases (degradation of nitroaromatic compounds), dehalogenases (degradation of chlorinated solvents and pesticides) and laccases (degradation of anilines) degrade or metabolize the organic contaminants or mineralize inside the plant cells. For example, *Populus* sp., *Myriophyllum spicatum*, Algae and stonewart (Katyayan 2019; Rylott and Bruce 2008; Schnoor et al. 1995).
- *Phytostabilization* (*phytoimmobilization*): Plant roots reduce the movement of contaminants (organic or inorganic) off-site.

  Root exudates act by precipitating the metals as insoluble forms and thus are subsequently trapped in the soil matrix. In this manner, the mobilization and diffusion of contaminants are restricted in the soil, e.g. *Haumaniastrum*, *Eragrostis*, *Ascolepis*, *Gladiolus*, *Alyssum*, Indian mustard (Ali et al. 2013; Berti and Cunningham 2000; Domínguez et al. 2009; Katyayan 2019; Prasad 2004).
- *Phytovolatilization*: Some plants have the ability to both absorb and volatilize certain metals and metalloids. Certain metals like Hg, Se and As can be absorbed by roots and transformed into non-toxic forms, thereby released into the atmosphere. Se can be absorbed and degraded by *Astragalus bisulcatus* and *Stanleya pinnata*. Plant species like *Nicotiana tabacum*, *Liriodendron tulipifera* or *Brassica napus* for reducing the toxic effect of Hg (Brooks 1998; Katyayan 2019; Pilon-Smits and LeDuc 2009; Pilon-Smits and Pilon 2000; Poschenrieder and Barceló 2004; Ruiz and Daniell 2009).
- *Phytoextraction* (*phytoaccumulation*, *phytoabsorption* or *phytosequestration*): In this method, plants accumulate metals and radionuclides and transport them to their harvest, i.e. aerial parts. Application of this technique can be applied to the metals, viz. Cd, Ni, Cu, Zn, Pb, Se, As etc. and other organic compounds. *Elsholtzia splendens*, *Alyssum bertolonii*, *Thlaspi caerulescens* and *Pteris vittata* are the hyperaccumulator plants which are known to carry out this process, which are known to store high concentrations of these metals in their aerial plants (this may vary from 0.01% to 1% dry weight, depending on the metal) (Blaylock and Huang 2000; Hernández-Allica et al. 2008; Ma et al. 2001; McGrath 1998; McGrath and Zhao 2003; Pedron et al. 2009; Van der Ent et al. 2013; Xie et al. 2009).

3.5 Parameters Affecting Bioremediation

Many factors combine in such a manner that the process of bioremediation can be taken care of systematically. The availability of the contaminants to the microbes possessing degradation abilities along with favourable conditions, viz., soil type, temperature, pH, O_2 or other electron acceptors and nutrient availability is essential (Abatenh et al. 2017).

Various chemical and physical wastes produced due to numerous human activities are degraded, removed, altered, immobilized and detoxified from the environment, after they are acted upon by bacteria, fungi and plants. Microorganisms work as biocatalysts and help in the process of biochemical and metabolic reactions that degrade the pollutant in question, although they perform against the pollutants only if they have support of compounds to help them generate energy and nutrients for the production of more cells. If the pollutants and microorganisms are not in contact, then the rate of reaction may slow down to a great extend (Abatenh et al. 2017).

3.5.1 Energy Sources

Bacteria have the ability of reducing the organic matter to behave as energy sources. Average oxidation state of carbon decides whether it can act as an energy source for an aerobic heterotrophic organism.

High oxidation states provide low energy yields, thus the process of degradation may be slow. There are various factors which are involved and result in the microbial degradation, viz. biomass concentration, microbial diversity and enzymatic or metabolic activities of the microbes. Acclimation period of the microbes are affected by the physico-chemical characteristics, molecular structure and concentration of the substrate along with a number of environmental factors like pH, temperature, moisture content, availability of electron acceptors and carbon and energy sources (Boopathy 2000).

3.5.2 Bioavailability

The rate of conversion of contaminants by the microbes during bioremediation is determined by the rate of contaminant uptake and metabolism (Boopathy 2000). The contaminated explosives would not undergo degradation even in 50 years, if mass factor is a limiting factor (Boopathy and Manning 1999). Conversion of larger soil particles into smaller ones by breaking and mixing of the soil particles increases the surface area to a great extent, hence enhances the degradation rate (Manning et al. 1995). Physico-chemical processes, viz. sorption, desorption, diffusion and dissolution, decide whether the contaminant is bioavailable or not. If the rate of mass transfer of contaminants is zero, then the contaminants are not available to the degrading microbes. This decrease in bioavailability is known as ageing or weathering (Boopathy and Manning 1999).

3.5.3 Bioactivity and Biochemistry

The processes in which microbiological processes are carried out is called bioactivity. The bioactivity can be improved by implying the conditions that can optimize biodegradation (Blackburn and Hafker 1993). Depending upon the requirement of the bioremediation, techniques may be configured to achieve the optimal required rate and adjustments of conditions. The organisms possess a diverse ability to transfer contaminants, both simple and complex molecules (Boopathy 2000).

3.5.4 Nontechnical Criteria

Along with various technical hurdles, there are also nontechnical norms that affect the process of bioremediation to achieve the required target of clean environment, reduced cost when compared under options, contaminants' residues if any left should be acceptable from the risk point of view, socially the technique should be acceptable, regulatory perception should be favourable, time limitations should be able to meet, the problem of space limitations should also be encountered (Boopathy 2000).

3.5.5 Nonscientific Factors

There are various nonscientific reasons which can hinder the development of bioremediation technologies as below.

3.5.5.1 Regulatory Factors

Regulations are the basis of any process; these both impel and constraint the process of bioremediation. The fact that what must be cleaned, how it must be cleaned and which methods should be used to clean up are decided on the basis of the regulations (Caplan 1993). There are additional regulations for the usage of genetically engineered microorganisms (GEMs). The microbes occurring naturally are considered over GEMs in the present scenario (Boopathy 2000).

3.5.5.2 Research and Technical Factors

Various industrial chemicals like PCBs, pesticides, coal tars, chlorinated solvents and polynuclear aromatic hydrocarbons are not degraded readily, but funds required for this research is less. Each bioremediation technique has to be standardized particularly for each polluted site, depending on the uniqueness of the polluted site (Boopathy 2000).

3.5.5.3 Human Resource Factor

Comparatively, bioremediation is a novel technology; hence, this field confronts lack of trained and experienced human resources. The combination of various faculties like microbiology, engineering, geology, hydrogeology, soil science and project management together is followed with a multidiscipline approach to carry out successful bioremediation programme.

3.5.5.4 Economic and Liability Factor

The complex process of bioremediation does not produce any high value-added products. This creates a low interest in the R&D process in comparison to other industrial sectors. The bioremediation techniques are being scrutinized by regulatory agencies more strictly than conventional technologies. Hence, the operating rules and regulations for bioremediation projects are much tighter, and performance standards are quite high. Thus, make the projects difficult to run from a practical point of view (Boopathy 2000).

3.6 Microbial Populations for Bioremediation Processes

Microorganisms are cosmopolitan in nature and are recorded from all possible environments all around the world. They exist in extreme heat, desert, water and anaerobic conditions. However, carbon and energy sources remain the essential requirements. Many microbes can easily adapt to the various harsh and hazardous conditions. Thus, they can be used to remediate environmental hazards.

3.7 Conclusions

Although techniques used in bioremediation may represent a slow response when compared to the conventional ones, bioremediation is the technique which would fully convert the properties of contaminants from the toxic properties to the environment friendly properties. It also enables the environmental habitats and habits to be reused by the human and others.

This may be costly project, but is worth investing when the pollutants can be eradicated from the system forever. Also there is a lack of professionals who can lead the project with not hurdles successfully. The regulations for the use of microbes have to be amended so that the process can be easily carried out successfully.

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Chapter 4
Phycoremediation: A Sustainable Biorefinery Approach

William Michelon, Aline Viancelli, Gislaine Fongaro, Lidiane Maria de Andrade, Helen Treichel, and Cristiano José de Andrade

Contents

W. Michelon
Department of Chemical Engineering & Food Engineering, Technological Center, Federal University of Santa Catarina, Florianópolis, SC, Brazil

Department of Environmental Engineering, University of Contestado, Concordia, SC, Brazil

A. Viancelli
Department of Environmental Engineering, University of Contestado, Concordia, SC, Brazil

G. Fongaro
Department of Microbiology, Immunology and Parasitology, Federal University of Santa Catarina, Florianópolis, SC, Brazil

L. M. de Andrade
Department of Chemical Engineering, University of São Paulo, São Paulo, SP, Brazil

H. Treichel
Department of Environmental Engineering, Federal University of Fronteira Sul, Erechin, RS, Brazil

C. J. de Andrade (✉)
Department of Chemical Engineering & Food Engineering, Technological Center, Federal University of Santa Catarina, Florianópolis, SC, Brazil

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Abstract This chapter addresses the phycoremediation as an alternative treatment process for the removal of pollutants from water and wastewaters. Simultaneously, the phycoremediation produces microalgae biomass that is a valuable source of feedstock. Microalgae are one of the most substantial examples of the biorefinery concept, since microalgae biosynthesis of high-added-value compounds such as long-chain polyunsaturated fatty acids, phenolic compounds, sterols, proteins, amino acids, peptides, vitamins, among others. In addition, microalgae can degrade/absorb pollutants such as heavy metals, drug residues (antibiotics and hormones), nitrogen, and phosphorus. Moreover, microalgae increase the degradation capacity of the local microbiota as bacteria, yeasts, and fungi by supplying them with oxygen and nutrients. Regarding the crucial current environmental problem (worldwide), it is essential to develop low-cost technologies that aim to significantly reduce the environmental impact of manufacturing, in particular, technologies that are related to integrated processes such as phycoremediation and production of high-added-value molecules.

Keywords Microalgae · Polluted water · Phycoremediation · Biorefinery

4.1 Introduction

High chemical organic demand wastewaters are inherently produced by industries, mainly the food industry. These wastewaters have high organic content, thus they can threaten the environment when disposed improperly, mainly due to eutrophication, color (it harms aquatic life), and phytotoxicity. Paddy rice, for instance, generates high volumes of yellowish wastewater (chemical organic demand ≈ from 400 to 4500 mg/L) (Umamaheswari and Shanthakumar 2019); whereas swine wastewater (chemical organic demand ≈ 500–60,000 mg/L) and dairy wastewater (chemical organic demand ≈ 900–38,000 mg/L) (Ansari et al. 2017). In this sense, phycoremediation (including seaweeds, microalgae, cyanobacteria, and lower plants) is one of the most promising alternatives for wastewater treatments, since they are virtually found throughout the earth. In addition, phycoremediation is an economically viable process that leads to greenhouse gas mitigation, can bioremediate metals, hydrocarbons, and pesticides and inherently produces high-added-value molecules (algae biomass) that can be used for multipurpose as bioenergy (biogas and biofuels), fertilizer, bio-ore for precious heavy metals, pharmaceuticals, cosmetics, and other valuable chemicals—biorefinery concept (Phang et al. 2015; Podder and Majumder 2016; Ansari et al. 2019).

An efficient wastewater treatment (phycoremediation) can be associated with high-added-value molecules such as fatty acids (long-chain polyunsaturated fatty acids), phenolics, sterols, proteins including amino acids and peptides, vitamins, pigments, among others (Andrade et al. 2018). Nevertheless, this remarkable microalgae potential should be, at least roughly, aligned to technical features of microalgae species, for instance, *Chlorella* spp. and *Spirulina* spp. are well-known for the protein production (qualitatively), *Dunaliella salina* for the pigment production, whereas *Ankistrodesmus spiralis* for mycosporine-like amino acids, among others. Similarly, Yee (2016) prospected microalgae from the genera *Hematococcus*, *Dunaliella*, *Botryococcus*, *Chlorella*, *Scenedesmus*, and *Nannochloropsis* for biodiesel production. Selenastraceae, family that includes *Monoraphidium* spp. and *Ankistrodesmus* spp., showed the highest lipid production.

The microalgae biomasses can be used for a wide range of application including the recovery of high-added-value compounds, antiviral, antibacterial, antifungal, fertilizer, among others. Nevertheless, microalgae biomasses are mainly used for the biofuel production, electricity generation, and animal feed.

The application of microalgae biomasses should be aligned to biomass harvesting and disruption systems. Regarding the most promising methodologies, autoflocculation can be useful strategy for biomass harvesting (low-cost, non-toxic, etc.), and non-mechanical techniques, in particular enzymatic ones, for disruption.

Therefore, phycoremediation is a promising biorefinery process in which wastewaters (high chemical organic demand values) can be efficiently treated, simultaneously, to production of microalgae biomass (high range of valuable molecules). This chapter aims to put a light on the main key features and drawbacks of phycoremediation.

4.2 Improper Wastewater Disposal and Its Consequences

Water quality improvement is a global concern (EPA 2004). Water pollution sources include industrial, domestic, or agricultural wastes, pesticides, fertilizer, urban development, chemicals, and human activities (Crini and Lichtfouse 2019).

The wastewater treatment is essential to reach high water quality (broad environmental sense). Wastewater can contain huge amounts of nutrients, pathogens, pharmaceuticals, and heavy metals. The physical, chemical, and biological wastewater characteristics are related to the effluent sources; however, it is mostly composed of water, nevertheless it has also solids. More than a decade ago, researchers highlighted those 1.3 billion L of sewage was discharged directly into rivers every day without any kind of treatment (Singh et al. 2004).

Discharge of high-nutrient concentration wastewater into water bodies can lead to undesirable phytoplankton blooms, and consequently eutrophication. Additionally, recent studies proved that the daily consumption of water containing more than 5 mg/L of nitrate is associated with congenital abnormalities as limb deficiencies or neural tube defects (Brender et al. 2013; Blaisdell et al. 2019).

In addition, wastewaters usually contain heavy metals such as Hg, Cd, Zn, Ni, Pb, Cr, Co, and Cu that have long persistence in the environment. The improper disposal of heavy metals in water bodies can lead to bioaccumulation by aquatic life and thus affect the entire food chain (human)—cancer and/or pathogens infection (Gochfeld 2003; Hadzi et al. 2018). In this sense, according to Eggers et al. (2018), there is a synergism among Pb and/or Cd content in blood and methicillin-resistant *Staphylococcus aureus* infection. Additionally, studies have shown a positive correlation between virus infectivity (HAdV and HAV) and iron concentration in water (Fongaro et al. 2019). Poole (2017) compiled more than 15 studies showing that in the presence of metals such as Cu and Zn, bacteria develop a resistance mechanism to these metals and simultaneously resistance to antibiotics.

Untreated wastewater can be a source of potentially pathogenic bacteria and viruses that can lead to diseases as cholera, diarrhea, and dysentery. These diseases are of strong concern not only due to the mortality and morbidity but also due to the high cost to treat patients. Most problems are associated with infection, more specifically *Salmonella typhimurium*, *Vibrio cholerae*, *Legionella*, *Escherichia coli* O157:H7, *Campylobacter jejuni*, and viruses such as adenovirus, astrovirus, hepatitis A and E viruses, rotavirus, norovirus, and enterovirus (Ashbolt 2015; Haramoto et al. 2018).

Other pollutants that have drawn attention recently are endocrine disruptors such as antibiotic, antiviral, analgesic, anti-inflammatory, psychiatric drugs, residual bioactive fractions of medicines, and personal care products (Santos et al. 2010; Boxall et al. 2012; Tijani et al. 2016). Additionally, many of these compounds have negative synergistic effects—broad environmental sense (Cizmas et al. 2015; Yu et al. 2019).

The wastewater treatments of these pollutants, generally, are performed using physical (sedimentation), chemical (coagulation-flocculation), and biological methods (activated sludge, nitrification-denitrification) (Kumar and Pal 2015; Whitton et al. 2015). These steps are sequential commonly: primary, secondary, and tertiary treatments (Fig. 4.1).

4.3 Wastewater Treatment

Physical-chemical processes, such as coagulation-flocculation, are preliminary treatments that can be applied on sedimentation of suspended solids and organic matters. After this process, the wastewater still contains considerable organic matter content (Rao et al. 2012).

The secondary treatment step consists of biological process based on aerobic and/or anaerobic metabolism of bacteria and/or fungi (biodegradation) (Tran et al. 2013). These processes occur in opened (lagoons) or closed reactors. After this process, the wastewater passes through disinfection and/or polishing process before disposal in the environment.

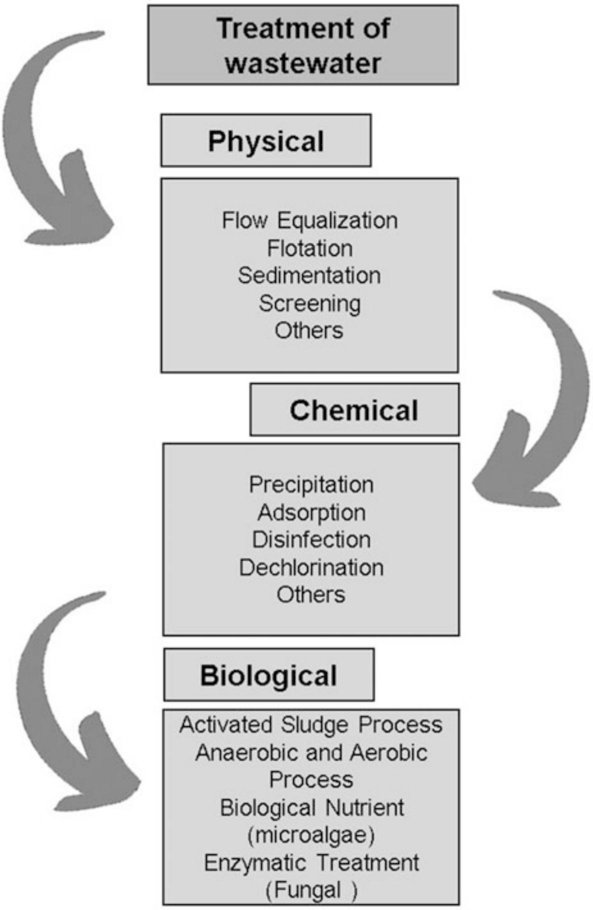


Fig. 4.1 Sequential wastewater treatment

Finally, the tertiary treatment process eliminates potentially pathogenic bacteria and/or viruses that are not removed with the previous treatment steps (Viancelli et al. 2013). These pathogens are structurally very different from one another (Cervero-Aragó et al. 2015). The most used methods include chlorination, UV irradiation (De Sousa et al. 2013), or electrochemical (Ghernaout and Ghernaout 2010; Simas et al. 2019). However, these processes are costly (Jin et al. 2014; Sun et al. 2016). A promising biological treatment process is the phycoremediation, since phycoremediation provides significant benefits such as (a) removal of nutrients, even those in low concentration; (b) microalgae could be transformed into biofuel, fertilizer, animal feed, among others (Whitton et al. 2015; Raheem et al. 2015)—(Fig. 4.2).

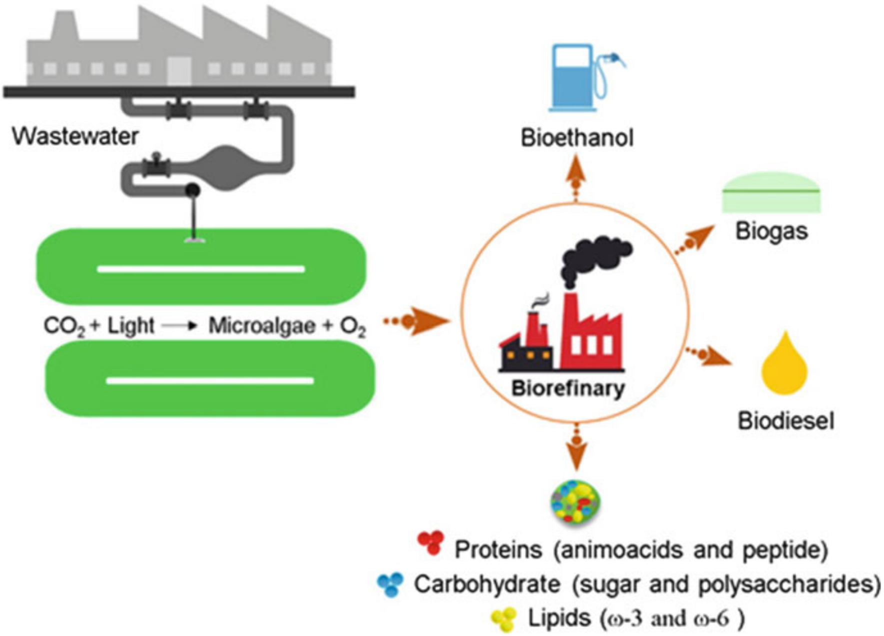


Fig. 4.2 Phycoremediation: a sustainable biorefinery approach

4.4 Phycoremediation

Microalgae are photosynthetic microorganisms (Pires et al. 2013), ubiquitous in natural, aqueous environment, as freshwater, marine water, and wastewaters (agroindustrial and from livestock). The microalgae growth requires carbon, nitrogen, phosphorus, and other essential trace elements, as well as light (Barsanti and Gualtieri 2014). For example, to produce 1 g of microalgae biomass is necessary mainly carbon (more than 50% of the mass weight), nitrogen (63 mg) and phosphorus (9 mg). Other compounds such as lipids, DNA, proteins, carbohydrates vary their proportion depending on algae species and also the cultivation conditions variation (Hongyang et al. 2012; Richmond and Hu 2013).

Microalgae have been classified as eco-friendly because they are more efficient than other methods for CO_2 mitigation (Chiu et al. 2009) and also produce high-added bioproducts such as lipids, biofuel, and enzymes (Wang et al. 2010; Lam and Lee 2012a). Microalgae have been recommended more than other tertiary processes for nutrient removal (Wang et al. 2010). In this sense, some microalgae are aligned to specific wastewater, for instance (items 1, 2 and 3):

1. Some *Chlorella* species as *Chlorella pyrenoidosa* can grow in polluted water, in particular, polluted water contaminated with arsenic either As(III) or As(V). Podder and Majumder (2016) studied the phycoremediation (arsenic) by *C. pyrenoidosa*, in which ≈81% and 85% of As(III) and As(V) were

bioremediated, respectively. The highest specific growth rate observed was 0.15/day.

2. *Scenedesmus* spp. are green algae (family Scenedesmaceae) commonly found in freshwater. *Scenedesmus* spp. are used often related to phycoremediation studies and also as a source of oil for biodiesel production. Ansari et al. (2019) reported an interesting data on the application of *Scenedesmus obliquus* for municipal wastewater phycoremediation and simultaneous production (w/w of dry weight) of lipids (26), proteins (28), and carbohydrate (27). In addition, the authors described an unusual economic analysis of wastewater phycoremediation. It is worth noting that high specific growth rate of 0.42/day and phycoremediation yields were obtained as 81% NH_4^+, 100% NO_3^-, and 94% PO_4^{3-}. Infrared spectroscopy analysis indicated functional groups as N-H, CH_3, CH_2, C=O, C-N, P=O, and Si-O on the biomass surface—accumulation of biochemical elements. When amortization, operating costs (including energy), and environmental benefits were taken into account, the net profit of phycoremediation was 16,885 US$/year.
3. *Spirulina* spp., in particular *Spirulina platensis*, can be used specifically for the phytoremediation of waters polluted by toxic compounds. Compared to other microalgae genera, *Spirulina* spp. have low generation time (fast biomass formation). Some specific metabolites of *Spirulina* spp. can induce heavy metals complexion. Other interesting advantage of *Spirulina* spp. is easier biomass separation from wastewater, since their vacuoles inflate (as aging), as a result *Spirulina* spp. float (Adamia et al. 2018).

Adamia et al. (2018) applied *Spirulina platensis* for the bioremediation of 2,4,6-trinitrotoluene—phycoremediation. The authors described that *S. platensis* adsorbed ≈90% of 2,4,6-trinitrotoluene (22.5 ppm) during 15 days, in addition it was observed that a relative low biomass accumulation decreases. The cultivation parameters are illustrated in Table 4.1. The lag phase lasted 4 days, whereas the log phase 13 days and the stationary phase 5 days (0.3 at 750_{nm}). Thus, *S. platensis* is an efficient and sustainable tool for the bioremediation of 2,4,6-trinitrotoluene.

Therefore, each microalga has an optimal growth rate, which should be related to its specific wastewater. Thus, a correlation between yield of phycoremediation and cultivation conditions is briefly described below (Table 4.1).

Since there is a wide structural diversity of microalgae species and also their cultivation condition, regarding phycoremediation, some criteria should be taken into account such as (a) growth rate, (b) key compounds removal rate, (c) cultivation adaptation, and (d) biomass and/or bioproducts production rate (Arita et al. 2015; Kesaano and Sims 2014). Phycoremediation should achieve removal rates higher as 56.5%, 68.5% and 90.6% of chemical organic demand, total nitrogen and phosphorus, respectively (Wang et al. 2010; Wang and Lan 2011). These nutrients (N and P) are removed through assimilation; on the other hand, heavy metal removal is performed through bioaccumulation and biosorption (Jais et al. 2017). Heavy metals are successfully removed from wastewater by microalgae, since microalgae have a wide range of polymers on their surface that are negatively charged (functional

Table 4.1 Phycoremediation: wastewater

Microalgae	Wastewater	Reactor	Cultivation mode	µ (1/day)	Productivity (mg/L day)	References
Scenedesmus obliquus	Domestic digestate effluent	Batch	16:8	0.35	89	Wang et al. (2016)
Chlorella vulgaris				0.37	115	
Scenedesmus obliquus	Domestic digestate effluent	Batch	12:12	–	112	Zhao et al. (2015)
Chlorella vulgaris				–	217	
Neochloris oleoabundans				–	86	
Chlorella sp.	Diluted domestic digestate	Batch		–	37.4	Zhao et al. (2013)
			12:12		58.6	
					52.5	
Mixed	Dairy wastewater	Continuous	Outdoor		321	Hemalatha et al. (2019)
Scenedesmus obliquus	Piggery anaerobic digestate	Batch	12:12	0.41	217.9	Xu et al. (2015)
Chlorella vulgaris	Digested swine	Batch	24	–	186	Franchino et al. (2016)
Neochloris aquatica	Wastewater treatment facilities	Batch	24	0.461	890	Wang et al. (2017)
Coelastrella sp.	Digested swine	Batch	12:12	0.298	50.1	Luo et al. (2016)

Table 4.2 Phycoremediation: heavy metals

Microalgae	Metal	Optimal pH	Removal maximum (mg/g)	References
Chlamydomonas reinhardtii	Cd	6	79.7	Bayramoğlu et al. (2006)
Chlamydomonas reinhardtii		6	2.9	Bayramoğlu et al. (2006)
Chlorella sorokiniana		5	192	Akhtar et al. (2003)
Chlorella vulgaris		6.9	2.6	Munoz and Guieysse (2006)
Chlorella vulgaris		4	85.3	Aksu (2001)
Chlamydomonas angulosa	Cr	8.2	5.3	Dwivedi et al. (2010)
Dunaliella sp.		2	58.3	Dönmez and Aksu (2002)
Dunaliella sp.		2	45.5	Dönmez and Aksu (2002)
Scenedesmus obliquus		2	15.6	Dönmez et al. (1999)
Chlorella sorokiniana		4	58.8	Akhtar et al. (2008)
Chlorella vulgaris	Cu	4.5	3.6	Tien et al. (2005)
Chlorella vulgaris		4.5	4.2	Tien et al. (2005)
Scenedesmus obliquus		7	1.8	Yan and Pan (2002)
Spirulina spp.		–	100	Doshi et al. (2007)
Chlorella vulgaris	Ni	5.25	29.3	Ferreira et al. (2011)
Chlorella vulgaris		5	15.6	Al-Rub et al. (2004)
Scenedesmus obliquus		5	18.7	Dönmez et al. (1999)
Chlamydomonas reinhardtii	Pb	5	96.3	Tüzün et al. (2005)
Chlorella vulgaris		5.25	131.4	Ferreira et al. (2011)
Scenedesmus subspicatus		6	38.7	Schmitt et al. (2001)
Phormidium spp.		5	13.6	Wang et al. (1998)
Scenedesmus obliquus	Zn	6.5	209.6	Monteiro et al. (2011)
Scenedesmus obliquus		6.5	836.5	Monteiro et al. (2011)
Chlorella vulgaris		5.25	43.4	Ferreira et al. (2011)
Spirulina platensis		5.25	33.2	Ferreira et al. (2011)
Chlorella pyrenoidosa	As (III)	9	81.74	Podder and Majumder (2016)
Chlorella pyrenoidosa	As (V)	9	85.08	Podder and Majumder (2016)

groups carboxyl, hydroxyl), and thus, they bind heavy metal ions as shown in Table 4.2 (Al-Gheethi et al. 2015).

Regarding pharmaceuticals, their removal is influenced by environmental factors, for instance when compared to colder seasons, warmer seasons show higher phycoremediation rate (Matamoros et al. 2015; Gentili and Fick 2017). It is worth noting that temperature is not the main factor, but higher and longer sunlight intensity/exposition (warmer seasons) (Khanam and Deb 2016). Additionally, the removal rate could be species dependent (Escapa et al. 2017), as shown in Table 4.3. On the other hand, the presence of some compounds such as antibiotics can affect algae growth and thus phycoremediation rate, for instance, wastewater that contains residues of tetracycline (an antibiotic) decreases phycoremediation rate, more specifically higher concentration than 30 mg/L eliminates ≈94% microalgae (Taşkan 2016; Yang et al. 2013; Xiong et al. 2018).

Conventional techniques for industrial wastewater treatments are composed of sequential steps that include oxidation, co-precipitation and adsorption, lime treatment, ion exchange resins, membrane, among others. Nevertheless, all of these techniques have technical drawbacks—toxic residual waste, limited efficiency, operational difficulty, and high operational cost. In this sense, microbial remediation processes, mainly those that use microalgae (phycoremediation), are the most promising alternative technologies—"eco-friendly nanofactories" (Madakka et al. 2019).

Phycoremediation is very versatile. It can be applied for wastewater (carbon, nitrogen, sulfur, etc., degradation), heavy metals (Cd, Cr, Pb, As, etc.), pharmaceuticals paracetamol, salicylic acid, diclofenac, carbamazepine, acetaminophen, ibuprofen, ketoprofen, naproxen, carbamazepine, diclofenac, triclosan, diclofenac, ibuprofen, paracetamol, metoprolol, carbamazepine, trimethoprim, estrone, ethinylestradiol, etc. Therefore, phycoremediation should be much more explored scientifically and technologically.

4.5 High-Added-Value Molecules

There is no consensus on the definition of biorefinery. According to IEA (2008), which is widely used, "Biorefining is the sustainable processing of biomass into a spectrum of marketable products and energy." Thus, microalgae bioprocesses are very much aligned to the biorefinery concept since there is an inherent and simultaneous production of high-added-value molecules such as phenolics compounds, fatty acids (long-chain polyunsaturated fatty acids), sterols, proteins including amino acids and peptides, vitamins, pigments, among others (Andrade et al. 2018).

4.5.1 Volatile Organic Compounds

Volatile organic compounds are compounds that have a high vapor pressure at room temperature and can be naturally produced by microalgae, mostly, acids, alcohols, aldehydes, carbonyls, esters, hydrocarbons, ketones, sulfuric compounds, and terpenes (Santos et al. 2016).

Table 4.3 Phycoremediation: pharmaceuticals

Microalgae	Wastewater	Pharmaceuticals	Removal mechanism	Efficiency removal (%)	References
Chlorella sorokiniana, *Chlorella vulgaris*, *Scenedesmus obliquus*	Synthetic (Mann and Myers medium)	Paracetamol, salicylic acid, and diclofenac	Biodegradation	82.5	Santos et al. (2017)
Chlamydomonas mexicana, *Scenedesmus obliquus*	Synthetic (Bold's basal medium)	Carbamazepine	Biodegradation, adsorption, and bioaccumulation	27	Xiong et al. (2016)
Consortium	Wastewater treatment plants	Acetaminophen, ibuprofen, ketoprofen, naproxen, carbamazepine, diclofenac, and triclosan	Biodegradation, adsorption, and bioaccumulation	66	Matamoros et al. (2015)
Chlorella sorokiniana	Synthetic (M-8a medium and urea-based synthetic urine)	Diclofenac, ibuprofen, paracetamol, metoprolol, carbamazepine and trimethoprim, estrone, 17β-estradiol, and ethinylestradiol	Biodegradation, photolysis, biosorption	65	Wilt et al. (2016)
Anabaena cylindrical Chlorococcus Spirulina platensis, *Chlorella Scenedesmus quadricauda Anaebena* var.	Synthetic (simulate the characteristics of effluent from anaerobic pond)	17α-ethinylestradiol, estrone, and 17β-estradiol	Adsorption biodegradation	87.5	Shi et al. (2010)

The volatile organic compounds occur in microalgae as a consequence of their primary metabolism, that is, the availability of carbon, nitrogen an energy supply, impacting the concentration of secondary metabolites, such as volatile organic compounds (Papaleo et al. 2013, Dudareva et al. 2013). Even some compounds such as alcohols, aldehydes, and ketones can be formed by the lipid degradation (Rzama et al. 1995) or alcohols can be oxidized to aldehydes and then to carboxylic acids, and ketones may be reacted with the hydroxyl radicals in the air to form aldehydes (Atkinson et al. 2000; Korpi et al. 2009).

In the recent years, volatile organic compounds, especially the volatile fatty acids which is usually based on non-renewable petrochemical sources, have attracted much attention due to the production of bioactive compounds, biodegradable materials, and energy by microorganisms through dark fermentation, by using volatile fatty acids as carbon source (Chalima et al. 2017).

The production of volatile fatty acids by using wastes as alternative culture media, such as food wastes, sludge, and similar biodegradable organic wastes, can be an alternative to reduce the production cost. Kim et al. (2006), in order to optimize volatile fatty acid production in dark fermentation, pretreated the raw food waste by commercial enzymes and thus the authors reported a 3.3 times higher production of volatile fatty acids.

Microalgae are able to use volatile fatty acids as carbon source producing high-added-products such as $\omega - 3$ and exopolysaccharides. In this sense, Kim et al. (2019) studied two processes, anaerobic fermentation (microalgae)—production of volatile fatty acids, and the cultivation of microalgae using synthetic volatile fatty acids more specifically acetate, propionate, and butyrate. Then they compared the yields of volatile fatty acids and their profile. They estimated that around 40% of the total carbon could be enhanced from the lipid-extracted algae that can be recovered for the production of algal biomass and an increase in the volatile fatty acids conversion yield beyond 60% by adopting pretreatment methods.

4.5.2 Fatty Acids

Fatty acids are composed of a carboxylic acid with a long aliphatic chain, which can be saturated or unsaturated. The long-chain polyunsaturated fatty acids, including essential fatty acids, play an important role in the brain and central nervous system.

Currently, there is an increasing demand for microalgae cultivation at industrial scale, mainly as a source of oils for biofuels production. In this sense, Makareviciene et al. (2019) reported the simultaneous production of *Ankistrodesmus* sp. oil and its transesterification using a lipase Lipozyme TL IM. BG 11 was used as a cultivation medium. The oil content was calculated by using a Soxhlet extraction (hexane). The authors have optimized the process by surface response methodology, precisely central composite design. In this sense, the moisture was inversely proportional to oil extraction and transesterification. The oil transesterification and oil extraction reached an impressive ≈98%.

Thus, microalgae can be a powerful source of oil (biodiesel production). However, microalgae biosynthesize long-chain polyunsaturated fatty acids in particular omega-6 family ($\omega - 6$) such as γ-linolenic acid, arachidonic acid, and omega-3 family ($\omega - 3$) as eicosapentaenoic acid and docosahexaenoic acid. These long-chain polyunsaturated fatty acids are essential fatty acids and also well-known for their nutraceuticals properties (healthy and disease prevention) (Andrade et al. 2018).

The investigation of the production of essential fatty acids, such as omega 3, 6, and 9, was studied by Abdo et al. (2015) by using the microalgae species *Chlamydomonas variabilis*, *Chlorella vulgaris*, *Haematococcus pluvialis*, and *Spirulina platensis*. The species *Chlamydomonas variabilis* showed the highest lipid content (21%) with 29.24% of omega 6, whereas *Haematococcus pluvialis* showed the lower lipid content (10%) with 14.83% of omega 6. The species *Chlorella vulgaris* and *Spirulina platensis* showed the presence of omega 3, respectively, 21.17% and 4.9%. *Spirulina platensis* was the only one that showed the presence of omega 9 (3.22%). Hence, the species *Chlamydomonas variabilis* and *Chlorella vulgaris* are recommended healthy range.

Considering the biorefinery concept, a recent study evaluated the growth of *Crypthecodinium cohnii*, a heterotrophic marine microalga, in the presence of volatile fatty acids, such as acetic butyric or propionic acids, which are released in high amounts through the dark fermentation by using biowastes. They also evaluated the ability of the microalgae to convert volatile fatty acids in high-added-value like docosahexaenoic acid, the most known omega-3 fatty acids. After 60 h of fed-batch cultivation, they observed the docosahexaenoic acid content of 29.8% of total fatty acids (Chalima et al. 2019).

4.5.3 Phenolic Compounds

Phenolic compounds are defined as low-molecular-weight compounds. Their structures have at least one phenol unit (Rosa et al. 2019; Sánchez-Salgado et al. 2019).

Recent studies are shown that phenolic compounds are produced by photosynthetic organisms (Wilson et al. 2017), and thus, microalgae have drawn attention as a source of high-value-added molecules. In this sense, *Spirulina* spp. are the most commercial sources of microalgae phenolics (Klejdus et al. 2009; Machu et al. 2019; Kumar et al. 2019). Table 4.4 shows some phenolic compounds obtained from different species of microalgae.

The humans' long life is also related to antioxidant-rich feed whose compounds are produced by photosynthetic organisms (Wilson et al. 2017), and phenolic compounds are a very important class of antioxidants molecules, such as flavonoids (isoflavones, flavanones, flavonols, and dihydrochalcones) that can be found in microalgae (Klejdus et al. 2010). Thus, microalgae molecules can play an important role in repairing or preventing oxidative damages caused by free radicals

Table 4.4 Phenolic compounds from microalgae (adapted from Sudhakar et al. 2019)

Microalgae	Phenolic compounds
Haematococcus pluvialis	p-OH benzoic acid Gallic acid Syringic acid Vanillic acid Protocatechuic acid Sinapic acid Ferulic acid Caffeic acid Chlorogenic acid
Spongiochloris spongiosa	p-OH benzaldehyde p-OH benzoic acid
Anabaena doliolum	3,4-Dihydroxy benzaldehyde
Spirulina maxima	Hydroxy-cinnamic acids Hydroxybenzoic acids Kaempferol Euganol Chrysin Galangin Pinostrobin
Isochrysis galbana	Brassicasterol Stigmasterol
Pavlova lutheri	
Skeletonema costatum	

(antioxidants) or other diseases such as cancer and cardiovascular and neurological diseases—nutraceutical (Scalbert et al. 2005).

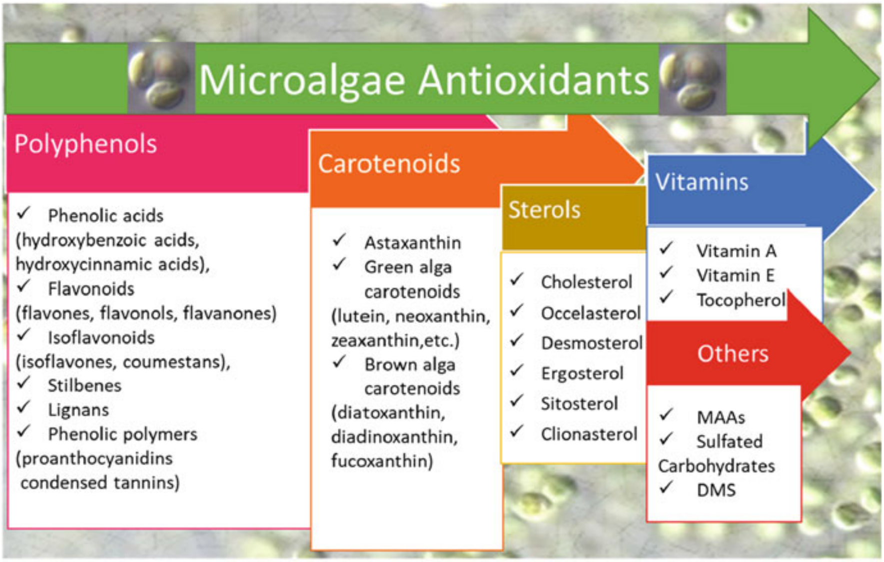
Figure 4.3 shows a wide range of antioxidants compounds of great industrial interest that can be produced by microalgae.

There are a few studies reported on phycoremediation (wastewaters) and further the use of its biomass for phenolic compounds production.

A very recent study, based on the biorefinery concept, applied the microalgae for waste treatment and also for the production of high-added-value molecules, such as phenolic compounds. Ferreira et al. (2019) used *Scenedesmus obliquus* for the treatment of brewery effluent and the use of the biomass to produce phenolic compounds. Through the subcritical water extraction of the biomass, they investigated the content of phenol and flavonoid, one subgroup of phenols. They found a range of 0.249–1.016 of gallic acid equivalents/mL extract for phenol and 0.050–0.167 of catechin equivalents/mL extract for flavonoids.

4.5.4 Sterols

Sterols are molecules that contain 27–29 carbon atoms. Among sterols, phytosterol is mainly found in the cell membranes of plants and also in microalgae. Phytosterols are one of the most promising sterols, with potential application in functional food and pharmaceutical industry, since it can be used in healthy diets, or as



*MAAs – mycosporine-like amino acids

†DMS - dimethylsulphite

Fig. 4.3 Wide range of antioxidants compounds of great industrial interest that can be produced by microalgae. *MAAs* mycosporine-like amino acids, *DMS* dimethylsulfite (Sansone and Brunet 2019)

Table 4.5 Sources of phytosterols

Source of phytosterols	Content (g/kg)	References
Corn oil	8.09–15.57	Piironen et al. (2000)
Wheat germ oil	19.7	
Rice bran oil	32.25	
Microalgae oil	7–34	Ryckebosch et al. (2014)

immunomodulatory, anti-inflammatory, anti-hypercholesterolemic, antioxidant, anticancer, antidiabetic (Xu et al. 2015), or cosmetic-based products (Rajakumar 2018).

Table 4.5 shows some sources of phytosterols, vegetable oil, and microalgae. It is worth noting that the microalgae oil has similar or higher phytosterols than the vegetable oils.

Among microalgae, the families Chlorophyceae, Rhodophyceae, and Phaeophyceae are the main sterol producers (Hernandez-Ledesma and Herrero 2013), and Ahmed et al. (2015) reported, after screening several Australian isolates, that *Pavlova lutheri*, *Tetraselmis* sp. M8, and *Nannochloropsis* sp. BR2 are the main phytosterol producers of microalgae.

Yasukawa et al. (1996) studied sterols in *Chlorella vulgaris* and found ergosterol peroxide, Prakash et al. (2010) identified the sterols 24-oxocholesterol acetate, ergost-5-en-3β-ol, cholest-5-en-24-1,3-(acetyloxy)-3β-ol in the species *Isochrysis galbana*, Francavilla et al. (2012) described ergosterol and 7-dehydroporiferasterol in *Dunaliella tertiolecta*, whereas Hetta et al. (2014) found campesterol, stigmasterol, and β-sitosterol in the *Spirulina platensis*, and those sterols showed bioactivity as anticancer, antituberculosis, neuromodulatory, and antimicrobial, respectively.

In the meantime, the potential of microalgae as sources of phytosterols remain to be fully explored in terms of phycoremediation.

4.5.5 Proteins, Amino Acids, and Peptides

Microalgae may represent innovative sources of proteins, amino acids, and peptides due to their high contents of those compounds. In view of the demand for food and the increase of the global population, microalgae have been proposed as a sustainable solution due to their high production of protein, essential amino acids, and peptides (Koutra et al. 2018).

Microalgae cell contains approximately 45% of protein, and its contents and the profile of amino acid depend on species and growth conditions (Soto-Sierra et al. 2018). Table 4.6 shows the protein content of some microalgae species and the main amino acids identified.

Protein is one of the main nutrients that will be in short supply in the future (Bleakley and Hayes 2017), and microalgae is an alternative source, rich in protein, in terms of content and quality of its composition.

The dietary guidelines specified the ingestion of high-quality protein. However, the quality of proteins can vary depending on the availability of essential amino acids

Table 4.6 Phenolic compounds from microalgae (adapted from Sudhakar et al. 2019)

Microalgae	Protein	Amino acids	References
Chlorella vulgaris	57.25%	Isoleucine, leucine, phenylalanine, and valine	Shim et al. (2008)
Isochrysis aff. galbana	13%	Glutamate, aspartate, histidine, methionine, tryptophan, cysteine, and hydroxyl-proline	Brown and Jeffrey (1992), Dörner et al. (2014)
Nannochloropsis sp.	45.2%	Arginine, lysine, leucine, asparagine, glutamic acid, alanine, glycine and valine	Valente et al. (2019)
Porphyridium cruentum	23.5%	Aspartic acid, threonine, serine, glutamic acid, glycine, alanine, cysteine, and valine	Becker (2007), Hempel and Maier (2012), Safi et al. (2014)
Spirulina platensis	53%	Leucine, valine, isoleucine, phenylalanine, tyrosine, Methionine, cysteine, and tyrosine	Becker (2007)
Tetraselmis sp.	64%	Leucine, asparagine, glutamine, glycine, proline, lysine, valine, and serine	Schwenzfeier et al. (2011)

and the digestibility, and thus, the dietary could be considered in terms of essential amino acids instead of total protein (Wolfe et al. 2016). It is worth mentioning that protein from microalgae, alternatively to the animal protein, is rich in essential amino acids that the human body cannot synthetize. Table 4.7 shows the essential amino acid profile of some of the most well-known microalgae species.

The main amino acids found in higher concentration levels in microalgae are aspartic acid and glutamic acid (MacArtain et al. 2007). On the other hand, in most of the microalgae species, the essential amino acids tryptophan and lysine are often limited (Dawczynski et al. 2007; Volkmann et al. 2008), leucine and isoleucine are at low concentrations (Dawczynski et al. 2007; Mišurcová et al. 2014), and cysteine is often even undetectable (Kakinuma et al. 2001).

Bioactive peptides are a sequence of specific amino acids that have health benefits such as antioxidative, antihypertensive, appetite suppression, hypocholesterolemic, antimicrobial, among others (Korhonen and Pihlanto 2006) and high nutritional value (Hayes 2013), whose main source is the milk proteins (Saito 2008), but also were identified in microalgae (Harnedy and Fitz Gerald 2011).

To obtain protein and amino acids from microalgae, it is necessary to carry out the disruption of the microalgae cell wall. Similarly, to obtain bioactive peptides, enzymatic hydrolysis should be carried out (González-López et al. 2010; Kim and Wijesekara 2010).

In this sense, *Chlorella vulgaris* and *Chlorella ellipsoidea* showed antioxidant peptides (Sheih et al. 2010; Ko et al. 2012). *Chlorella pyrenoidosa* presented anticancer peptides such as *Chlorella pyrenoidosa* antitumor polypeptide (CPAP) (Wang and Zhang 2013) and anti-inflammatory peptides, such as *Chlorella* 11-peptide (Shih et al. 2013).

Spirulina platensis showed anticancer peptides as polypeptide Y2 (Zhang and Zhang 2013) and the peptides (Leu-Asp-Ala-Val-Asn-Arg and Met-Met-Leu-Asn-Phe) which have also anti-inflammatory and anti-atherosclerosis properties (Vo and Kim 2013; Vo et al. 2013).

4.5.6 Vitamins

Vitamins are organic molecules that are essential in small quantities for good functioning of the metabolism of the organisms.

Microalgae appear as a valuable source of vitamins such as A, B1, B2, B6, B12, C, E, biotin, folic acid, and pantothenic acid (Villarruel-López et al. 2017) and can be easily used for humans as a supplement food.

Chlorella genus is a very rich source of vitamins such as vitamin B1, B2, B3, B5, B6, E, and K, and also, but in minor quantities, folic acid, biotin, inositol, choline, and vitamin B12 (Rani et al. 2018). *Spirulina* is known as a source of vitamin A, B1, B2, and B12. *Tetraselmis suecica* is an excellent source of vitamin B1, B3, B5, B6, and C, and *Dunaliella tertiolecta* are rich in vitamin B2 and B12 (Fabregas and Herrero 1990).

Table 4.7 Essential amino acid profile of some species of microalgae (g/100 g of dry matter) (adapted from Koyande et al. 2019)

Microalgae	Histidine	Isoleucine	Leucine	Lysine	Methionine	Phenyl-alanine	Threonine	Tryptophan	Valine
Aphanizomenon sp.	0.9	2.9	5.2	3.5	0.7	2.5	3.3	0.7	3.2
Arthrospira maxima	1.8	6.0	8.0	4.6	1.4	4.9	4.6	1.4	6.5
Chlorella sp.	2.4	4.4	9.2	8.9	2.2	5.5	4.7	–	6.1
Chlorella vulgaris	2.0	3.8	8.8	8.4	2.2	5.0	4.8	2.1	5.5
Dunaliella sp.	2.6	4.5	9.4	6.8	2.4	5.5	4.9	–	6.0
Dunaliella bardawil	1.8	4.2	11.0	7.0	2.3	5.8	5.4	0.7	5.8
Nannochloropsis sp.	2.6	4.7	9.4	6.8	2.3	5.5	4.8	–	6.0
Scenedesmus obliquus	2.1	3.6	7.3	5.6	1.5	4.8	5.1	0.3	6.0
Scenedesmus sp.	2.5	4.7	9.3	6.2	2.5	6.0	5.0	–	6.0
Spirulina platensis	2.2	6.7	9.8	4.8	2.5	5.3	6.2	0.3	7.1
Spirulina sp.	2.0	5.8	9.0	5.1	2.9	4.8	5.1	–	6.4

Vitamin K1, majority produced by chemical synthesis, is essential for blood coagulation and bone health (Russell and Suter 2015; Yaegashi et al. 2008). Tarento et al. (2018) studied seven species of microalgae and found that the richest in vitamin K1 is *Anabaena cylindrica*, reaching up to 200 μg/g on a dry-weight, which is around six times more than traditional vitamin K1 sources (spinach and parsley).

Tarento et al. (2019) described the scale-up (50 L photobioreactor) for the synthesis of vitamin K1 by *Anabaena cylindrica,* and they reach 330 μg/L on dry-weight, which means ten times more than rich dietary sources.

The influence of cobalt chloride salt in vitamin B12 production by *Chlorella vulgaris* was investigated by Jalilian et al. (2019), and they found 173.32 μg/100 g of dry biomass with 2.5 μM of the salt, which means around 12% more than the control.

Tossavainen et al. (2018) studied the potential of the consortium composed by *Euglena gracilis* and *Selebastrum* sp. to grow in aquaculture wastewater and then to produce tocopherol (vitamin E). They showed that the aquaculture wastewater can be used to increase the microalgae biomass and, due to the reduction in terms of nutrients and carbon organic dissolved, also to treat this wastewater. Additionally, the vitamin E content (total tocopherol) was superior to common plant oils, holding up to 1358 μg/L, depending on the type of aquaculture wastewater used.

4.5.7 Pigments

Natural pigments are colored compounds that have anti-cancer, anti-oxidative, and antihypertensive properties, enabling its application food industry, pharmaceutical industry, cosmetics industry, and textile industry (Mobin et al. 2019). Presenting higher content of pigments than some plants (Koyande et al. 2019), microalgae can contain pigments such as carotenoids (orange), xanthophylls (yellowish shade), phycobilins (red or blue), and chlorophylls (green) (Villarruel-López et al. 2017).

The most important class of pigments are the carotenoids. Carotenoids can be divided into carotenes, molecules containing only oxygen and carbon, and xanthophylls, which are carotene oxidized (Soares et al. 2019).

There are more than 400 already known carotenoids, nevertheless only β-carotene, astaxanthin, lutein, zeaxanthin, lycopene, and bixin are commercially available (Suganya et al. 2016).

The content of carotenoids in microalgae is around 0.1–2% dwt (Suganya et al. 2016). However, the environmental parameters can influence the carotenoid composition of microalgae (Mobin et al. 2019). *Haematococcus pluvialis*, under stress condition, such as salt stress, elevated temperature, heterotrophic media, among others, can accumulate up to 2–3% dwt of astaxanthin (Rao et al. 2007; Sarada et al. 2002).

Soares et al. (2019) identified and quantified major carotenoids in nine microalgae species and found that *Desmodesmus protuberans*, *Desmodesmus denticulatus* var. *linearis*, and *Chlamydomonas planctogloea* are lutein productors, and *Coelastrum*

Table 4.8 Carotenoid composition in different microalgae species (Soares et al. 2019)

Microalgae	Carotenoid contents (mg/g)						
	Lutein	Astaxanthin	Cryptoxanthin	Fucoxanthin	Canthaxanthin	β-Carotene	α-Carotene
Chlamydomonas planctogloea	7.4	0.14	0.99	–	1.49	–	–
Chlorella zofingiensis	0.49	5.65	0.11	–	0.18	0.29	0.09
Coelastrum sphaericum	2.75	15.29	0.42	–	0.21	0.11	–
Desmodesmus denticulatus var. *linearis*	8.46	–	–	0.07	–	0.40	0.21
Desmodesmus protuberans	10.53	–	–	–	0.14	–	–
Eutetramorus fottii	1.72	–	–	–	0.38	–	–
Mougeotia sp.	1.56	3.48	0.73	0.92	–	0.14	–
Parachlorella kessleri	1.40	22.96	0.26	–	–	–	–
Selenastrum bibraianum	1.73	0.41	1.34	0.41	0.03	0.16	0.08

sphaericum and *Parachlorella kessleri* are good for astaxanthin production. Table 4.8 shows the contents of carotenoids from each studied microalga species.

Among the microalgae, *Chlorella* genus is one of the major sources of chlorophyll pigment which can provide health benefits such as healing of sores, ulcers, hemorrhoids, regulation of menstruation, helpful in hemophilia, and improves diabetes and asthma (Rani et al. 2018).

Kulkarni and Nikolov (2018) studied a selective extraction of carotenoids and chlorophylls from *Chlorella vulgaris*, and they identified lutein and chlorophyll (a and b), respectively 5.4 mg/g dry mass and 15.4 mg/g dry mass.

Regarding astaxanthin pigment, the green microalgae *Haematococcus pluvialis* is one of its most important biological sources (Cuellar-Bermudez et al. 2015), representing around 90% of total carotenoids (Borowitzka 2013), and Fig. 4.4 shows the metabolic pathway of astaxanthin production from β-carotene in the microalgae.

In addition, in many applications that were already cited, microalgae can also be used in aquaculture products, for instance, as feed for salmon (Spolaore et al. 2006).

4.5.8 Polysaccharides

Polysaccharides are polymeric carbohydrate molecules that are commonly applied in food industry (Andrade et al. 2018) which can present anti-inflammatory, antiviral, anticancer, and antioxidant properties (Dufossé et al. 2005; Herrero et al. 2005; Sheng et al. 2007).

Similar to pigments, stress conditions can influence the biosynthesis of the polysaccharides, in this case increasing its content (Dufossé et al. 2005).

Pugh et al. (2001) identified three polysaccharides from *Spirulina platensis*, *Aphanizomenon flos-aquae*, and *Chlorella pyrenoidosa*: immulina, immunon, and immurella, respectively. Comprising between 0.5% and 2% of the microalgal dry weight, those polysaccharides are between 100 and 1000 times more active than that are currently used for cancer immunotherapy.

Bernaerts et al. (2018) studied the cell wall–related polysaccharides of ten microalgae species (*Arthrospira platensis*, *Chlorella vulgaris*, *Diacronema lutheri*, *Tisochrysis lutea*, *Nannochloropsis* sp., *Odontella aurita*, *Phaeodactylum tricornutum*, *Porphyridium cruentum*, *Schizochytrium* sp., and *Tetraselmis chuii*) with potential as functional food ingredients. They observed that *Arthrospira platensis* and *Chlorella vulgaris* are mainly composed of proteins and polysaccharides. The polysaccharides correspond to 10% of the biomass and containing uronic acid and sulfate groups that provide anionic characteristics. Table 4.9 shows the characteristics of the monosaccharide and uronic acid composition in cell wall polysaccharides of microalgae.

For many years, *Nostoc* genus microalgae have been used as food and medicine. Its composition rich in polysaccharides provides a very good resistance to several environmental stresses, as oxidative stress. Li et al. (2018) isolated a polysaccharide

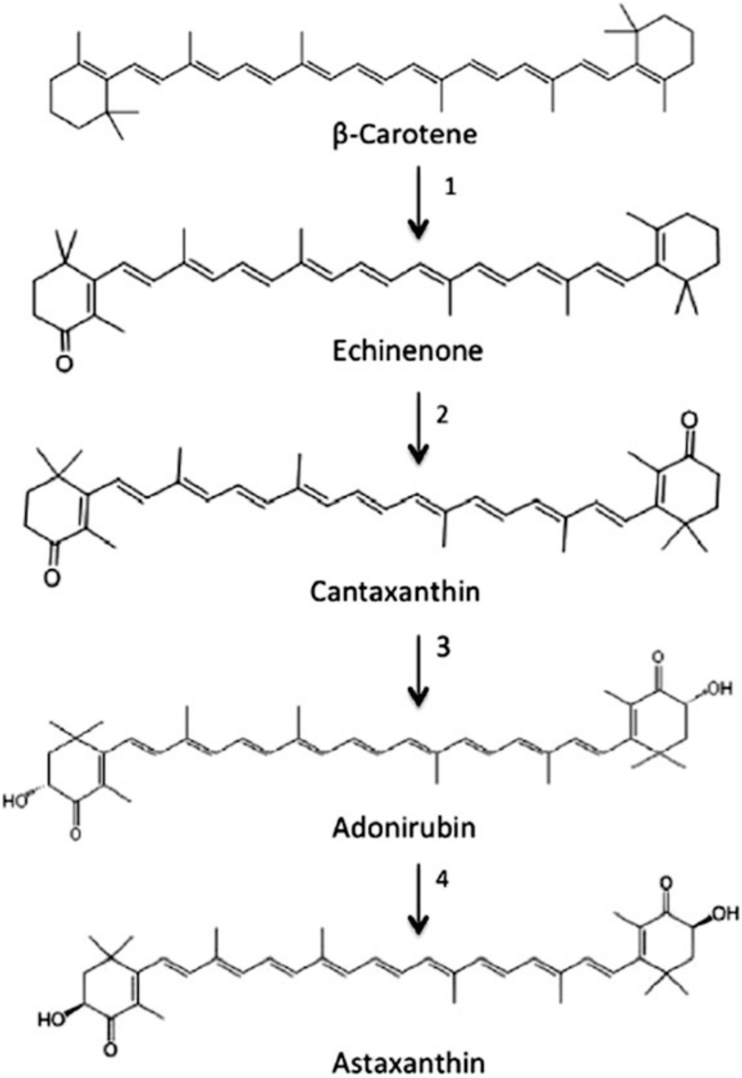


Fig. 4.4 Metabolic pathway of astaxanthin in the microalgae *Haematococcus pluvialis*. Enzymes are 1: 4,4′-ketolase, 2: 4,4′-ketolase, 3: 3′3-hydroxylase, 4: 3′3-hydroxylase (Cuellar-Bermudez et al. 2014)

nostoglycan from *Nostoc sphaeroides* and demonstrated that this compound is capable of reducing the reactive oxygen species and also can inhibit the growth of numerous tumor cells.

Thus, polysaccharides have a great potential to be applied in food and pharmaceutical industries.

Table 4.9 Polysaccharides: characterization of their monosaccharide and uronic acid composition in cell wall polysaccharides of microalgae (Bernaerts et al. 2018)

Composition	*P. cruentum*	*C. vulgaris*	*T. chuii*	*P. tricornutum*	*O. aurita*	*Nannochloropsis* sp.	*Schizochytrium* sp.	*T. lutea*	*D. lutheri*	*A. platensis*
Glucose	30.5	41.5	28.9	4.4	11.1	75.8	33.1	22.7	82.2	49.8
Galactose	22.4	8.6	5.7	3.8	35.7	6.4	29.0	14.8	–	3.8
Xylose	27.8	–	5.3	14.3	9.3	3.5	14.3	9.6	4.9	–
Mannose	9.3	34.8	41.3	46.4	17.1	4.7	20.5	16.4	6.2	29.8
Rhammose	–	2.7	1.0	8.9	3.2	3.0	–	1.3	–	6.7
Arabinose	–	–	0.8	–	–	–	–	20.3	3.4	–
Fucose	–	–	0.7	2.5	12.4	2.1	–	4.2	0.8	–
Ribose	–	1.9	–	2.9	1.3	4.5	–	4.0	1.3	–
Glucosamina	1.8	2.9	–	–	–	–	3.1	–	–	2.1
Galacturonic acid	3.9	3.3	15.1	2.9	4.0	–	–	4.1	–	5.6
Glucuronic acid	4.3	4.3	1.2	13.9	5.9	–	–	2.6	1.2	2.2

4.6 Drying and Disruption Techniques

Photoautotrophic microalgae usually have a low concentration of biomass (0.5–4 g/L, dry basis) suspended in a large volume of water (Chatsungnoen and Chisti 2016). Thus, biomass harvesting is a process that involves the separation of microalgae from the water media, where it is considered successful when achieving up to 20% of solids at the end of the process (Kadir et al. 2018). The harvesting can be performed using solid–liquid separation processes, such as physical, chemical, and biological methods (Fig. 4.5). However, the disadvantages of these techniques are related to the high cost, the high energy consumption, and the long extraction period (Wang et al. 2015). On the other hand, autoflocculation can occur similarly to bioflocculation, having the advantages of being a low-cost method, with no cell damage, non-toxic to microalgae biomass, high separation efficiency; the disadvantages are related to the occurrence of alterations in cellular composition or microbiological contamination (Christenson and Sims 2011; Zhou et al. 2012). After harvesting, the microalgae biomass needs to be submitted to a disruption process to obtain the bioproduct for subsequent application.

After the separation, to obtain the bioproduct for subsequent application, the microalgae cell biomasses need to be disrupted (intracellular molecules). Currently, a variety of cell disruption processes are available. In general, they can be categorized into mechanical and non-mechanical techniques of microalgal cellular disruption (Fig. 4.6). Cell wall destruction by a nonspecific technique is usually achieved by mechanical forces such as solid-shear forces (Yap et al. 2015), liquid-shear forces (Halim et al. 2012), energy through waves (Zheng et al. 2011), and currents (Goettel et al. 2013). Non-mechanical methods frequently involve cell lysis with chemical compounds (Kim et al. 2016) or enzymatic agents (Zheng et al. 2011). These methods are considered more advantageous than mechanical processes since cells are often only perforated or permeabilized rather than being shredded. Chemical and enzymatic methods depend on selective interaction of the cell wall or membrane

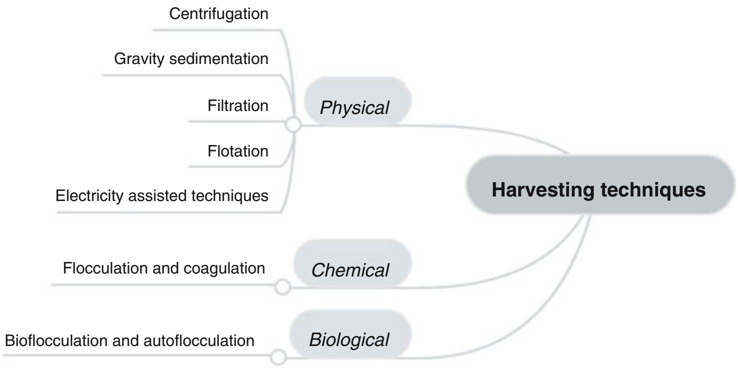


Fig. 4.5 Separation methods applied on microalgae harvesting processes

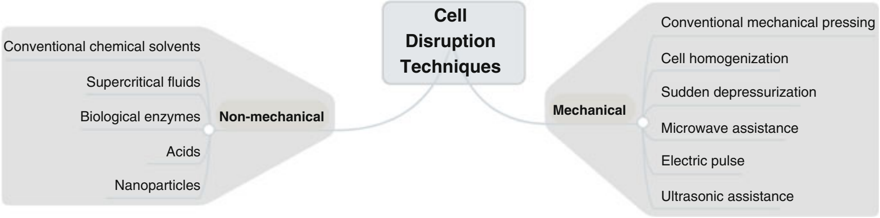


Fig. 4.6 Microalgae cell disruption methods for bioproducts recover

constituents that changes the cell boundary layer, to permit extraction of bioproducts (Günerken et al. 2015; Show et al. 2015).

4.7 Application of Microalgae Biomass

In addition to the benefits generated in the effluent polishing processes, the biomass produced at the end of the process has been receiving special attention currently, serving as raw material for several biotechnological products, and makes them very attractive for bioprospecting and potential exploitation of byproducts (Cheng et al. 2019). For example, the bioethanol and biogas production, as a protein source for human and animal nutrition, antimicrobial products, antioxidant, antitumoral, and anti-inflammatory features (Rizwan et al. 2018). Furthermore, microalgae biomass can be pyrolyzed to produce sequestered carbon in the form of biochar, which holds value as a soil enhancer, aiming to recover of nutrients (Kruse and Hankamer 2010; Wang et al. 2013) (Table 4.10).

4.8 Conclusion

Phycoremediation is one of the most promising alternatives for wastewater treatments. Phycoremediation leads to lower cost of microalgae cultivation. Microalgae are composed by many high-added-value molecules including volatile organic compounds, fatty acids, phenolic compounds, sterols, proteins, amino acids and peptide, vitamins, pigments, polysaccharides, among others. After phycoremediation, these molecules can be purified (biorefinery approach). In addition, the microalgae biomasses can also be used for a wide range of applications such as bioenergy—biogas and biofuels, fertilizer, pharmaceuticals, cosmetics, and bio-ore for precious heavy metals, among others. Therefore, phycoremediation is a sustainable biorefinery approach.

Table 4.10 Applications of microalgae biomass harvested from phycoremediation

Microalgae	Applications		References
Chlorella vulgaris	Biofuel	Biodiesel	Alam et al. (2019)
Chlorella vulgaris			Wong et al. (2017)
Nostoc linckia		Biohydrogen	Mona and Kaushik (2015)
Scenedesmus sp.			Ren et al. (2019)
Scenedesmus obliquus		Bioethanol	Ho et al. (2017)
Spirulina			Tourang et al. (2019)
Chlorella sp.		Biogas	Dębowski et al. (2017)
Scenedesmus spp.			Perazzoli et al. (2016)
Dunaliella tertiolecta		Bio-oil	Shuping et al. (2010)
Chlorella protothecoides			Miao and Wu (2004)
Chlorella vulgaris		Biochar	Wang et al. (2013)
Spirulina sp.			Chaiwong et al. (2013)
Scenedesmus	Electricity production	Microbial fuel cell	Rashid et al. (2013)
Scenedesmus and *Chlorella vulgaris*			Cui et al. (2014)
Spirulina platensis	Animal feeding	Ruminants	Kulpys et al. (2009)
Spirulina platensis			El-Sabagh et al. (2014)
Spirulina and *Chlorella*		Swine	Furbeyre et al. (2017)
Chlorella spp.			Bañoch et al. (2013)
Chlorella vulgaris		Poultry	Oh et al. (2015)
Spirulina			Bonos et al. (2016)
Spirulina platensis		Rabbits	Peiretti and Meineri (2008)
Schizochytrium sp.			Mordenti et al. (2010)
Haematococcus pluvialis and *Dunaliella salina*	Others	Antiviral	Santoyo et al. (2012)
Porphyridium sp.			Huleihel et al. (2001)
Pseudokirchneriella subcapitata		Antibacterial	Yang et al. (2008)
Spirulina platensis			Abedin and Taha (2008)
Chlorella vulgaris		Antifungal	Ghasemi et al. (2007)
Scenedesmus quadricauda			Abedin and Taha (2008)
Isochrysis sp.		Antioxidant	Goiris et al. (2012)

(continued)

Table 4.10 (continued)

Microalgae	Applications		References
Neochloris oleoabundans			Goiris et al. (2012)
Porphyridium		Anti-inflammatory	Matsui et al. (2003)
Chlorella vulgaris		Antitumoral	Ogawa et al. (1999)
Botryococcus sudeticus		Enzyme (lipase)	Yong et al. (2016)
Spirulina platensis		Enzyme (protease)	Nanni et al. (2001)
Chlorella vulgaris		Fertilizer	Lam and Lee (2012b)
Nannochloropsis sp.			Coppens et al. (2016)

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Chapter 5
Cyanobacteria-Mediated Bioremediation of Problem Soils

Kuttalingam Gopalasubramanian Sabarinathan, Muthukrishnan Gomathy, D. Arun Kumar, R. Kannan, and K. Eraivan Arutkani Aiyanathan

Contents

Abstract Cyanobacteria (BGA) are prokaryotic photoautotrophs capable of doing photosynthesis and nitrogen fixation simultaneously. The nitrogen fixing blue green algae are well documented for their efficiency of keeping the rice fields fertile. Cyanobacteria is a versatile organism possess different mechanisms to adapt to a broad range of environmental factors. Cyanobacteria are unique microorganisms which occupy and predominate diversified habitats as a result of many general characteristics; some cyanobacteria are like bacteria and others unique to higher plants. Agricultural productivity is greatly enhanced through cyanobacterial biofertilizer technology. The adverse effects of different uses of chemical fertilizers, pesticides and agrochemicals lead to a reduction in soil productivity and environmental quality. As a substitute for chemical fertilizers, and to bioremediate the problem soils caused by various agrochemicals, cyanobacteria are economically viable and sustainable technology in modern agriculture. Cyanobacteria are also recognized as an important agent in the stabilization of soil surfaces by different

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K. G. Sabarinathan (✉) · D. A. Kumar · R. Kannan · K. E. A. Aiyanathan
Department of Plant Pathology, Tamil Nadu Agricultural University, Agricultural College & Research Institute, Killikulam, Vallanadu, Tamil Nadu, India

M. Gomathy
Department of Soil Science and Agrl. Chemistry, Tamil Nadu Agricultural University, Agricultural College & Research Institute, Killikulam, Vallanadu, Tamil Nadu, India

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mechanisms which are prominent agents in the process of aggregate formation and increase in soil fertility. This chapter deals with the ability of cyanobacteria and their mechanisms on reclamation of wide range of problem soils such as saline, alkaline and acid soils.

Keywords Cyanobacteria · Photoautotroph · Bioremediation · Problem soils · Economical

5.1 Introduction

Soil pollution is a serious issue due to extensive industrialization and agrochemical practices. These pollutants render harmful effects to humans, crop plants and animals, causing ecological changes leading to a collapse in natural biodiversity. Reclamation of these contaminated soils for better life habitation is always an intriguing factor in the minds of scientists all over the world. Many physical and chemical conventional methods were enforced to remove and reduce the soil contaminants, but they were remarked with certain drawbacks and risks. Scientists in search of eco-friendly and feasible approaches resulted in the evolution of bioremediation. Application of microorganism to eliminate the anthropogenic and non-anthropogenic contaminants from soils was widely studied, especially the role of bacteria and fungi. However, bioremediation not only limited to the use of bacteria and fungi, but the positive impact of cyanobacteria in remediation is currently getting more attention. Cyanobacteria are often called blue-green algae, which is a prokaryote that belongs to bacteria. Rather than the presence of chloroplast, it is no way related to eukaryotic algae. Among the prokaryotes, cyanobacteria were noted with degradation nature of versatile contaminants and other benefits; hence with the help of modern scientific approach, cyanobacteria could be adapted as a new strategic technique to overcome soil-related problems.

5.2 Why Cyanobacteria?

The soil-related problems include (1) excessive usage of agrochemicals, (2) heavy metal contamination and (3) alkalinity and salinity. The ability of cyanobacteria to withstand extreme conditions made it a superordinate one in a prokaryote. Cyanobacteria can also develop in hypersaline and alkaline condition, tolerate xerophilic conditions, desiccation and high temperature and affirm high metal concentration. However, cyanobacteria are excluded from acidic environments at pH below 5 (Rampelotto 2013).

5.3 Agrochemicals

5.3.1 Pesticides

Pesticides were widely used in agricultural ecosystem which include herbicide, insecticide, nematicide, molluscicide, piscicide, avicide, rodenticide, bactericide, insect repellent, animal repellent, antimicrobial and fungicide. These pesticides were used in order to reduce the yield loss due to pest organisms. As the pest organism gets resistance, the excessive usage of pesticides to protect crop plants was encountered. In adverse, it has serious impact on farmers' health, human on food consumption, contamination of air, soil and water, non-target and beneficial organism and soil fertility (Aktar et al. 2009). In the current scenario, although the usage of chemical pesticides was reduced and replaced with eco-friendly management tools, the residues in the soil still remain without proper degradation (Gupta and Dikshit 2010). Application of cyanobacteria as bioremediation approach for contaminated soil would reduce contaminant residues as well as improve soil fertility.

Cyanobacteria have the natural ability to degrade most of the pesticides, and Kuritz and Wolk (1995) acknowledge that *Anabaena* sp. and *Nostoc ellipsosporum* could naturally degrade lindane (g-hexachlorocyclohexane) and also studied that the genetic engineering results in increased lindane degradation of these cyanobacteria. In addition to lindane, engineered cyanobacteria were evidenced to degrade chlorinated pollutant 4-chlorobenzoate. Similarly, degradation of lindane residue by *Oscillatoria*, *Synechococcus*, *Nodularia*, *Nostoc*, *Anabaena*, and *Microcystis* were also reported (El-Bestawy et al. 2007). Detoxification of endosulfan pesticide by *Anabaena* species were reported (Lee et al. 2003). Utilization of organophosphorus pesticide malathion as phosphorous source was reported in *Anabaena oryzae*, *Nostoc muscorum* and *Spirulina platensis*, which results in biodegradation of malathion. In the presence of malathion, a significant increase in biomass was also noted in these cyanobacterial strains (Ibrahim et al. 2014). *Anabaena* sp. and *Nostoc* sp. were able to detoxify the organophosphorus pesticide Fenamiphos through hydrolysis and oxidation approach. Hydrolysis of Fenamiphos leads to stable non-toxic products while oxidation gives the products which are toxic to aquatic invertebrates (Cáceres et al. 2008).

Glyphosate is a common organic phosphorus herbicide used all over the world and sold in the market name 'Round-up'. Accumulation of glyphosate and its degradation product aminomethylphosphonic acid (AMPA) in several environments has been identified which results with consequences like emergence of antibiotic-resistant microorganisms and shift in microbial community composition of soil, plants and animal guts (Van Bruggen et al. 2018). Basically, cyanobacteria grow well in excess phosphorous condition in order to fix nitrogen and has the ability to accumulate phosphorous. Cyanobacteria could break down glyphosate using alkaline phosphatase enzyme and utilize it for metabolism process, and the mechanism has been studied in *Nostoc* sp. L. ACN 101 and *Westiellopsis* sp. L. ACW 101 (Balakumar and Ravi 2001). Tolerance to glyphosate was reported in cyanobacterial species *Synechocystis* PCC 6803 and *Anabaena variabilis* ATCC 29413 (Powell et al. 1991). Next to glyphosate, atrazine is a commonly used nitrogen-rich herbicide which inhibits the photosynthesis of weed plants. Atrazine is only weakly adsorbed to soil particles during treatment and thus leaves the field mainly as runoff water. Moreover, they can migrate from upper soil surface to lower ground and reach underground water. Hence, it pollutes soil as well as water. Atrazine basically inhibits photosystem II (PSII) in many plant species, algae and cyanobacteria. Novel atrazine-resistant gene has been identified in naturally occurring cyanobacteria, but they lack degradation and catabolism mechanism. However, trace amount of ammonia compounds was identified in cyanobacteria tank treated with atrazine (Sajjaphan et al. 2002).

Cyanobacteria not only degrade and accumulate pesticides in soils but also replace that use by their potential bioactive compounds. Cyanobacteria were able to produce a wide variety of bioactive compounds which possess antagonistic activity against competitive organisms. Hence, these bioactive compounds can be commercially utilized for pest and disease management in agriculture. Novel metabolites from *Fischerella* sp. were found to have insecticidal activity against larval grazers (Becher and Jüttner 2006). Ethanolic extract of *Nostoc carneum* showed insecticidal activity against cotton leafworm *Spodoptera littoralis*, and the crude was found to comprise of fatty acids and terpenes. Extracellular metabolites of *Nostoc muscorum* and *Oscillatoria* sp. reduce the severity of purple blotch disease of onion caused by *Alternaria porri* through its antifungal activity, and their metabolites were mostly comprised of phenols and alkaloids. Precisely the most prevalent compounds in their filtrates were identified to be beta ionone, norharmane and α-iso-methyl ionone, and other trace compounds were piperazine derivatives, isocyclocitral, α-trans-sequicyclocitral, phytol, oleic acid, methyl palmitate, linoleic acid methyl ester, myristic alcohol and palmityl chloride (Abdel-Hafez et al. 2015). Antagonistic activity of two commercially available cyanobacterial compounds, viz. oligo-mix and weed-max on root rot fungal pathogens *Alternaria solani*, *Fusarium solani*, *F. oxysporum*, *Rhizoctonia solani*, *Sclerosium rolfsii*, *Sclerotinia sclerotiorum*, and *S. minor*, was tested individually and also co-inoculated with antagonistic bio-control agent. Under both the situations, it showed efficient growth suppression of fungal pathogens (El-Mougy and Abdel-Kader 2013).

5.3.2 *Chemical Fertilizers*

Chemical fertilizers are used to enhance the plant growth and yield in farm fields. Massive application of fertilizers leads to several soil-related problems such as soil acidification, ground water pollution and depletion in soil microorganism. In general, soil possess tremendous amount of natural nutrients, and it is sufficient to have good plant growth. Plant could not uptake all forms of nutrients, there are certain nutrient kind present in unavailable form. Clever utilization of nutrients present in farm soil will reduce the risk of soil contamination. Microbes are used as biofertilizers, and many cyanobacteria are potentially used as nitrogen fixer and phosphate solubilizer (Rai 2006; Sahu et al. 2012).

Excessive use of nitrogen fertilizer is a major reason for soil acidification which leads to soil deterioration. Plants generally uptake nitrogen in the form of ammonia, but excessive use of ammonia-based nitrogen fertilizers such as ammonium nitrate, ammonium sulphate, monoammonium phosphate, and diammonium phosphate than adequate level increases the soil pH through the conversion of ammonia into nitric acid (Wallace 1994). Instead of chemical fertilizers, nitrogen fixing microbes can be employed to eliminate ammonia residues in soil. Generally, nitrogen fixers use nitrogenase enzymes to fix atmospheric nitrogen into ammonia in soil. All heterocystous and many non-heterocystous cyanobacteria are capable of fixing atmospheric nitrogen. *Anabaena* and *Nostoc* sp. are the model organism for heterocyst-based nitrogen fixing cyanobacteria (Kumar et al. 2010). *Anabaena azollae* symbiotic relation is the most commonly known nitrogen fixing symbionts, especially in irrigated rice fields.

Similarly, non-heterocystous cyanobacteria were also reported to fix nitrogen in soil. The important nitrogen fixing genera are *Gloeocapsa*, *Gloeothece*, *Cyanothece*, *Synechococcus*, *Synechocystis*, *Lyngbya*, *Symploca*, *Oscillatoria* and *Trichodesmium*. Nitrogen fixation by non-heterocystous cyanobacteria under aerobic condition is an amazing fact as nitrogenase is irreversibly inhibited by oxygen, non-heterocystous cyanobacteria utilize diverse mechanism to fix nitrogen, the intracellular location of nitrogenase and the supply of ATP, reductant and carbon skeletons to support N_2 fixation. Moreover, they fix during dark period (Gallon and Stal 1992).

Apart from nitrogen fixation, under anaerobic condition, cyanobacteria also reduce the free ammonia content in soil through denitrification and anaerobic ammonia oxidation process (Chen et al. 2012). Moreover, over heterocyst cyanobacteria are able to recognize the presence and absence of nitrogen source. In the absence of nitrogen source like nitrate or ammonia, it forms heterocyst in between 10 and 20 vegetative cells for nitrogen fixation. While in the presence of ammonia or nitrate, it just forms a long filament containing stretch of hundred photosynthetic vegetative cells (Kumar et al. 2010). Thereby, cyanobacteria could perform the combined activity of diazotrophic bacteria and denitrifying bacteria, hence relevantly replace the usage of ammonia fertilizers in farm lands.

Many reports have been made on mineral phosphate solubilization by cyanobacteria. Phosphorus is highly essential for nitrogen fixation by cyanobacteria, hence they generally withstand under excess phosphorus condition. Under phosphorus limitation condition, they undergo mineral phosphate solubilization. Common mechanism involved in solubilization are organic acid production and enzyme activity. Phosphate solubilization ability of two diazotrophic cyanobacteria, *Westiellopsis prolifica* and *Anabaena variabilis*, was assessed, and it was found that among many organic acids, phthalic acid plays the major role in phosphate solubilization (Yandigeri et al. 2011). Anabaena was reported with phosphate solubilization by using phosphatase enzyme under phosphorus-deficit condition

(Natesan and Shanmugasundaram 1989). Cyanobacteria could replace the nitrogen and phosphate fertilizer efficiently.

5.4 Heavy Metal Contamination in Soil

Heavy metals are naturally present in biosphere, hydrosphere, lithosphere and lithosphere. Due to urbanization and industrialization, heavy metals have been included in almost all materials that are used in day-to-day life and result in anthropogenic activity. Improper disposal of heavy metals leads to soil and water contamination while irrigation of such contaminated water further affects the agricultural ecosystem. Apart from irrigation, pesticides and herbicides also serve as a source of heavy metal contamination (Li et al. 2019a). Consumption of heavy metal–contaminated food results in neurotoxicity, carcinogenesis, cell damage and loss of cellular functions in humans (Engwa et al. 2019). Microbial bioremediation has different processes which include bioaccumulation, bioleaching, biosorption, biotransformation and biomineralization, and the principle behind this process includes binding, immobilization, oxidation, transformation and volatizing of heavy metals (Verma and Kuila 2019).

Cyanobacteria has a major role in bioremediation of heavy metals. Photosynthetic organism generally requires metals which act as cofactors for several metabolism and in turn maintains metal homeostasis. Role of copper, nickel, cobalt, zinc, iron, manganese and magnesium in cyanobacterial metabolism was clearly studied; hence, the cyanobacteria undergo accumulation and transformation of heavy metals for their metabolism, thereby reduce the heavy metal contaminants in soils (Huertas et al. 2014). Biosorbent capability of Fe, Ni, Cr, Cd and Zn by *Nostoc* sp. was reported. Similarly, adsorption of Cr and Cu by *Spirulina* sp. and *Spirogyra* sp. was also studied (Igiri et al. 2018).

Waste effluents from industries include large amount of heavy metals. EPS producing microorganisms are used to remove heavy metal contents as the EPS are negatively charged molecules which act as biosorption of heavy metals. Further on extraction of EPS from effluents removes the heavy metals, thus the effluents are heavy metal free. Unique feature of cyanobacterial EPS is complex polysaccharide with more than six monomer types, which results in versatile EPS production. Hence the cyanobacterial EPS can used to remove or accumulate heavy metals in contaminated soil and water (Bhunia et al. 2018). *Nostoc muscorum* isolated from polluted water was reported with the potential to remove Zn^{2+}, and it is evidenced that the negative charge of hydroxyl, carbonyl, alcohol, amine, phosphoryl, sulfhydryl and carboxyl on surface of EPS is produced by *Nostoc muscorum* (Diengdoh et al. 2017). Consortium of algae with *Spirulina platensis* showed effective bioremediation in waste water and agricultural drainage water containing organophosphorus pesticide malathion and heavy metals, viz. nickel, lead and cadmium (Abdel-Razek et al. 2019).

5.5 Reclamation of Alkaline and Saline Soil

Alkaline and saline soil sets an unfavourable condition for plant growth; hence, reclamation of such soil could increase the cultivation area, thereby increasing the crop production. Cyanobacteria could tolerate and thrive on high pH and saline condition and thus promote plant growth under unfavourable conditions.

Merely, the agricultural soils are in the different physiochemical combined state of alkaline, saline, abundant nutrients, rich cations and a high percentage of organic matter. Diversified heterocyst and non-heterocyst cyanobacterial species occupy different kinds of agricultural soil condition. *Spirulina platensis* and *Spirulina maxima* were reported to thrive in alkaline lakes of Africa and Mexico at pH ranging from 8.0 to 11.0, which made them cyanobacterial monospecies devoid of other cyanobacteria. Thus, these cyanobacteria could be used in alkaline agricultural soil to improve fertility (Alghanmi and Jawad 2019; Habib 2008).

Salinity is one of the most prevalent agricultural problems in the arid and semi-arid regions of the world, affecting approximately 1 billion ha of land (Latef and Chaoxing 2011). Estimations indicate that increased salinization of arable land will result in 30% land loss within the next 25 years, and up to 50% within the next 40 years (Porcel et al. 2012). High salt depositions in the soil generate a low water potential zone in the soil, making it increasingly difficult for the plant to acquire both water and nutrients. In Tamil Nadu, 4.7 lakh ha is salt-affected saline soil in which 2.0 lakh ha is alkali soil confined to inland. The ESP of soils range between 26 and 45. In general, higher sodicity (>15%) leads to severe structural degradation due to high degree of dispersion of clay particles. The basic physiology of high salt stress and drought stress overlaps with each other. Therefore, salt stress essentially results in a water-deficit condition in the plant and takes the form of a physiological drought (Mahajan and Tuteja 2005). Most of the crops, commonly used for food production, are sensitive to salinity stress and vary in their response to salt stress tolerance (Flowers and Colmer 2008). Among cereals, rice (*Oryza sativa*) is the most sensitive, while barley (*Hordeum vulgare*) is regarded as the most tolerant. Bread wheat (*Triticum aestivum*) is comparatively more tolerant than durum wheat (*Triticum turgidum* ssp. durum). High salt concentrations lead to a decline in soil fertility by adversely affecting the soil microbial flora, including nitrogen-fixing cyanobacteria and therefore further decreasing rice productivity.

Cyanobacteria are capable of not only surviving but thriving in conditions which are considered to be inhabitable, tolerating desiccation, high temperature, extreme pH and high salinity with high sodicity, illustrating their capacity to acclimatize to extreme environments. Until recently, the responses of cyanobacteria to salinity stresses were poorly documented as compared to heterotrophic bacteria and phototrophic eukaryotic algae. These organisms evolved about 3000 million years ago and are considered to be the primary colonizers of the inhospitable ecosystems. The physiological aspects for the adaptation of cyanobacteria to high salinities include (a) synthesis and accumulation of osmoprotective compounds, (b) maintenance of low internal concentrations of inorganic ions and (c) expression of a set of salt-stress proteins. Cyanobacterial biofertilizers have been reported to be very useful in ameliorating various physico-chemical properties of marginal soils, and the EPS produced by the cyanobacteria seems to play an important role (Nisha et al. 2007).

The high sodium content in the soils leads to clogging of clay particles and reduce the soil porosity in turn reflect on plant respiration and absorption of nutrients. The extracellular polysaccharides excreted by cyanobacteria had been reported to be responsible for binding of soil particles, thus leading to the formation of a tough and entangled superficial structure that improves the stability of soil surface and protects it from erosion. Certain cyanobacteria have been found not only to grow in saline ecosystems but also to improve the physiochemical properties of the soil by enriching them with carbon, nitrogen and available phosphorus. The potential impact of these organisms on agriculture through their use as soil conditioners, plant growth regulators and soil health ameliorators has been well-recognized. The mechanism used by cyanobacteria to reclaim the saline soils are active export of ions through K^+/Na^+ channels and Na+/H+ antiporters, extracellular polymeric substance (EPS) production, the accumulation of compatible solutes, defence enzyme productions, phytohormone production and nitrogen fixation (Li et al. 2019b).

Consortia of EPS-producing cyanobacteria results in the improvement of growth in rice, maize and wheat under salt stress. It was found that the salt stress increases the EPS production and showed significant removal of Na+ ions from solution thus reduces the negative effect of salt concentration on crop plants (Arora et al. 2010). Gene expression of salt stress related proteins were profiled in *Synechocystis* sp. strain PCC 6803. It was found that genes responsible for PSI, PSII, phycobilisomes, and synthesis of compatible solutes, such as ion homeostasis were expressed well under salt-stressed condition and positively correlated with its physiological process (Arora et al. 2010).

Desertification is another serious soil deterioration challenge for agriculture. Desert soils are generally not suitable for cultivation due to less water activity and abiotic stress factors. Inoculation of cyanobacteria in such lands could reverse the state to crop cultivation. Through the formation of biological soil crust (BSC), it is possible to restore the semi-arid and arid soils for agricultural practice. Biological soil crust is a consortium of cyanobacteria, algae, fungi, bacteria, liches and mosses. Such BSC plays an important role in stabilizing and predominantly colonizing desert soil by increasing the quality of nutrients and moisture (Rossi et al. 2017). Though the cyanobacteria forms BSC and retrieve the arid soils, it is a retard process. Hence a novel technique was presented by Park et al. (2017), where cyanobacteria were integrated with biopolymers and tackifiers such as polyvinyl alcohol (PVA) and Tacki-Spray (TKS7) chemicals and added to the soils. As a result, it improves the soil aggregation and pave the way for BSC formation. Beyond this, it promotes cyanobacteria growth.

Cyanobacterial species were identified in different arid regions were reported *Chroococcidiopsis* sp. from hyper arid zone, *Chloroflexi* sp. and *Microcoleus vaginatus* from arid zone, *Microcoleus vaginatus*, *Nostoc punctiforme* and *Chroococcus* sp. from semiarid zone and *Chloroflexus* sp. from dry sub-humid zone (Perera et al. 2018). Apart from plant growth promotion, desert cyanobacteria were noted with industrial value products. *Nodularia sphaerocarpa* PUPCCC 420.1 from cold desert of Himachal Pradesh, India has the ability to produce Phycobiliprotein pigment which is used as food colourant (Kaushal et al. 2017). *Chroococcidiopsis* sp. from hyper-arid zone of Atacama Desert could produce Scytonemin pigment under stress conditions which is yellow-green ultraviolet sunscreen pigment. Similarly, Calothrix and Scytonema from Wadi Al-Khoud in Muscat was reported with scytonemin pigment production (Abed et al. 2018; Vítek et al. 2017). Carotenoid production by *Chroococcidiopsis* sp. from the eastern edge of the Qubqi desert, Negev desert, Israel was studied (Baqué et al. 2013).

Application of cyanobacteria has an immense role in paddy field. Paddy field contributes fairish amount of greenhouse gases, resulting in global warming. The important gases are carbon dioxide, methane and nitrous oxide and are mainly due to microbial activity in rice fields. Cyanobacteria in flooded condition enhance the oxygen concentration by photosynthetic activity, thereby create aerobic condition in rice rhizosphere which may consequently cut down the methane emission by methanogens. However, improves the activity of methanotrophs which could possibly utilize the methane source. Additionally, cyanobacteria fix atmospheric carbon dioxide during the oxygenic photosynthesis process. Apart from methane reduction, it reduces the nitrous oxide emission from the field. Inordinate use of nitrogen fertilizer in flooded fields results in emission of nitrous oxide gas, contrastingly deployment of cyanobacteria fixes nitrogen in the rice fields. Overall, cyanobacteria crucially reduce the greenhouse gas emission from rice fields. Consortium of cyanobacteria and methanotrophs can be an innovative strategy to for an eco-friendly rice cultivation (Prasanna et al. 2002; Singh et al. 2016).

5.6 Conclusion

Soil and water are indispensable natural resources for our domesticated food production systems based on animals and plants. Desirable physiochemical properties and biological activity decides the better agricultural ecosystem. Increase in population and climatic factor increases the usage of agrochemicals, but poor farmers were unable to afford for this. However, agrochemicals have results in deterioration of ecosystem. Cyanobacteria in this circumstance can be efficient for improving soil organic carbon matter and also enhances nitrogen and phosphorus availability to crop plants. Cyanobacterial mat or colonies in alkaline and saline soils accumulate ions and create suitable environment for plant growth. However, cyanobacteria under unfavourable condition improves nutrient availability and produce phytohormones. While in desert soil agriculture, it improves water activity and soil aggregation for a better cultivation. Cyanobacteria are excellent bioremediatory which effectively accumulates and degrades agrochemicals, xenobiotics and heavy metals in soils. Apart from soil treatments, bioremediation of industrial waste water by cyanobacteria increases the irrigation source for agriculture and also supports during drought season. Cyanobacteria are the rice source of bioactive compound production which potentially acts as biocontrol agents against pest and diseases. Considering the decrease in soil health and productivity caused by increased human activity, preserving environmental sustainability is the challenge ahead. Utilization of multifarious beneficial properties of cyanobacteria is highly necessary for healthy and efficient agriculture and environmental sustainability. Having understood their importance, a number of key issues relating to the exploitation of cyanobacteria have to be addressed immediately. In future, genome editing/engineering will play an essential role in bettering the economical utilization of cyanobacteria for soil-related problems.

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Chapter 6
VAM: An Alternate Strategy for Bioremediation of Polluted Environment

Poonam Verma, Suneel Kumar, Mridul Shakya, and Sardul Singh Sandhu

Contents

Abstract Soil remediation is a term that involves a numerous processes designed to get rid of contaminants like hydrocarbons (petroleum and fuel residues), heavy metals, pesticides, cyanides, volatiles, or semi-volatiles from soil. Remediation is required to control the pollution in soil, water, and air that can consequently benefit commercial cultivation or for wild flora and fauna. AM fungi are ubiquitous in soil habitat and form beneficial symbiosis with the roots of angiosperms and other plants. Their life cycle is often obligate in nature. So, use of mycorrhiza in mycoremediation techniques has generated many productive and long needed studies that examine the exact mechanisms that are at work. This chapter includes a review of basic remediation techniques and methods for soils and their limits and benefits for environment. We also discussed the uses of mycorrhiza for phytoremediation processes and

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P. Verma · S. Kumar · M. Shakya · S. S. Sandhu (✉)
Bio Design Innovation Centre, Ekatm Bhawan, Rani Durgavati University, Jabalpur, MP, India

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observed that more research is needed in order to fully understand the mechanisms of VAM fungi.

Keywords Anthropogenic pollution · Bioremediation · Degradation · Fungi · Phytoremediation

6.1 Introduction

In the third report of Royal Commission on Environmental Pollution U.K., the term "Pollution" is defined as the introduction of hazardous matter by human being into the environment that is liable to cause hazardous impact on living organism, damage to structure or amenity, or interference with legitimate uses of the environment (Appannagari 2017). Pollution can be broadly classified as natural pollution and man-made pollution or anthropogenic (Negev et al. 2010). In case of natural pollution, nature pollutes the environment through different activities like earthquakes, floods, drought, and cyclones, but in case of anthropogenic pollution, human beings spread pollution in air/water/land/food through their different activities like generation of toxic gasses, percolate waste in water and land, and producing radioactive compounds from nuclear reactor. The main pollutants produced by anthropogenic activities that drastically affect the environment are (Anand 2013; Holliger et al. 1997) polyaromatic hydrocarbons (PAHs) (Deshmukh et al. 2016), polychlorinated biphenyls (PCBs) (Akcil et al. 2015), polychlorinated dibenzo-*p*-dioxins (PCDDs) (Passatore et al. 2014), polychlorinated dibenzofurans (PCDFs) (Megharaj et al. 2014), heavy metals, etc. (Verma et al. 2016b; Das and Chandran 2010).

In the present scenario, the pollution caused by heavy metals is the primary concern around the world because heavy metal toxicity in the environment is a serious threat to the health of animals, plants, and humans (Ayangbenro and Babalola 2017). Contaminants of heavy metals cannot be degraded by chemical, physical, or biological processes. Hence, only level of toxicity is reduced (Chaturvedi et al. 2015). Heavy metal is dumped in environment through various modes like disposal of industrial metals waste and mining activity, etc. In the presence of heavy metal, soil properties are adversely affected. When high concentration of heavy metal such as Pb, Cd, Zn show reduced level of eco-friendly microbes like phosphorous solubilizing bacteria and nitrogen fixing bacteria (Fliessbach et al. 1994; Giller et al. 1998; Verma et al. 2016b) and affect the soil's physical properties like pH, temperature, and chemical properties like organic matter, clay mineral, and inorganic ion content (Baath 1989; Giller et al. 1998). Usually, copper, nickel, zinc, manganese, and iron present in trace amounts as natural constituents are harmless, but overlimiting the percentage has toxic effects on plants and animals as it gets accumulated in the food chain (Panda and Choudhary

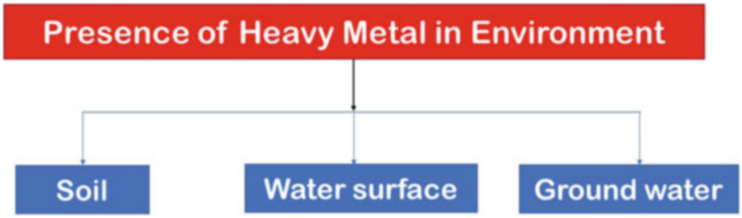

Fig. 6.1 Presence of heavy metal in environment

2005). Among all the heavy metals, cadmium, arsenic, lead, chromium, and mercury explore chief pollutants worldwide (Bempah et al. 2011). Figure 6.1 shows the spreading of heavy metal on the earth.

Toxic metals affect plants, animals, and microorganisms in different way through change in the metabolism processes of the organism (Verma et al. 2017). The development of different approaches and the method for elimination of toxic metal from the contamination site are a great matter of soil remediation importance to eliminate contamination from different location (Verma and Verma 2017) because pollution destroys ecosystems quality and pattern of land uses (Burlakovs and Vircavs 2011). Heavy metals decrease the fertility of soil as well as the natural eco-friendly microbes occurring (Directive 2008; Chandrakar et al. 2012).

6.1.1 Effect of Heavy Metal Toxicity on Plants

Different types of heavy metals show diverse effect on plant, which depends on concentration and types of metal (Verma et al. 2016a). Baker (1981) reported that these plants are able to tolerate these metals via three mechanisms, namely (1) exclusion: restriction of metal transport and maintenance of a constant metal concentration in the shoot over a wide range of soil concentrations; (2) inclusion: metal concentrations in the shoot reflecting those in the soil solution through a linear relationship; and (3) bioaccumulation: accumulation of metals in the shoot and roots of plants at both low and high soil concentrations.

Kibra (2008) recorded soil contaminated with 1 mgHg/kg reduced length of rice plants. Hg-contaminated soil also reduced the tiller and panicle formation. Soil contaminated with Cd 5 mg/L reduced shoot and root growth in wheat plants (Ahmad et al. 2012). Heavy metal also reduced plant mineral nutrition, photosynthetic activities, and reduced activity of some enzymes (Kabata-Pendias 2001). Nolvak et al. (2013) concluded that in the presence of Pb, soil productivity was decreased, and also vital processes of plants like photosynthesis, mitosis, and water absorption with toxic symptoms of dark green leaves, wilting of older leaves, stunted foliage, and brown short roots are also affected. Jayakumar et al. (2013) recorded that 50 mgCo/kg metal concentrations in soil increase nutrient content of tomato plants compared with the control. On the other hand, when concentrations were increased 100–250 mgCo/kg, plants show lower nutrient contents. Similar results were observed in radish and mung bean, when 50 mgCo/kg of heavy metal present in soil increase plant growth as well as physiochemical properties, whereas reductions were recorded at 100–250 mgCo/kg of heavy metal present in soil concentration (Jayakumar et al. 2008, 2007). Enhancement in growth of cluster beans has also been reported at lower (25 mg/L) Zn concentration of the soil solution, and opposite results were observed when the concentration of Zn (50 mg Zn/L) was increased (Manivasagaperumal et al. 2011). Nicholls and Mal (2003) reported that the mixture of Pb and Cu at high (1000 mg/kg each) and low (500 mg/kg) concentrations resulted in a rapid and complete death of the leaves and stem of *Lythrum salicaria*. Some related data are shown in Table 6.1.

6.1.2 Effect of Various Heavy Metals on Fungi

Heavy metals can alter major mechanisms of fungi. Due to metal toxic effect, many biological important molecules were unfunctional, for example, reaction of enzyme, transfer of nutrients and ions, the dislocation and/or exchange of essential metal ions, structural change, denaturation and inactivation of biomolecule, and interruption of cell function and organellar membrane integrity (Ochiai 1987). Fungi and metal show broad spectrum toxic interaction at every aspect of metabolism, development, and differentiation may be affected, depending on the individual, types of metal, concentration, and soil properties (Ross 1975; Gadd 1986; Gadd and White 1989). We all know that heavy metals are essential for the cultivation of filamentous fungi on synthetic media. Fungal continuous existence generally depends on different characteristics: biochemical and structural properties, physiological and/or genetical adaptation, morphological changes, and environmental alteration, availability, and toxicity (Gadd and Griffiths 1978; Gadd 1992a). Metal resistance is a word which means organisms have the ability to grow in the presence of metal by means of a mechanism produced in direct response to the metal species consumed, e.g., metallothionein or γ-glutamyl peptide synthesis (Mehra and Winge 1991). "Metal tolerance" depends on intrinsic properties and/or environmental modification of toxic metal (Gadd 1992b, c, 1993). Intrinsic properties include permeability of cell walls, extracellular biomolecule (polysaccharide), and secretion of metabolite, which help in detoxification of the metal species by binding or precipitation. However, distinctions are complicated in many cases because of the participation of several direct and indirect physico-chemical and biological mechanisms in survival. Biological mechanisms are altered (including extracellular precipitation, complexation and crystallization, transformation of metal species by oxidation, reduction, methylation, and dealkylation, biosorption to cell walls, pigments, and extracellular toxicity) for fungal survival (as distinct from environmental modification of toxicity). Some related data are shown in Table 6.2.

Table 6.1 Effect of heavy metal toxicity on plants

S. No.	Heavy metal	Toxic effect on plant	Reference
1	As	Reduced seed germination; decrease height of seedling; it also reduced area of leaf and dry biomass production that effect fruit yield. The other effect of heavy metal are stunted growth and chlorosis	Marin et al. (1993), Cox et al. (1996), Abedin et al. (2002), Barrachina et al. (1995)
2	Cd	Reduced seed germination; lower plant nutrient content; decrease shoot and root length; Cd accumulation in plant part	Jiang et al. (2001), Wang et al. (2007), Yourtchi and Bayat (2013)
3	Co	Plant nutrient content decrease; antioxidant enzyme activities were decreased; decrease concentration of plant sugar, starch, amino acids, and protein content; reduction in height (shoot, root) and leaf area; decrease in chlorophyll content	Jayakumar et al. (2013), Jayakumar et al. (2008), Jayakumar et al. (2007)
4	Cr	Reduced height of plant (shoot and root); reduction in plant nutrient acquisition; decreased rate of germination; reduction of plant biomass	Sharma and Sharma (1993), Panda and Patra (2000), Moral et al. (1996), Nematshahi et al. (2012)
5	Cu	Cu concentration increased in plant root; root malformation and reduction; plant death; decreased biomass and seed production; root growth inhibited	Cook et al. (1997), Kjer and Elmegaard (1996), Sheldon and Menzies (2005)
6	Hg	Reduction in plant length; formation of tiller and panicle decreased; yield reduced; Hg concentration increased in shoot and root of seedlings; germination percentage decreased; flowering decreased; fruit weight reduced; chlorosis	Du et al. (2005), Shekar et al. (2011)
7	Mn	Mn concentration increased in plant; reduced shoot and root length; chlorosis; decrease photosynthetic content (chlorophylls a and b); relative growth rate decreased; O_2 evolution activity decreased; decline plant growth	Arya and Roy (2011), Asrar et al. (2005), Doncheva et al. (2005), Shenker et al. (2004)
8	Ni	Chlorophyll content reduced and stomatal conductance; reduction in enzyme activity which affected Calvin cycle and CO_2 fixation; reduced plant nutrient; decrease in shoot yield; chlorosis; decreased root growth	Sheoran et al. (1990), Khalid and Tinsley (1980), Pandolfini et al. (1992), Barsukova and Gamzikova (1999), Lin and Kao (2005)
9	Pb	Decreased seed germination percentage; plant growth inhibited; plant biomass decreased; plant chemical	Hussain et al. (2013), Kabir et al. (2009), Moustakas et al. (1994)

(continued)

Table 6.1 (continued)

S. No.	Heavy metal	Toxic effect on plant	Reference
		content reduced; area of leaf and number of leaves decreased; inhibition of plant height; decrease in plant biomass; enzyme activity decreased which affected CO_2 fixation	
10	Zn	Seed germination percentage decreased; reduction in plant length and biomass; photosynthesis content decrease, carotenoid, sugar, starch, and amino acid content; variation in structure of chloroplast; accumulation of Zn in plant leaves; decrease in plant nutrient content; reduced efficiency of photosynthetic and energy conversion	Manivasagaperumal et al. (2011), Doncheva et al. (2001)

Table 6.2 Effect of various heavy metals on fungi

S. No.	Metal	Fungal sp.	Increase activity	Decrease activity	Reference
1	Cd	*Aspergillus flavus*	Total RNA, aflatoxin, O-methylsterigmatocystin	None	Cuero et al. (2003)
2	Cr	*Agrocybe praecox*	None	Enzyme production	Hartikainen et al. (2013)
3	Cu	*Aspergillus flavus*	Total RNA, aflatoxin, O-methylsterigmatocystin	None	Cuero et al. (2003)
4	Fe				
5	Zn	*Coniothyrium* sp.	None	Enzyme production on ABTS malt extract agar plates	Hartikainen et al. (2012)
		Sordaria sp., *Pyrenophora* sp., *Alternaria* sp., *Chaetomium* sp., *Fusarium* sp., *Epicoccum* sp., *Gliocladium* sp., *Mortierella* sp., *Cylindrocarpon* sp.	Enzyme production on ABTS malt extract agar plates	None	

6.1.3 *Effect of Heavy Metal on Invertebrates*

Metal concentrations in invertebrates showed considerable variation between individual species. Scientists observed that earthworms, oribatid mites, and carabid beetles and low in springtails, centipedes, and spiders have higher metal concentration. Metal accumulating capacity is not depended on trophic level (Straalen et al. 2001). Primary producer first consumes heavy metal and then accumulates in invertebrates that live in soils (Schipper et al. 1996). In ecotoxicological studies, invertebrates are mostly used due to their distribution, diversity, abundance, play important role in biogeochemical cycle, represent first tropic level, and close contact with soils (Heikens et al. 2001; Zaitsev and Straalen 2001; Migliorini et al. 2004). For example, Gramigni et al. (2013) recorded toxic heavy metal (Zn, Ni, Mn, Cd, and Pb) accumulated in ants (*Crematogaster scutellaris*) intestines, Zn accumulated specifically in Malpighi tubules, and low Zn concentrations were found in fat tissue. Heavy metals are accumulated at specific target organ in invertebrate. Spiders (*P. amentata*, *L. triangularis*, *M. segmentata*, *A. diadematus*, and *A. marmoreus*) had higher bioaccumulation of heavy metals (Cu, Zn, and Cd) in their hepatopancreas and gonads (Wilczek and Babczyńska 2000). Some other heavy metals like Ni, Pb, and Cd did not bioaccumulate specifically in target organs. Some related data are shown in Table 6.3.

6.2 Remediation Techniques

Remediation is defined in terms of procedures used to clean up, mitigate, or avoid to release pollutant into the environment in order to protect animals and plants (Marques et al. 2009). Nowadays, many types of remediation techniques and approaches are available. But the selection of remediation approaches depends on the physical properties of soil, type of contaminant, feasibility of contaminant isolation, handling intensity, economic value, etc. (Wuana and Okieimen 2011). Hence, on the basis of the above conditions, remediation process is broadly classified into the following groups.

6.3 Types of Remediation Technology

There are three options where remediation can take place.

6.3.1 On the Basis of Site

In this process, the treatment of contaminated soil or water in the dump site is known as in situ bioremediation (Abramovitch et al. 1999a, b). The treatment of contaminated soil or water once it has been dug out of the site at which it was present is known as ex situ bioremediation (Gomes et al. 2013) (Figs. 6.2 and 6.3). In addition, remediation techniques are also performed as a “singular method approach” or, in combined with other procedure, as part of a “multiple method approach.”

Table 6.3 Effect of heavy metal on invertebrates

S. No.	Metal	Invertebrata	Increase activity	Decreases activity	Reference
1	Cd	*Phormia regina*	Mean percent pupation, stage specific death	Mean % emergence, pupae death	Nascarella et al. (2003)
			Pupae death, stage specific death	Mean % pupation, mean % emergence	Nascarella et al. (2003)
		Eisenia fetida	Catalase (CAT), sodium dismutase (SOD)	None	Nascarella et al. (2003)
			None	CAT, SOD	Zhang et al. (2009)
2	Cu	*Folsomia candida*	Survival	None	Ardestani and Van Gestel (2013)
3	MeHg	*Caenorhabditis elegans*	Expression of glutathione S-transferases (gst-4): GFP (green fluorescence protein)	Heat shock proteins (hsp-4):GFP, metallothioneins (mtl-1):GFP and mtl-2:GFP	Helmcke and Aschner (2010)
4	Ni	*Eisenia fetida*	Microbial biomass carbon, soil basal respiration	Dehydrogenase activity	Giovanetti et al. (2010)
			None	Urease (UA) and dehydrogenase activity	Xia et al. (2018)
5	U	*Eisenia fetida*	Natural red retention time, DNA breaks	Toxicity factor	Giovanetti et al. (2010)
			DNA breaks		

6.3.2 *On the Basis of Separation Method*

6.3.2.1 Physical Method

In this method, only contaminants are separated from the site, degradation does not takes place (Bento et al. 2005; Gong et al. 2005), and posttreatment requirements for proper treatment are water solutions, solvents, or vegetable oils. Like regeneration of the solvent by distillation (Khodadoust et al. 1998), UV-degradation (Isosaari et al. 2001, 2005) or adsorption of contaminants by activated carbons (Ahn et al. 2007), through soil replacement method, dilutes the concentration of heavy metal(loid)s in soil and increases soil fertility (Yao et al. 2012). High-temperature treatment is used for the removal of heavy metal(loid)s from contaminated site (Mallampati et al. 2015) which leads to the formation of vitreous material. In vitrification, some metallic species (along with Hg) can be volatilized under excessive temperature

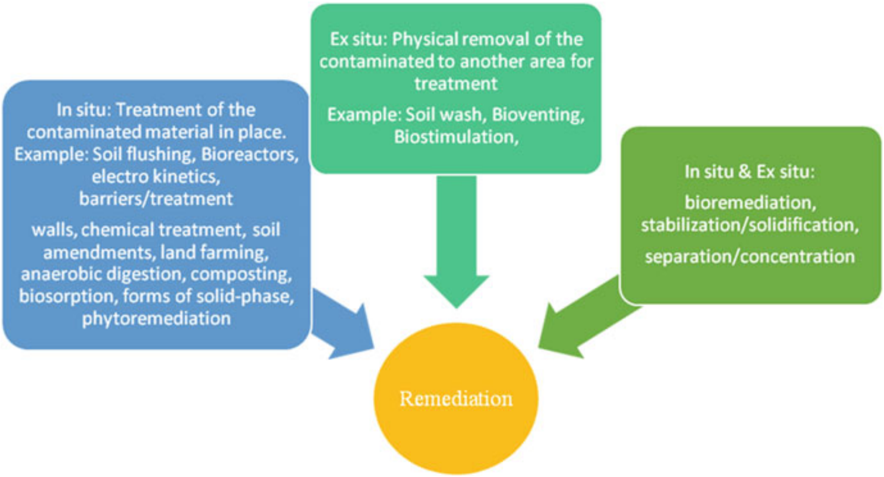

Fig. 6.2 Type of remediation technology

that ought to be accrued for disposal or remedy. In soil electrokinetic remediation processor, electric field gradient of appropriate intensity is established on two facets of the electrolytic tank containing saturated infected soil. Combinations of electrokinetic remediation techniques were also used:

1. Combined remediation by electrokinetic microbe method (Yu et al. 2009)
2. Electrokinetic-chemical dual remediation techniques (Vocciante et al. 2016)
3. Joint remediation by electrokinetic oxidation/reduction (Yang et al. 2015)
4. Phytoremediation coupled with electrokinetic (Mao et al. 2016)
5. Electrospun polyacrylonitrile nanofiber membrane with electrokinetics (Peng et al. 2015)
6. Electrokinetic remediation coupled with permeable reactive barrier (Rosestolato et al. 2015).

6.3.2.2 Chemical Method

Oxidation, reduction, and neutralization techniques are used for remediation of pollutant. The oxidation state of the metal can change through the loss of electrons called chemical oxidation reaction (Collins et al. 2009; Roach et al. 2009). Examples of commercially available oxidizing agents for chemical reaction are potassium permanganate, hydrogen peroxide, hypochlorite, lime, apatite chlorine gas, and Fenton's reagent (Masten and Davies 1997; Scanferla et al. 2009; Collins et al. 2009; Venalainen 2011). By adding electrons, the oxidation state of metals is changed and the reaction is called reduction reactions (Evanko and Dzombak 1997). Chemical remediation is rather a costly process, and some chemicals may react with soil and altered the soil capacity to promote plant growth.

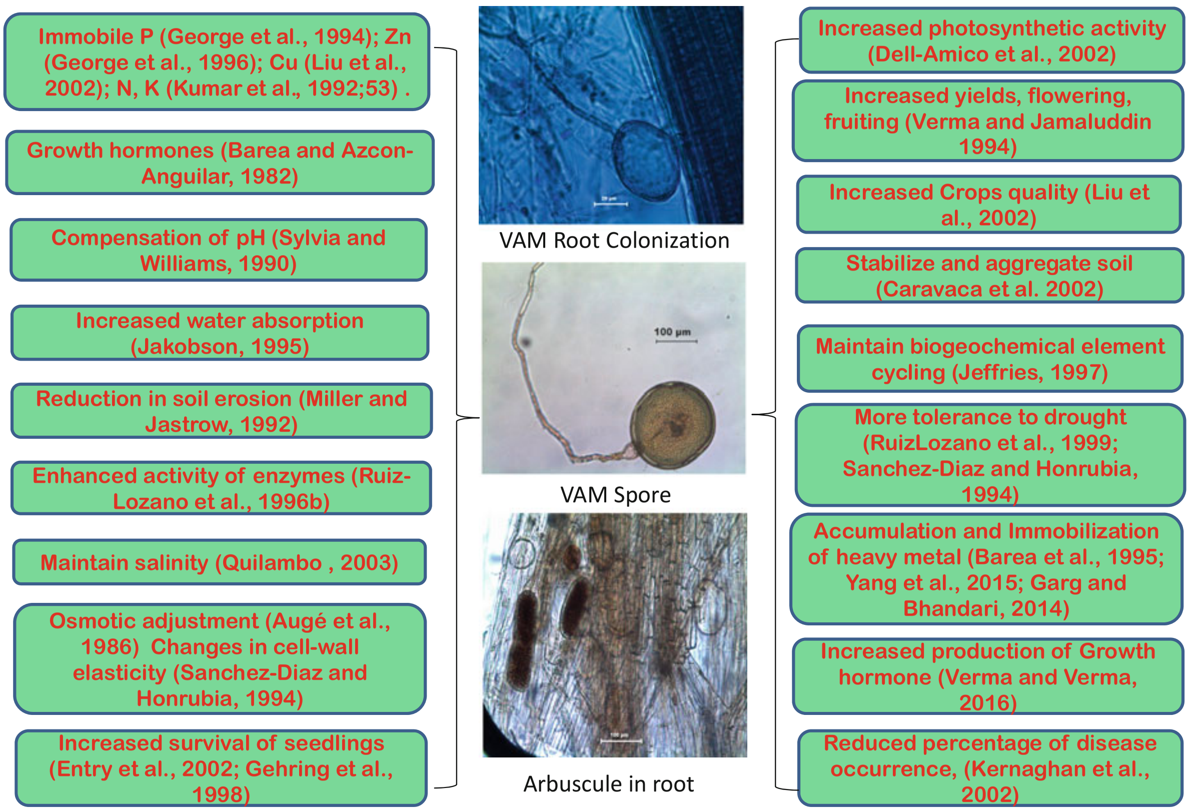

Fig. 6.3 Role of VAM fungi

6.3.2.3 Biological Method

Microorganisms (MO) and plants are used to degrade harmful contaminants, and this process called biological treatments. The beneficial effect of plant roots is dual: firstly—root execrations can provide energy for microorganisms, secondly—the presence of roots can alter the physical and chemical conditions in polluted soil in a manner that promotes microbial degradation (Malachowska-Jutsz and Kalka 2010).

But the above two techniques, physical and chemical methods, of bioremediation have some limitation or other side effect on environment. The selection of any method might rely upon the form of pollutant to be remediated, the proposed use of the contaminated area, required time periods and money (Chibuike 2013). But biological method is a unique system used for the elimination and/or recovery of contaminant from spoiled environments. The technique utilizes microorganisms and plants, or their products, to recover spoiled environments to their original condition (Tak et al. 2013; Mani and Kumar 2014). These methods are eco-friendly and cheap for the elimination/recovery of toxic metal, when compared to the conventional chemical and physical techniques, which are often more costly and unsuccessful, especially for low metal concentrations (Akcil et al. 2015; Verma and Verma 2016).

6.4 Importance of Biological Method

Biotechnology has amazing capability to cater for the need and holds hope for environmental safety, sustainability, and manageable (Hatti-Kaul et al. 2007; Azadi 2010). Therefore, bioremediation and phytoremediation are also an application of biotechnology (Koenigsberg et al. 2005). Because, they are able to metabolize, immobilize, or absorb toxic compounds from the surrounding. However, principal benefits of systems are that they are much less dangerous to surroundings with minimum or no through-products (Dowling and Doty 2009). Hyperaccumulators are plants that are able to accumulate, degrade, or render less poisonous pollutants present in ecosystem such as soils, water, and air. Bioaccumulation of Cs was observed by Lasat et al. (1998). In case of bioremediation, the consortia or microbial (bacteria and fungi) processes are used to degrade and detoxify environmental toxins (Dixit et al. 2015; M'rassi et al. 2015). This method has been used for decontamination of different horizon of soils and different types of water body (freshwater and marine) (Baker et al. 1994). It has positive effects upon soil composition and fertility. Another biological method of heavy metal removal is phytoremediation. Plants are used in phytoremediation techniques to remove, sequester, and/or detoxify toxic from polluted soil (Meagher 2003; Raskin et al. 1997). The method is completely cost-effective, green technology, and efficiently eliminate contaminants like metals, hydrocarbons, and chlorinated solvents from soil (Susarla et al. 2002; Jadia and Fulekar 2008; Zhang et al. 2010a, b).

6.5 Role of Fungi in Bioremediation

Biological remediation for the removal of heavy metal fungi plays an important role because they can chiefly bloom in the soils, and they have the capacity to grow in different type of weather like in unfavorable condition multiplied through dispersion of spores in the air and also maintaining the biogeochemical cycle (Eom et al. 2000). Fungi have the ability to secrete multiple enzyme and makes fungi potential for bioremediation as well as phytoremediation at various sites. So this type of remediation is also called mycoremediation. Successful treatments have been carried out on industrial wastewater sludge (Zeyayllah 2009), petroleum hydrocarbons (Prince et al. 2003), dyes (Mohsin et al. 2013), paper and pulp effluents (Afroz and Singh 2014), and mine land (Verma et al. 2016b). Much literature is available in the scientific society that fungi have capacity to survive everywhere as well as capable to modify or detoxify environmental pollutant and other anthropogenic pollutants including mining waste, nondegradable agriculture waste, industrial discharge, human hair, and petroleum product (Deshmukh et al. 2016). The utilization of Arbuscular mycorhizal fungi is a boon for the scientists as it not only causes sequestration of heavy metals but also enhances the nutrient content of soil (Barea et al. 2005). AMF are vital components of soil diversity of microorganisms because of increased yields, crops quality, flowering, fruiting, increased chances of survival of seedlings, reduced percentage of disease occurrence, more tolerance to drought, salinity, temperature, amplified utilization of NPK, and reduced erosion in soil. In environment different types of fungi are present, but VAM play an essential role in the removal of heavy metal from the different sites.

6.6 What Is VAM Fungi?

Mycorrhizal fungi show mutual beneficial symbiosis relationship between fungi and roots of plants (Sieverding 1991).

Two types of mycorrhiza are known today: ectomycorrhiza fungi and endomycorrhiza fungi. Fungi can absorb macro and micronutrients (N, P, K, Ca, S, Cu, and Zn) from the soil and transfer them to connected plants (Tinker and Gilden 1983). Mycorrhizal hyphae have the capacity to degraded bulky biomolecules into smaller molecules like N or P (George et al. 1995). Hyphae have the capacity to increase root surface area and absorbed nutrients from up to 12 cm away from the root surface (Cui and Caldwell 1996; Pacovsky 1986; Manjunath and Habte 1988). AM fungi also absorb non-motile nutrients from the soil and transfer them to host plants, as well as harmful heavy metal ions also absorb, help in inter plant relocation of nutrients and altered plant–water relationship (Smith and Read 1997). AM fungi in plant increase chlorophyll number in leaves, increased disease tolerance capacity, tolerance against parasites, improved water stress mechanism and salinity, and heavy metal toxicity (Bethlenfalvay 1992). AM fungi also help in the development of soil aggregates and soil conservation (Miller and Jastrow 1992). Assimilation and transfer of nitrogen from ammonium can also enhance biomass production in soils with low nutrients (K, Ca, and Mg) (Liu et al. 2002). Role of VAM fungi was shown in Fig. 6.3.

6.7 Role of VAM Fungi in Bioremediation

Mycorrhiza and plant show mutual relationship, and due to this, they help to immobilize heavy metal. In this process, both plant and mycorrhiza play a vital role in the removal of toxic metal and detoxification in plant cell as well as in VAM. Mycorrhiza cannot survive without a plant; hence, mycorrhizal remediation techniques are also called modified form of phytoremediation that utilizes the advantage derived from mycorrhizal fungi. In mycorrhizal remediation, some phytoremediation techniques such as phytoextraction and phytostabilization were utilized. Mycorrhizal remediation shows faster results as compared to phytoremediation because fungal hyphae cover larger area (Gao et al. 2010). Rufyikiria et al. (2004) recorded mycorrhizal remediation decrease transfer of contaminants from roots to the shoots of plants. AM fungal spore can survive in the soil up to 6 years (Nguyen et al. 2012); hence, they easily replicate and help in the growth of any crop planted on the soil. Thus, mycorrhizal remediation certifies the quick growth of vegetation on remediated soils.

6.7.1 Process of Detoxification

In different metabolic reactions, plants secrete chelating agents like histidine and organic acids in soil. These chemicals bind to heavy metals, which are present in soil. Plasma membrane has selective transportation capacity, as well as active and passive transportation system; through transportation system, specific and nonspecific metals are transported also from the pores of the plasma membrane (Fig. 6.4).

In intracellular detoxification, plant cells produced chelating agents like phytochelatins and metallothionein which have high affinity for heavy metals. Plant cells also secrete organic acids, amino acids, and specific metal chaperons. These secretary molecules react with heavy metals and form a complex structure. Heavy metal complex structures are exported from cytoplasm to tonoplast and then finally to vacuole. Heavy metal complex compounds are stored in vacuole, inside endoplasmic reticulum, and chloroplast (Briat and Lobreause 1997) (Fig. 6.5).

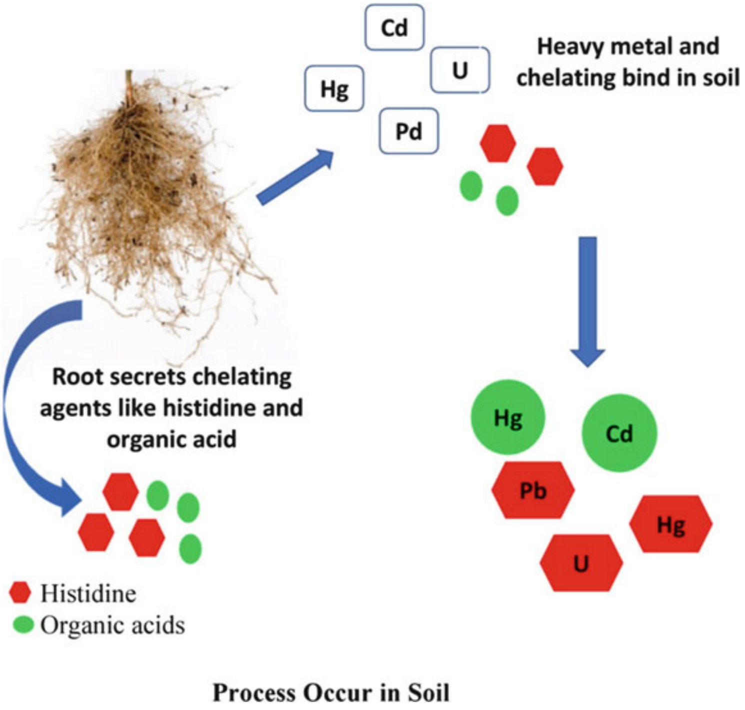

Fig. 6.4 Detoxification process occurs in soil

Host plants improve their nutritional status with the help of arbuscular mycorrhizal fungi by absorbing phosphorus, essential nutrients, and water. AM fungi secrete different types of proteins called glomalin which bind with toxic heavy metal to form a complex, and this complex binds with plant cell wall (Wright et al. 1996; Gadkar and Rillig 2006; Rillig and Mummey 2006; Gonzalez Chavez et al. 2004; Cornejo et al. 2008; Meier et al. 2012). Further heavy metal complexes are transported in the hyphae of the fungus (Preger et al. 2007). Plant cells secrete chelating agents like phytochelatins and metallothinein, and organic acids, amino acids, and specific metal chaperons have high affinity for heavy metals (Curaqueo et al. 2011). Later, molecular studies have observed that the structure of some protein molecule is a homolog of certain heat-shock proteins (Gadkar and Rillig 2006), which in general are related to environmental stresses. Nowadays, different types of VAM fungi were used for sequestration of heavy metal, and the data are shown in Table 6.4.

VAM fungi absorbed heavy metals from the soil in the process of phytostabilization and phytoextraction. AM fungal strain Glomeromycota is present in soil and absent in host plant. Generally, AM fungal spores and hyphae show response for heavy metals. A specific concentration of heavy metal affects the germination and growth of hyphae (Shalaby 2003). Gohre and Paszkowski (2006) assumed that plant and fungal vacuoles have similar structure, which are involved in

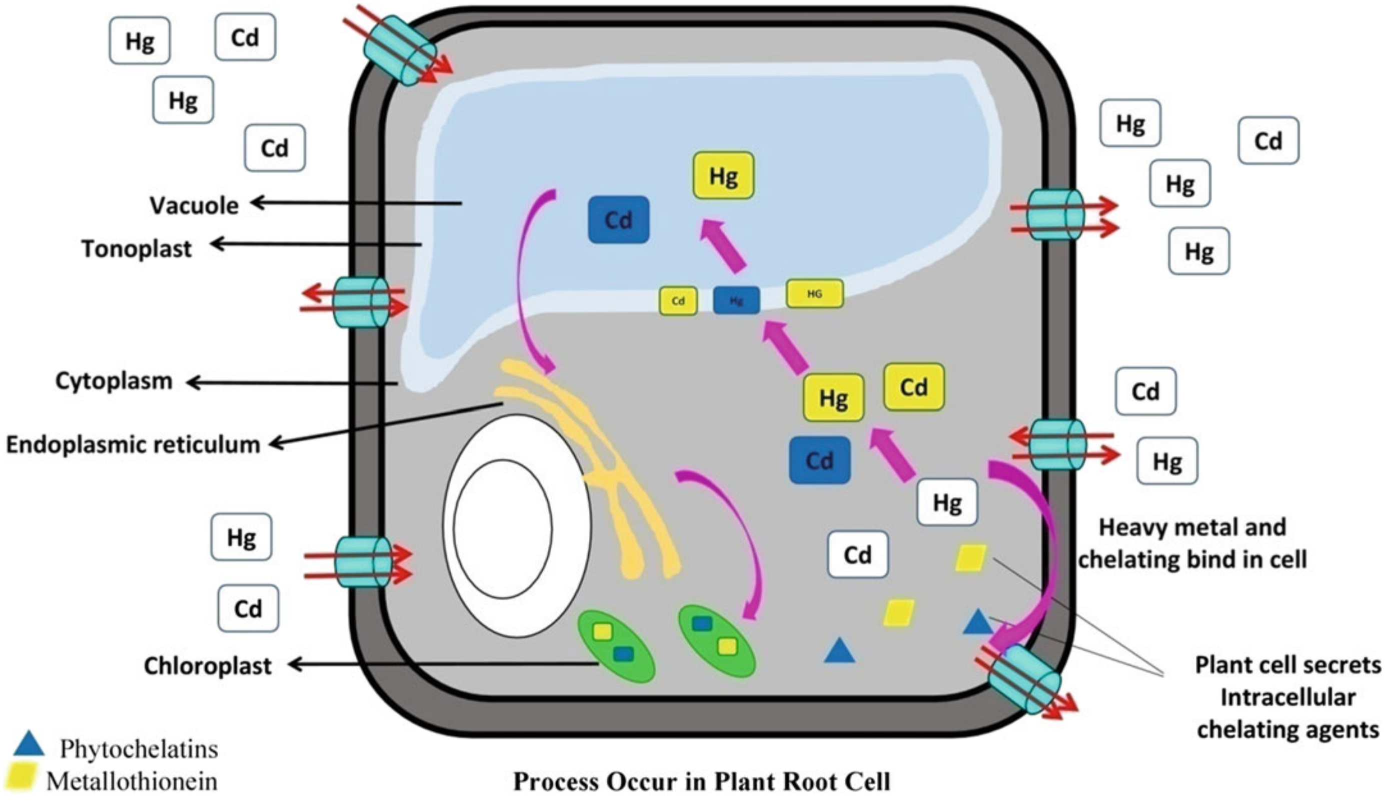

Fig. 6.5 Detoxification process occurs in plant cell

Table 6.4 Name of AM fungi used for bioremediation

S. No.	Name of Heavy Metal	Name of AM fungi	Name of plants	References
1	Cadmium (Cd)	*Glomus mosseae*; *Glomus* spp.; *Gigaspora* sp.; *Glomus intraradices*; *Suillus bovinus*; *Rhizopogon roseolus*; *G. constrictum*; AMF	*Trifoliumsub terraneum*; *Allium porrum*; *Zea mays L.*; *Trifolium repens*; Bean; *Hordeum vulgare*; *A. capillaries*	Joner and Leyval (2001), Weissenhorn et al. (1993), Vivas et al. (2003), Guo et al. (1996), Tullio et al. (2003), Liao et al. (2003), Lingzhi et al. (2014), Souza et al. (2013), GilCardeza et al. (2014)
2	Zinc (Zn)	*Glomus mosseae*; *Glomus intraradices*; *Glomus constrictum*; *G. ambisporum*; *G. scutellospora*; *G. dipurpurescens*; *G. fasiculatum*; *Glomus claodeum*; AMF	*Lygeum spartum*; *T. subterraneum*; *Solanum nigrum*; *Andropogon gerardii*; *Festia rubra*	Diaz et al. (1996), Joner (2000), Paula et al. (2006), Weissenhorn et al. (1994), Cornejo et al. (2008), Dueck et al. (1986)
3	Nikel (Ni)	*Gigaspora* species; *Glomus tenue*; *G. macrocarpum*	*Berkheya coddii*; maize; bean	Turnau et al. (2006), Guo et al. (1996)
4	Copper (Cu)	*Glomus intraradices*; AMF	*Zea mays*; *A. capillaries*; *Trifolium repens*; *Coreopsis drummondii, Pteris vittata*; *Oenothera picensis*	Liao et al. (2003), Chen et al. (2007), Cornejo et al. (2017), Cornejo et al. (2008)
5	Mercury (Hg)	AMF	*Nauclea orientalis*	Hanna et al. (2014)
6	Lead (Pb)	AMF; G. macrocarpum	*Lygeum spartum*	Vodnik et al. (2008), Diaz et al. (1996)
7	Aluminum	AMF		Seguel et al. (2015, 2016a, b)

storing of toxic heavy metal compounds and give additional detoxification mechanism for host plants.

The previous studies done by many workers have reported two methods of phytoremediation:

1. Phytoextraction (removal of heavy metals through plants)
2. Phytostabilization (Chaney et al. 1997; Garbisu and Alkorta 2001; Lasat 2002; Ernst 2000; Azaizeh et al. 1995; Baker and Brooks 1989; Kinnersley 1993; Welch 1993; Salt et al. 1995; Ghosh and Singh 2005).

Phytostabilization This method reduces the mobility of heavy metals in soil (Blaylock et al. 1999), for example, decreasing wind-blown dust, reduced soil erosion, and reducing pollutant solubility or bioavailability to the food chain (Radziemska et al. 2007). Solubility of metals in soil is decreased by the addition of soil amendments (organic matter, phosphates, alkalizing agents, and biosolids). Plant roots accumulate the contaminants and reduce the mobility of contaminants.

Phytoextraction Phytoextraction is the extraction of dangerous elements or compounds from soil or water with the help of plants. Hyperaccumulators of plants are used for phytoextraction method that absorbed extremely large amounts of heavy metals (Garbisu and Alkorta 2001). Absorption of heavy metal is completed by following five steps:

1. The metal must dissolve in some chemical (rhizospheric chemical).
2. The heavy metal is absorbed by plant root.
3. The plant must chelate the metal to protect itself and increase the mobility of the metal (this can also happen before the metal is absorbed).
4. Chelated metal is stored at safe place.
5. Finally, the plant recovers the damages caused during transportation and storage (Suman et al. 2018). Systems that transport and store heavy metals are the most critical systems in a hyperaccumulator. Sometimes, heavy metals are stored in leaves by hyperaccumulators.

Stored heavy metals were digested by the phytoremedation process like phytotransformation (Chaudhry et al. 1998; Broyer et al. 1972; Malone et al. 1974). In this method, plants also decrease toxicity and sequester the xenobiotics. The trinitrotoluene phytotransformation method has been widely studied, and a transformation pathway has been projected (Subramanian et al. 2006). Other bioremediation techniques are phytovolatilization (Lewis et al. 1966; Terry et al. 1992; Banuelos et al. 1993a, b; Wilber 1980; Suszcynsky and Shann 1995; Brooks 1998b), phytodegradation or rhizoremediation (Hoagland et al. 1994; Jacobsen 1997; Zablotowicz et al. 1994), and bioaugmentation (Fig. 6.6; Table 6.5).

6.8 Factors Responsible for Remediation

1. Type of soil: Uptake and tolerance of heavy metal depend on physiochemical properties of soil and soil microbes.
2. Gene expression: In legume plants, appearance of phytochelatin synthase gene (*PCS1*) also increased the heavy metal accumulation (Zhang et al. 2010a, b; Xu et al. 2014).
3. pH: The active uptake of cations via plasma lemma of roots includes H^+ excretion while anion uptake involves OH^- or HCO^{3-} excretion (Bolan et al. 1991). In symbiotic association with rhizobia, plants accumulate most of their N through N_2 fixation method. In this process, legume plants consume more cations than anions and discharge more H^+ ions from roots to soil and create acidic environment for the rhizosphere and bulk soil (Zhao et al. 2009). HM mobility and

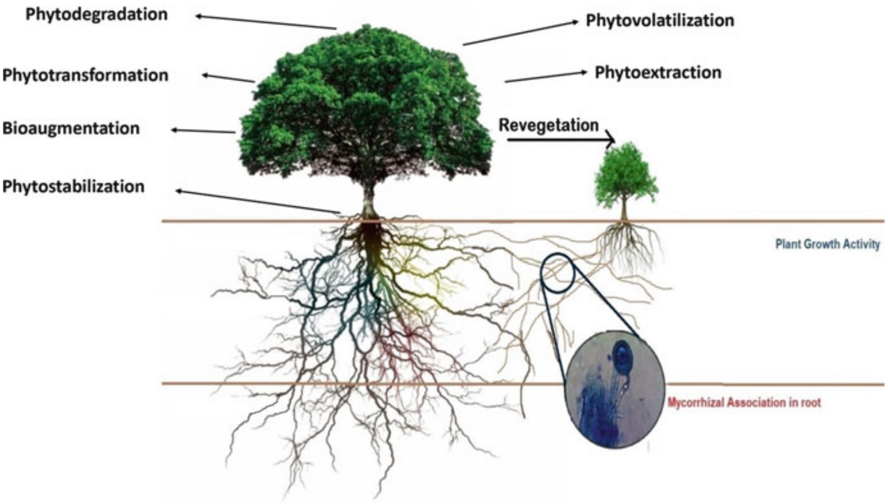

Fig. 6.6 Process of bioremediation by using plant and VAM fungi

availability totally depend on environmental factors as well as soil pH (Zhao et al. 2009). When soil pH is low, it could significantly increase the heavy metal concentrations in both the shoots and roots of plants.

4. Intercropping: Intercropping with legume tree shows higher efficiency for the removal of heavy metal. However, precaution is essential in screening of suitable legume neighbor plants because nitrogen fixing legume plants produced acid in variable amount; hence, pH of soil is altered (Tang and Chen 1999).
5. Transportation: Combination of heavy metal and mycorrhiza is known to affect acquisition and distribution of macronutrient in plants (Bati et al. 2014; Allen and Shachar-Hill 2009). Heavy metal concentration blocks ion absorption at the cell membrane and struggle for ion binding legends on the cell wall (Małkowski et al. 2005; Godbold and Kettner 1991), which show negative effect on plant nutrient uptake from soil. Uptake of phosphorus by non-mycorrhizal plants is by the direct pathway via Pi transporters in the epidermis, while AMF-associated plants can acquire phosphorus through root epidermal cells as well as phosphorus transporters in hyphae of mycorrhizal fungi (Smith et al. 2011; Tang and Chen 1999). Subramanian and Charest (1999) reported that the hyphae of VAM fungi were capable to consume and transfer inorganic nitrogen efficiently from soil to plant roots.
6. Legume plant: Legume plants show root–root interactions or root–AMF–root interactions (Teste et al. 2014). Association of legume plants, neighbors plant, and AM fungi increase the uptake of nitrogen and phosphorus as well as enhance plant heavy metal resistance, because the excess phosphorus simply create metastable compounds with toxic heavy metals (Andrade et al. 2004) and drastically reduce the bioavailability of heavy metals.

Table 6.5 Process of bioremediation by using plant

S. No.	Application	Process	Media	Contaminants	Plant	Disadvantage
1	Phytodegradation	Aquatic and terrestrial plants take up, store, and biochemically degrade selected organic (Newman et al. 1998)	Soil, groundwater, landfill leachate, land application of waste water	Herbicides (atrazine, alachlor); aromatics (BETX); chlorinated aliphatics (TCE); nutrient; ammunition waste (TNT, RDX)	Phreatophyte trees (poplar, willow, cotton wood, aspen); grasses (rye, Bermuda, sorghum, fescue); legumes (clover, alfalfa, cowpeas)	
2	Phytoextraction or phytoaccumulation or phytoabsorption or phytosequestration	Uptake of contaminants from soil into roots or harvestable shoots (Salt et al. 1995)	Soil, brownfields, sediments (Brooks 1998a)	Metals (Pb, Cd, Zn, As, Cu, Cr, Se, U) with EDTA addition for Pb, selenium, inorganics, radionuclides (Kumar et al. 1995)	Sunflower; Indian mustard; rapeseed plants, barley, hops; crucifers; serpentine plants; nettles, dandelions; alyssum, brassica, thelaspi (Cornish et al. 1995)	Metal hyperaccumulators are generally slow growing, and bioproductivity is rather small and shallow root system. Phytomass after process must be disposed off properly (Banuelos et al. 1999)
3	Rhizodegradation	Plant exudates, root necrosis, and other processes provide organic carbon and nutrients to soil bacteria growth by two or more orders of magnitude. Exudates stimulate degradation by mycorrhizal fungi and microbes. Live roots can pump oxygen to	Soil, sediments, land application of waste water	Organic contaminants (pesticides) aromatic and polynuclear aromatic hydrocarbons such as PAHs, petroleum hydrocarbons, TNT, pesticides	Phenolics releasers (mulberry, apple, Osage orange); grasses with fibrous roots (rye, fescue, Bermuda); aquatic plants for sediments	

(continued)

Table 6.5 (continued)

S. No.	Application	Process	Media	Contaminants	Plant	Disadvantage
		aerobes while dead roots may support anaerobes				
4	Phytovolatilization (Erakhrumen 2007)	Volatilization by leaves; plants evapotranspirate metal (Freestone 2006; Smit et al. 2009)	Soil, groundwater, landfill leachate, land application of waste water (Temperton et al. 2007)	Herbicides (atrazine, alachlor); aromatics (BETX); chlorinated aliphatics; ammunition waste (TNT, RDX) (Baumeister and Callaway 2006)	Phreatophyte trees (poplar, willow, cottonwood, aspen); grasses (rye, Bermuda, sorghum, fescue); legume (clover, alfalfa, cowpeas)	The contaminant or a hazardous metabolites might accumulate in vegetation and be passed on in later products such as fruit or lumber. Low levels of metabolites have been found in plant tissues (Adler 1996)
5	Phytostabilization	Plant control pH, soil gases and redox conditions in soil to immobilize contaminants, humification of some organic compound is expected (Alkorta et al. 2004)	Soil, sediments, metal, groundwater	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	Often requires extensive fertilization or soil modification using amendments, long term maintenance is needed to prevent leaching (Prasad 2004).
7	Phytotransformation	Sorption, uptake, and transformation of contaminants (Subramanian et al. 2006)		Organics, including nitroaromatics and chlorinated aliphatics	Tress and grasses	

6.9 Conclusion

The pollution of soils with heavy metals symbolized a worldwide ecological problem of great concern. Conventional methods for metal-contaminated soils are usually very costly and regularly induce undesirable effects on physico-chemical properties of soil and biological activity. Chemical methods have many drawbacks in the elimination of contaminants because they generally utilize chemical catalysts, and applying them in larger polluted sites is complicated. Physical remediation methods can totally eliminate heavy metal(loid)s from infected soil but can cause negative effect in nature and are highly expensive. The utilization of microbial cultures which destroy or alter heavy metal to less toxic compounds has become gradually more famous in recent years. Bioremediation is a biological mechanism of recycling wastes into another form that can be used by other organisms. Mycorrhizal fungi can enhance nutrient uptake and also have degradation capacities for heavy metal. Maximum research on fungal bioremediation has been carried out on laboratory. So further work is required to account the natural variables and increase their applicability in large-scale polluted fields. This chapter may further contribute to the substantial potential offered by fungal diversity in various habitats and their bioremediation potential.

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Chapter 7
Strategies to Improve Remediation Technology Using Fungi

Darshan M. Rudakiya, Yogesh Patel, and Urvish Chhaya

Contents

Abstract Fungi have competence to degrade hazardous contaminants by excreting the enzymes and other metabolites which decrease the risk associated with the toxicants and heavy metals. Furthermore, they have capability to form the mycelial networks which influence the remediation process. In fungal kingdom, ascomycetes, basidiomycetes, deuteromycetes, and zygomycetes are the major fungi which are mainly involved in the remediation process. These fungi can degrade wide array of hazardous contaminants such as heavy metals, pesticides, nitroaromatics, endocrine disrupting chemicals, antibiotics, and polycyclic aromatic hydrocarbons. This chapter also describes different strategies like utilization of multi-omics tools, screening the fungal isolates, genetic modification, and development of consortia for multiple

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D. M. Rudakiya (✉)
Bioconversion Technology Division, Sardar Patel Renewable Energy Research Institute, Anand, Gujarat, India

Y. Patel
College of Basic Science and Humanities, Sardarkrushinagar Dantiwada Agricultural University, Gujarat, India

U. Chhaya
Department of Microbiology, N. V. Patel College of Pure and Applied Sciences, Anand, Gujarat, India

D. G. Panpatte, Y. K. Jhala (eds.), *Microbial Rejuvenation of Polluted Environment*, Microorganisms for Sustainability 25, https://doi.org/10.1007/978-981-15-7447-4_7

pollutants. Thus, different strategies can enhance the rate of degradation or transformation of metabolites which can be further utilized for the large-scale application of myco-remediation.

Keywords Bioremediation · Dyes · Fungi · Heavy metals · Pesticides · Polycyclic aromatic hydrocarbons

7.1 Introduction

Vast numbers of fungi (more than 1.5 million species) inhabit on the Earth, living on the soil and rocks, under the soil and/or associated with different plant bodies. Fungal kingdom comprises single cellular and multicellular fungi that are spread all over the world wherein four major phyla are divided, namely chitridiomycetes, zygomycetes, ascomycetes and basidiomycetes (Hibbett et al. 2007). Generally, fungal decay types mainly categorized into three parts based on their type of degradation that comprises white rot, brown rot, and soft rot (Rudakiya and Gupte 2017, 2019a). Fungi can convert various organic and inorganic contaminants wherein dyes, metals, polycyclic aromatic hydrocarbons, and phenols are included. Fungal kingdom possesses more than 1 lakh known species which are capable enough to degrade the hazardous contaminants. Among these fungi, Ascomycetes, Basidiomycetes, and subphylum mucoromycotina are the potential degraders of the contaminants; however, other fungi are very few times reported for the bioremediation (Fig. 7.1).

Brown Rot Fungi Brown rot fungi show the effective degradation of cellulose and hemicellulose in lignocelluloses, but the degradation of lignin is limited. It is caused by majority of ascomycetes and some of basidiomycetes (Schwarze 2008). As brown rot fungi favor the degradation of carbohydrates such as cellulose and hemicellulose, the decayed wood shows the brittle nature. *Fomitopsis pinicola* and *Laetiporus sulphureus* are the example of typical brown rot (Schwarze et al. 2003).

Soft Rot Fungi Soft rot decay is generally caused by Ascomycetes and deuteromycetes fungi (Raberg et al. 2009). Compositional study shows that lower methoxy content of wood lignin is observed in soft rot decay (Rabinovich et al. 2004). Ligninolytic enzymes presented in soft rot fungi are not efficient to degrade the guaiacyl lignin; however, they can efficiently attack on the syringyl lignin and degrade it efficiently (Nilsson et al. 1989).

White Rot Fungi Fungi causing the white rot mainly belong to Basidiomycetes and Ascomycetes. Traditionally, the term “White rot” is used to describe the type of wood decay, wherein wood has a bleached appearance as majority of lignin is degraded by the fungi, and the remaining mass is made up of cellulose and

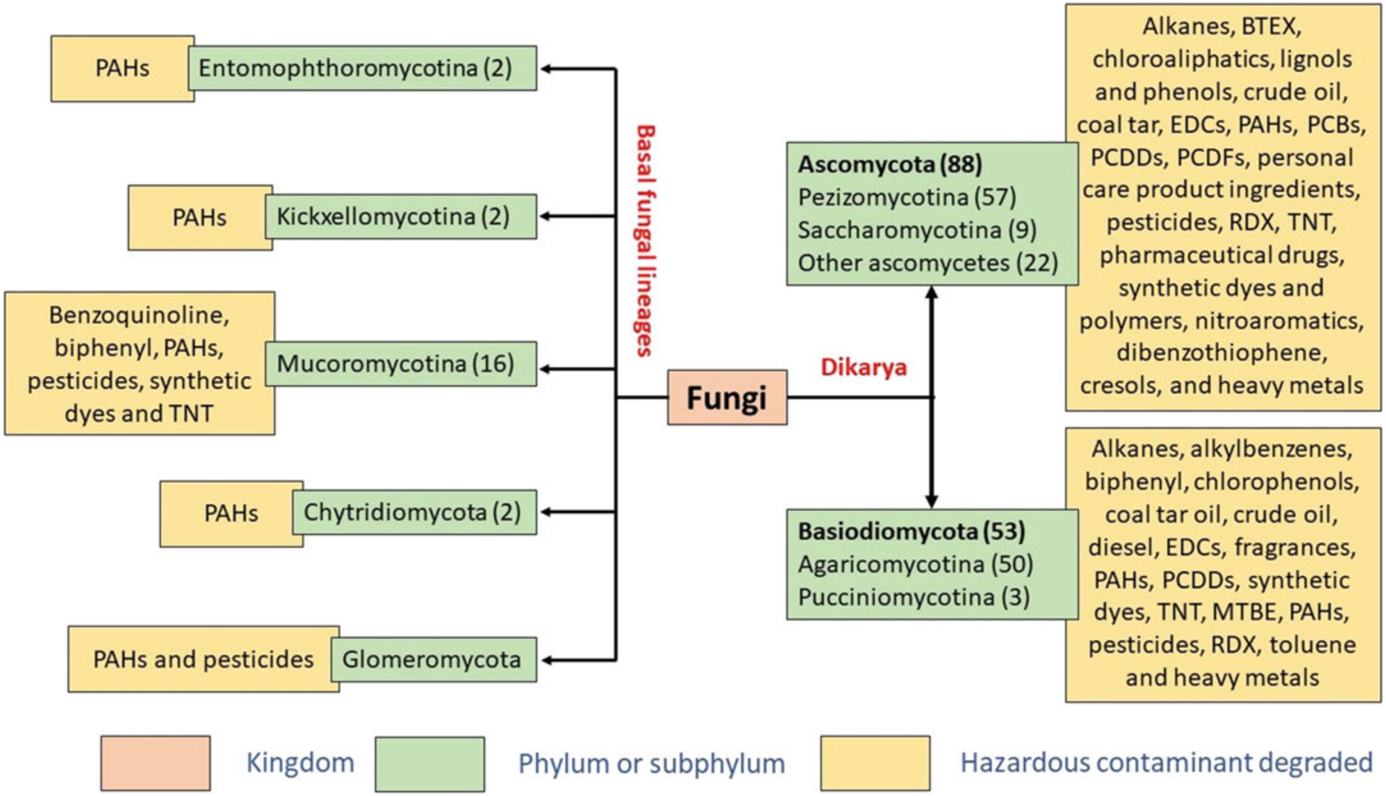


Fig. 7.1 Major hazardous contaminants are degraded by various phylum and subphylum of fungi

hemicellulose that are white in color (Garg and Chandel 2012; Kamei et al. 2012). On the contrary, the rate of degradation of lignin and cellulose is relative which can vary based on the fungal species, degradation condition, and wood types (Schwarze 2004). White rot fungi are subdivided into two types based on the degradation time of lignin which are selective delignification and simultaneous rot.

Since last three decades, fungi have been investigated for the degradation of broad spectrum organic contaminants. They were also exploited for the degradation of organic contaminants, including dyes, PAHs, TNT, pesticides, PCBs, chlorinated hydrocarbons, and other toxic organic compounds (Gupte et al. 2016; Patel et al. 2016; Gahlout et al. 2017; Shankar and Nill 2015; Khambhaty et al. 2015). Fungi are efficient remediation agent of organic contaminants than bacteria due to the efficient extracellular nonspecific ligninolytic enzyme system, which can degrade various hazardous contaminants (Christian et al. 2005). In addition, some white rot fungi and their enzymes are utilized to synthesize the bioactive compounds, which have potential anticancer, anti-HIV, antimicrobial, and antifungal activities (Mikolasch and Schauer 2009; Kudanga et al. 2017).

7.2 Fungal Components

7.2.1 Enzymes

White rot fungi secrete a wide array of enzymes in order for the degradation of lignocellulosic biomass, which comprise the ligninolytic, hydrolytic, and accessory enzymes.

7.2.1.1 Lignin Degrading Enzymes

White rot fungi produce various ligninolytic enzymes in large amounts which secrete externally. The enzymes are laccase, lignin peroxidase, Mn peroxidase, and versatile peroxidase (Li et al. 2018). The role of these enzymes in fungi is presented in Table 7.1.

7.2.1.2 Laccase

Laccases are benzenediol:oxygen oxidoreductases (EC 1.10.3.2) and these enzyme belongs to the of multicopper oxidases and blue oxidases (Rudakiya et al. 2020). It was first discovered in the Japanese lacquer tree *Rhus vernicifera*. Thereafter, laccase has been found in various plant species, insects, bacteria, and fungi (Rudakiya and Gupte 2019b). Fungi belonging from basidiomycete phylum are the most efficient laccase producers, some of the fungi are as follows: *Coriolopsis polyzona*, *Trametes*

Table 7.1 List of enzymes that assist the biomass degradation and their mechanism of action

Enzyme	Mechanism of action	Reference
Lignin degrading enzymes		
Laccase (1.10.3.2)	It catalyzes the oxidation reactions that lead to the free radical formation which can be intermediate substrates	Li et al. (2018)
Mn peroxidase (1.11.1.13)	It acts only on the phenolic structures of lignin	Kinnunen et al. (2017)
Lignin peroxidase (1.11.1.14)	It reacts in the presence of hydrogen peroxide with catalysis of the oxidative depolymerization of non-phenolic lignin, β-O-4 non-phenolic lignin, and phenolics	Houtman et al. (2018)
Versatile peroxidase (1.11.1.16)	It oxidizes the substrates of Mn peroxidase and lignin peroxidase	Kinnunen et al. (2017)
Cellulose degrading enzymes		
Endoglucanase (3.2.1.4)	Random catalysis of internal cellulose chain by releasing cellulose subunits, i.e., cellobiose and cello-oligosaccharides	Pamella et al. (2017)
Exoglucanase (3.2.1.91)	Catalysis of cellulose chains specifically at terminal part by releasing the cellulose subunits	Parafati et al. (2017)
β-Glucosidase (3.2.1.21)	Catalysis of cellulose subunits which release the glucose and other metabolites	Boudabbous et al. (2017)
Hemicellulose degrading enzymes		
α-L-Arabinofuranosidase (3.2.1.55)	Catalysis of terminal α-L-arabinofuranoside which can be converted into α-L-arabinosides	Bastos et al. (2018)
α-D-Glucuronidase (3.2.1.131)	Catalysis of α-1,2 glycosidic bond of hemicellulose which converts into D-glucuronic acid, 4-O-methyl-D-glucuronic acid, and D-xylose	Manavalan et al. (2015)
Acetyl xylan esterase (3.1.1.72)	Catalysis with deacetylation reaction of xylans and xylo-oligosaccharides	Komiya et al. (2017)
β-xylosidase (3.2.1.37)	Catalysis of xylobiose which convert the D-xylose	Bastos et al. (2018)
Endo xylanase (3.2.1.8)	Catalysis of β-1,4-xylan which convert xylose	Manavalan et al. (2015)
Ferulic acid esterase (3.1.1.71)	Catalysis of COOH- bonds of feruloyl-polysaccharide which converts into ferulic acid and polysaccharide	Manavalan et al. (2015)
Accessory enzymes		
Cellobiose dehydrogenase (1.1.99.18)	It reduces aromatic radicals preventing repolymerization, demethoxylation, or hydroxylation of nonphenolic lignin and reduction of precipitated MnO_2	Ma et al. (2017)
Aryl-alcohol oxidase (1.1.3.7)	Aromatic alcohols oxidized to aldehydes, which generates the H_2O_2	Houtman et al. (2018)
Glyoxal oxidase (1.2.3.15)	Glyoxal oxidized to glyoxylic acid, which produces the H_2O_2	Manavalan et al. (2015)
Oxalate decarboxylase (4.1.1.2)	It degrades the oxalic acid and converted into CO_2	Manavalan et al. (2015)

hirsuta, *Trametes ochracea*, *Trametes villosa*, *Trametes versicolor*, *Lentinus tigrinus*, *Trametes gallica*, *Cerrena maxima*, and *Pleurotus eryngii* (Ruiz-Duenas et al. 2013; Munir et al. 2015). Possible roles of laccase in fungi are in pigment formation, lignin degradation, and detoxification (Kim et al. 2008a, b).

7.2.1.3 Peroxidases

Lignin peroxidases (LiPs) are glycoproteins of approximately 30–50 kDa with pI ranging from 3.2 to 4.0. It oxidizes the most phenolic compounds through the generation of phenoxy radicals. Mn peroxidases are glycosylated proteins with pI ranging from 4.2 to 4.9 and molecular masses ranging from 45 to 47 kDa (Kirk and Cullen 1998). Mn peroxidase shows the catalytic cycle which is similar to lignin peroxidases. The reaction involves two-electron oxidation of the heme by H_2O_2, which is further carried out by reduction of two electrons to the native enzyme (Hatakka 1994). Versatile peroxidase is the enzyme which comprises the heme component with peroxidase activity with hybrid molecular structure of lignin peroxidase and Mn peroxidase. It was first described from the white rot fungus *Pleurotus eryngii* (Martinez et al. 1996).

7.2.1.4 Cellulose Degrading Enzymes

Cellulases or cellulose degrading enzymes from ascomycetes or basidiomycetes are categorized into three enzymes which are endoglucanase, exoglucanase, and β-glucosidases. The mechanism of action of all cellulolytic enzymes is shown in Table 7.1.

Cellulases are the enzymes which act on the cellulose in sequential manner which degrade or depolymerize step by step. Terminology for each enzyme: endoglucanase is endo-1,4-β-glucanase (E.C.3.2.1.4), exoglucanase is exo-1,4-β-D-glucanase (E.C.3.2.1.176), and β-glucosidase is β-D-glucoside glucohydrolase (E.C.3.2.1.21). First, the endoglucanase enzyme catalyzes the reaction with cellulose which cleaves the glycosidic bonds that forms the long chains of the different oligo- and/or disaccharides. Furthermore, the other enzyme called exoglucanase acts on the long chain of the oligomers which also acts on the either reducing or nonreducing ends. Finally, β-D-glucoside glucohydrolase catalyzes the reaction wherein oligo- or disaccharides are involved and convert into glucose subunits. The glucose molecules are directly used for the fungal growth and metabolism (Rudakiya 2019; Narra et al. 2020).

7.2.1.5 Hemicellulose Degrading Enzymes

Hemicellulases are the major group of enzymes which catalyze the degradation of hemicellulose. Specifically, endo-xylanase, β-xylosidase, α-glucuronidase, α-L-arabinofuranosidase, acetyl xylan esterase, and ferulic acid esterase are the enzymes which degrade the xylan components. All enzymes act in the sequential manner to degrade the hemicellulose to xylose (Shallom and Shoham 2003; Satyanarayana et al. 2019).

7.2.2 Exopolysaccharides

Various biotechnological applications are mainly focused on the natural and biopolymers which have huge demand in the market. Various fungi produce the extracellular polymeric substances such as exopolysaccharides (EPS) which have huge demand in recent times. Various types of polysaccharides are produced by plants which are cellulose, starch, and pectin. Likewise, algae produce the agar, alginate, and carrageenan type of polysaccharides which have huge biotechnology demand. Bacteria also produce the dextran, alginate, gellan, xanthan gum, and pullulan which are commonly used as food additives for their gelling, stabilizing, or thickening properties. Polysaccharides comprise higher capacity for the chelation and entrapment of hazardous contaminants (Kumar et al. 2007). Increasing interest to resolve the environmental issues and its production using green or environmental friendly procedures leads to the production of such substances that are mainly important for the global market for microbial products to about 250 billion US dollars by 2016. This carbohydrate product is the metabolite which secrete by the fungi on the cell surface which plays a critical role in various industries. EPS gained attention in the pharmaceutic industries due to their involvement in various biological mechanisms such as signal transduction, adhesion, infection, and immune response (Sutherland 2002; Kumar et al. 2007). Microbial polysaccharides are the polymer which comprises higher molecular weight. It is generally presented at lipopolysaccharides or capsular polysaccharides (Taylor and Roberts 2005).

An important distinction of polysaccharide is based on their charge properties; they may be naturally anionic and neutral. Microbial EPS like xanthan, phosphomannan and alginate belong to anionic group while EPS like levan, scleroglucan, pullulan, and dextran belong to neutral group. Some polysaccharides have anionic properties, and they contain acidic groups, such as carboxyl, phosphate, or sulfate. The diversity of various EPS produced by microorganisms is often stressed. At present, a considerable number of bacteria, lactic acid bacteria (LAB), higher basidiomycetes, lower filamentous fungi and yeasts from different ecological niches are known for their ability to synthesize EPS in nature as well as in laboratory culture system. Important EPS is produced by various fungi which is shown in Table 7.2.

Polysaccharide that comprises single glucose subunits is called as glucans (Murray et al. 2002). These types of carbohydrates include the glycogen, cellulose, and dextran. General formula of these polysaccharides are $(C_6H_{12}O_5)_n$ (Duchon 1985). Other polysaccharides called β-glucans, β-1,3-D-glucans, or β-1,4-D-glucans are generally present in higher plants.

Table 7.2 List of exopolysaccharides produced by the fungi

Exopolysaccharide	Fungi
Pullulan	*Aureobasidium pullulans*
Scleroglucan	*Sclerotium glutanicum*
Schizopyllan	*Schizophyllum commune*
Lentinan	*Lentinula edodes*
Grifolan	*Grifola frondosa*
Pleuran	*Pleurotus ostreatus*
Krestin	*Coriolus versicolar*
Ganoderan	*Ganoderma lucidum*

Natural products, i.e., β-glucan are generally utilized for several centuries. Countries such as China and Japan utilize the EPS to obtain the antioxidant and anticancer compounds. The fungi used for the medicinal purpose is now utilized in pharmaceutical industries. Fungi show favorable dietetic properties with respect to their low fat and caloric value and high levels of proteins, minerals, and certain polysaccharides (Borchers 1999).

7.2.3 *Organic Acids*

Fungi are known to secrete the organic acids in high amounts, which are mainly oxalic acid, citric acid, malic acid, gluconic acid, etc. Among them, oxalic acid is the most commonly produced in fungi and is thought to play an essential role in wood degradation, lignin degradation, plant pathogenesis, and metal transformation (Dutton and Evans 1996; Gadd 1999). Oxalic acid, a simplest dicarboxylic acid, has greater ionic strength than that of acetic acid. It is produced as a secondary metabolite from glyoxylate cycle. Oxalic acid typically occurs in di-hydrate form with molecular formula $C_2H_2O_4 \cdot 2H_2O$. It reduces the viscosity of cellulose and hemicellulose, lowers the pH (Rhee et al. 2012), and provides H_2O_2, which increases the accessibility of cellulose fibers to cellulases (Kim et al. 2008a, b).

The biosynthesis of oxalic acid mostly originates from intermediates of the tricarboxylic acid (TCA) cycle and the glyoxylate cycle, which are involved in the hydrolytic cleavage of oxaloacetate to oxalate and acetate by oxaloacetase (EC 3.7.1.1) (Dutton and Evans 1996). Several white rot fungi oxidize the glyoxylate and convert to oxalate using glyoxylate oxidase (Dutton et al. 1993). Degradation of oxalic acid is mainly caused by oxalate decarboxylase, oxalate oxidase, Mn peroxidase system, and lignin peroxidase system. Oxalate oxidase cleaves the oxalic acid using atmospheric oxygen and converts into the carbon dioxide and hydrogen peroxide. Oxalate decarboxylase degrades the oxalic acid to formic acid and carbon dioxide. In Mn peroxidase system, Mn(III) is reduced using oxalic acid and converted into Mn(II) by forming the two molecules of carbon dioxide (Fig. 7.2). Similarly, veratryl alcohol is reduced using oxalic acid and converted into reduced form of veratryl alcohol by forming the two molecules of

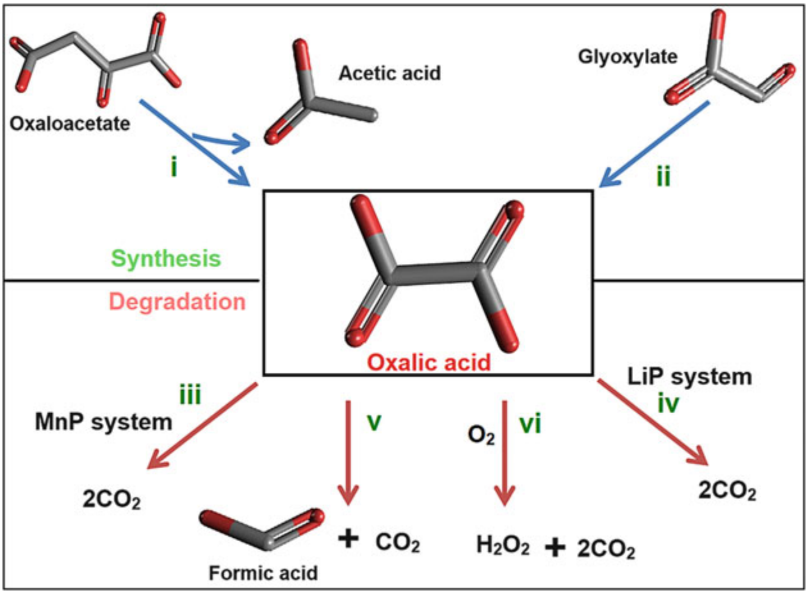


Fig. 7.2 Pathway for the synthesis and degradation of oxalic acid observed in white rot fungi (adapted from Dutton et al. 1993; Mäkelä et al. 2002) (i = oxaloacetase; ii = glyoxylate oxidase; iii = Mn peroxidase; iv = Lignin peroxidase; v = oxalate decarboxylase; and vi = oxalate oxidase)

carbon dioxide in lignin peroxidase system. So, oxalic acid plays a crucial role in regulating the level of oxalate concentrations inside the fungal cells (Dutton et al. 1993; Mäkelä et al. 2002; Arnstadt et al. 2016).

Oxalic acid is organic di-acid, which is the most oxidized carbon compound after carbon dioxide. It is a strong acid and has the ability to form complex metals, which result to precipitate the insoluble metal oxalate. However, the metal oxalate formation depends on the metal and chemical conditions (Arnott 1995; Gadd 1999). Oxalates are generally crystalline or amorphous in nature, and its solubility lie between 10^{-5} and 10^{-15}. Production of oxalic acid by white rot fungi causes the metal mobilization from solid metal substrates which can be proceeded by acidolysis and complex formation or metal immobilization which can form the insoluble oxalate minerals. Produced oxalate minerals have central role in several geomicrobiological processes, and they have been applied for the various biotechnological applications (Gadd et al. 2014; Gadd 2017).

7.2.4 Reactive Oxygen Species (ROS)

White rot fungi secrete the ligninolytic and cellulolytic enzymes which participate in the lignocellulose degradation; however, the activity of enzymes is hampered due to

Table 7.3 Production and mechanism of action of ROS by white rot, brown rot, and soft rot fungi

ROS	Mechanism of action	Reference
$OH^{\bullet}$	Radicals cleavage the β-O-4, the nonphenolic lignin hydroxylation causes the phenolics metabolite formation, demethoxylation, Cα-oxidation of nonphenolic structures	Hildén et al. (2000), Hammel et al. (2002)
$ROO^{\bullet}$	These radicals cleave the Cα–C3 and β-O-4 which metabolite the nonphenolic lignin	Hammel et al. (2002)
$O_2^{-\bullet}$	It produces the hydrogen peroxide via dismutation, Mn^{2+} oxidation to Mn^{3+}	Gierer et al. (1994)

their large size. So, fungi have to secrete smaller radicals which are the primary agents to start the degradation process, wherein reactive oxygen species play an important role in initiating the wood decay. There are three types of ROS, which includes hydroxyl radical ($OH^{\bullet}$), peroxyl radicals ($ROO^{\bullet}$), and superoxide radicals ($O_2^{\bullet -}$). The role of ROS in lignin degradation is shown in Table 7.3. Various white rot fungi show the production of $OH^{\bullet}$ radicals before producing the lignocellulolytic enzymes (Barr et al. 1992; Kutsuki and Gold 1982; Tanaka et al. 1999). Among ROS, $OH^{\bullet}$ radicals are very reactive that causes the cleavage of lignin by reducing the aliphatic Cα-H and by adding to aromatic rings (Hammel et al. 2002). Mn peroxidases of white rot fungi act on the unsaturated fatty acids, which generates $OH^{\bullet}$ and $ROO^{\bullet}$ (Moen and Hammel 1994). However, superoxide radical does not play any role in the degradation of lignin units, but it produces H_2O_2 via dismutation (Gierer et al. 1994).

7.2.5 Other Molecules

White rot fungi also produce the low molecular weight chelators, which are able to penetrate into the cell wall. For instance, *G. trabeum* produces low molecular weight peptide that cleaves the cellulose into short fibers (Wan and Li 2012). Fungi also produce various organo-halogen metabolites, which are mainly chlorinated anisyl metabolites (CAM) and chlorinated hydroquinone metabolites (CHM). CAM serves as substrates for aryl alcohol oxidase that is responsible for the H_2O_2 production. On the contrary, some of the CHMs serve as a substrate for lignin peroxidase (de Jong and Field 1997).

7.3 Remediation of Hazardous Toxicants

Due to the secretion of enzymes and other metabolites, fungi have been employed to mineralize and/or degrade the hazardous contaminant as they have wide range of lignocellulolytic enzymes that act on the contaminants. Fungal enzymes have been applied to degrade the polycyclic aromatic hydrocarbons (PAHs), chlorinated

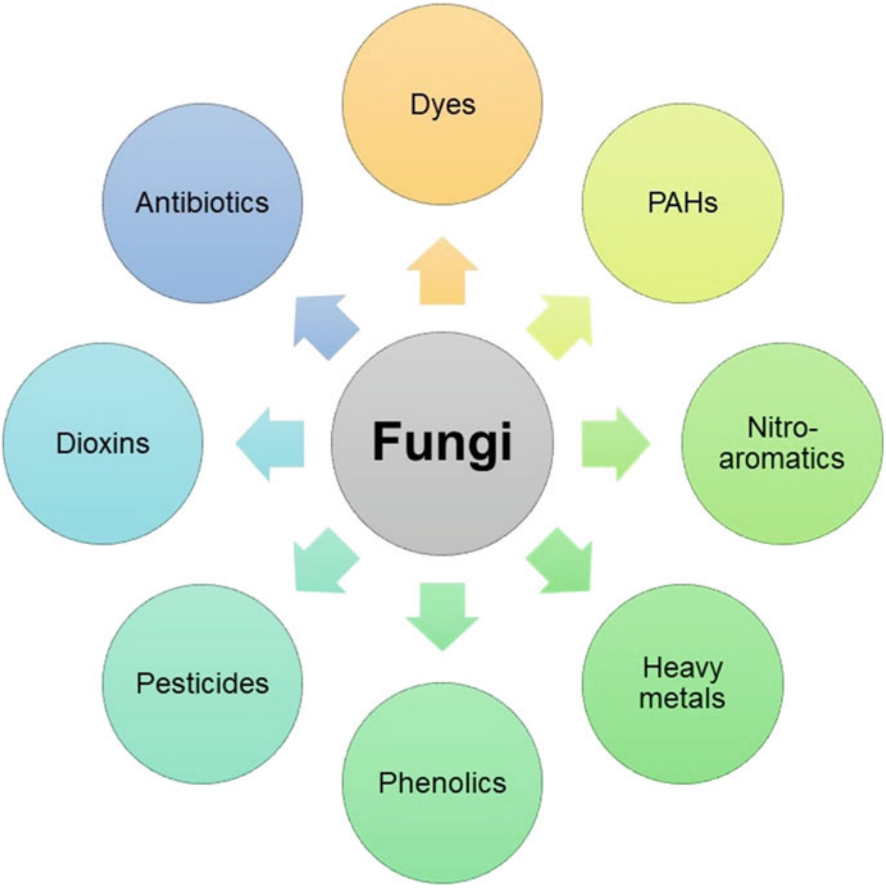


Fig. 7.3 Fungal remediation of different toxicants by biomass, organic acids, enzymes, and other metabolites

phenols, azo dyes, bisphenol A, pesticides, aflatoxin B1, imiprothrin, triclosan, and diclofenac (Rudakiya and Pawar 2014; Rudakiya et al. 2019). List of degraded compounds by fungi have been shown in Fig. 7.3.

Fungi mainly utilizes the organic pollutants to degrade it, wherein they initially use carbohydrates and proteins for the growth which is further used under contaminant-stressed condition. Furthermore, the fungus metabolizes the organic compounds and cleaves the initial aliphatic or aromatic compounds which comprise volatile organic compounds. Initially, contaminant is degraded by the extracellular enzymes, organic acids, or hydroxyl radicals which is further followed by intracellular enzymes. Intracellular enzymes such as P450 monooxygenase enzymes oxidize the cyclic structure of the organic oxidative coupling. The reaction proceeds for the further mineralization and converted into mineralized compounds (Fig. 7.4) (Harms et al. 2011; Rudakiya and Pawar 2013a, b, 2017; Rudakiya 2018).

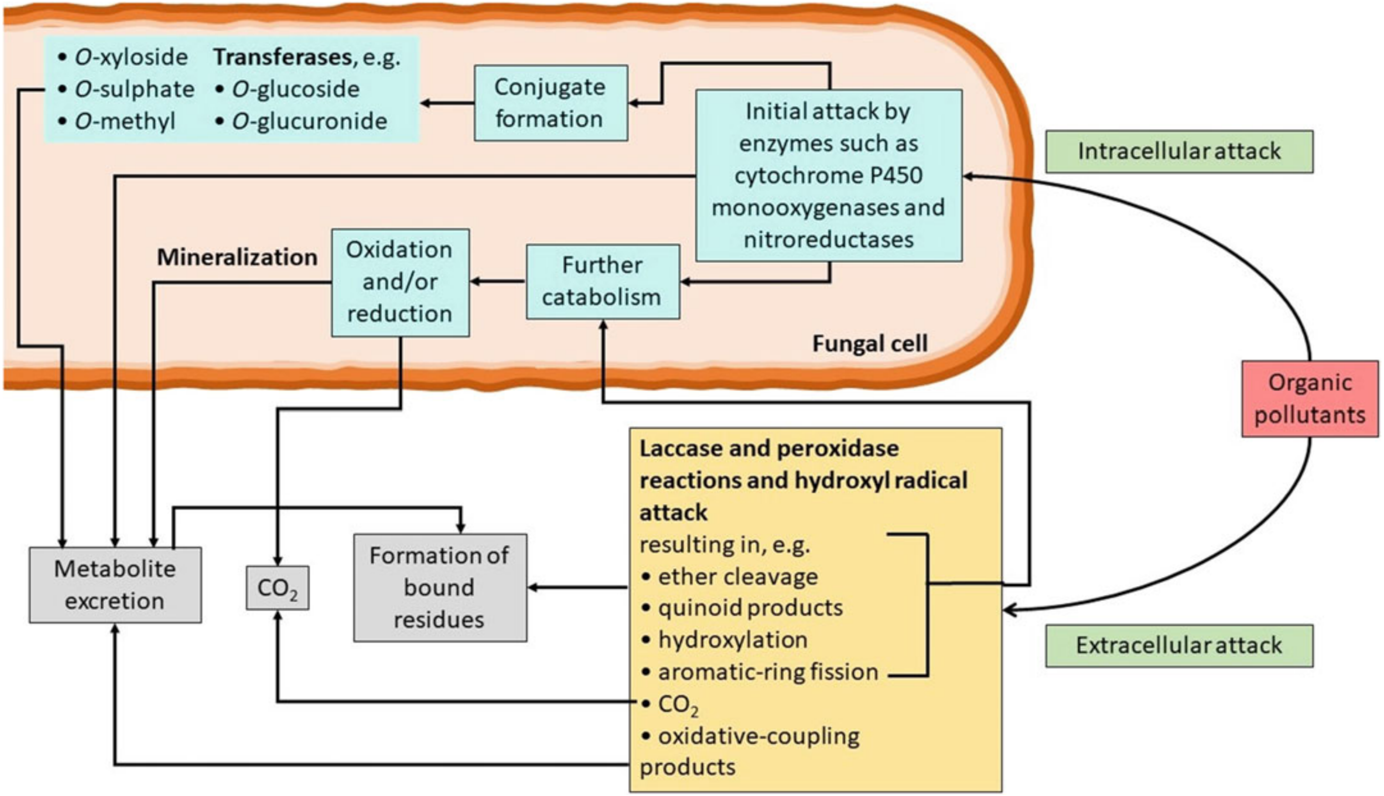


Fig. 7.4 Various methods have been used to degrade the organic hazardous toxicants

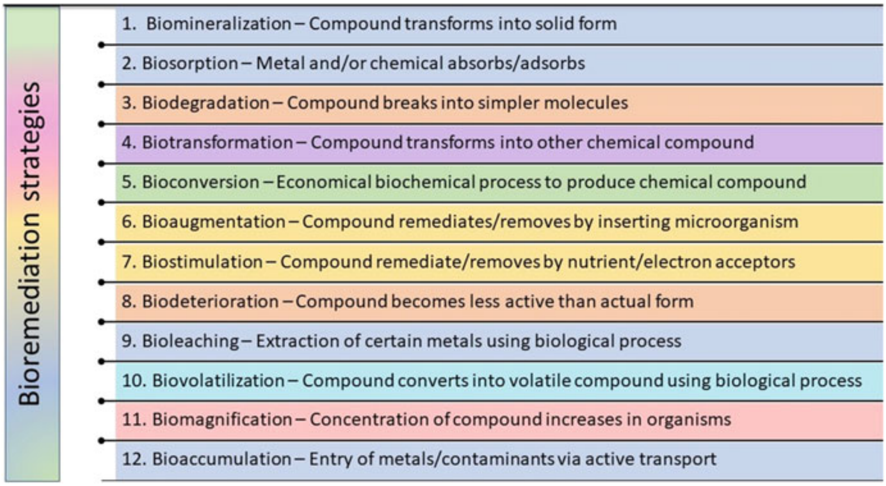
Fungal cell wall comprises various proteins, fatty acids, and carbohydrates that comprise certain functional groups in which COOH-, NH_3-, and PO_4- groups are involved in the metal chelation. In addition to that, white rot fungi and brown rot fungi produce extra polymeric substances such as exopolysaccharides in the environment which is effective to binding of various toxicants, metal chelation, reduction of heavy metals, and tolerance to metals. Anionic property of exopolysaccharides carries out the electrostatic interactions with heavy metals (Shah et al. 2018). Some fungi show the efficient biosorption efficacy with different heavy metals (Rudakiya et al. 2018).

7.4 Strategies to Improve Bioremediation Technology

Bioremediation technology has been improved by using various tools and techniques which can be considered as its strategies. Fungi can do biomineralization, biosorption, biodegradation, biotransformation, bioconversion, bioaugmentation, biostimulation, biodeterioration, bioleaching, biovolatization, biomagnification, and bioaccumulation to degrade or remediate various hazardous contaminants and heavy metals. Figure 7.5 shows that these strategies can be used for the remediation of hazardous contaminants.

Based on the above discussion, following strategies should be carried out to remediate various hazardous contaminants.

1. **Screening strategy:** Screening of fungal isolates is required to achieve higher degradation efficiency.



1. Biomineralization – Compound transforms into solid form
2. Biosorption – Metal and/or chemical absorbs/adsorbs
3. Biodegradation – Compound breaks into simpler molecules
4. Biotransformation – Compound transforms into other chemical compound
5. Bioconversion – Economical biochemical process to produce chemical compound
6. Bioaugmentation – Compound remediates/removes by inserting microorganism
7. Biostimulation – Compound remediate/removes by nutrient/electron acceptors
8. Biodeterioration – Compound becomes less active than actual form
9. Bioleaching – Extraction of certain metals using biological process
10. Biovolatilization – Compound converts into volatile compound using biological process
11. Biomagnification – Concentration of compound increases in organisms
12. Bioaccumulation – Entry of metals/contaminants via active transport

Fig. 7.5 Various bioremediation strategies that can be employed by fungi

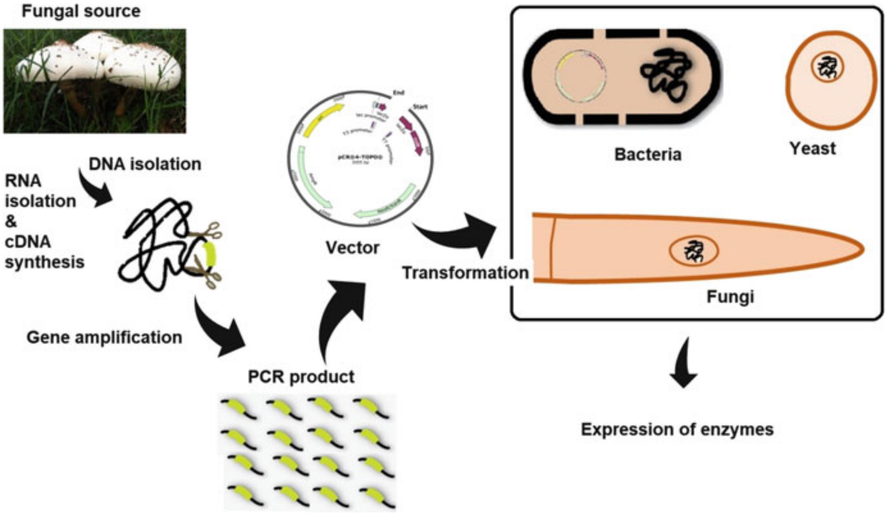


Fig. 7.6 Bioremediation of hazardous contaminants can be influenced by modifying the gene and expressing it into other organisms. Sometimes overexpression of gene can be done to increase the enzyme activity which influences to the bioremediation

2. **Optimization strategy:** Optimization of culture conditions like C and N sources, surfactants using single factorial, and statistical optimization should be carried out to increase degradation.
3. **Co-culture strategy:** Microcosm or growing two or more fungi together is the best way to achieve the higher degradation.
4. **Genetic modification strategy:** Modification and expression of gene to another organism and overexpression of gene within the same organism are known as molecular modification strategies to increase the bioremediation process. Figure 7.6 depicts the whole process wherein fungal DNA as well as RNA is isolated and expressed in the other organisms or overexpressed in the same organisms to increase enzyme production which ultimately leads to higher degradation.
5. **Multi-omics strategy:** This work can be done by isolating bacteria, genome, gene, and proteins from environmental samples by performing metagenomics, metatranscriptomics, and metaproteomics analysis. So, the potential gene and/or protein can be used for bioremediation process.

7.5 Conclusion

Fungi have been employed to remediate the hazardous toxicants for four to five decades wherein various white rot basidiomycetes can utilize and metabolite the toxicants which can further convert into non-toxic form. So, the strains can be utilized for the commercial treatments for the remediation of dyes, metals, and other hazardous toxicants. Many studies show that the bacteria and fungi can efficiently remediate the contaminants when they are processed together, i.e., microcosm under in vitro and under in situ conditions. However, more work is focused on the various pesticides, polycyclic aromatic hydrocarbons, and dyes which are present in soil and water which has to be remediated. Still, it is quite surprising that few studies were conducted to address this aspect wherein degradation of multiple hazardous contaminants were focused for remediation using fungi (Gouma et al. 2014). In addition to this, metagenomics, metatranscriptomics, and metaproteomics should be used to isolate and explore new genes, proteins, and enzymes that can be useful for the bioremediation.

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Chapter 8
Bioremediation of Polluted Soil by Using Plant Growth–Promoting Rhizobacteria

Manoj Kumar Chitara, Sadhna Chauhan, and Rajesh Pratap Singh

Contents

M. K. Chitara (✉) · S. Chauhan · R. P. Singh
Department of Plant Pathology, College of Agriculture, GBPUA & T, Pantnagar, India

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Abstract Soil pollution generally causes huge losses in the world's agricultural output, and therefore, soil pollution control is essential in agriculture crop production system. For soil pollution management, we usually reduce the use of chemical fertilizers, manures, and pesticide, reuse the domestic waste product materials such as glass containers, plastic bags, paper, and cloth, and recycle the materials such as some kinds of plastics and glass cane, but their indiscriminate use causes environmental problems and human health hazards. Moreover, the continuous use of those products without safe disposal leads to soil pollution. Thus, bioremediation of soil pollution is an alternate eco-friendly method for soil pollution management, in which plant growth–promoting rhizobacteria are used in alleviating the contaminated soil. Many rhizosphere microorganisms including *Azotobacter* spp., *Pseudomonas aeruginosa*, *Glomus* spp., *Acaulospora* spp., *Scutellospora* spp., *Streptomyces* spp., *Klebsiella* spp., *Lysobacter* spp., *Rhizobium leguminosarum*, *Burkholderia* spp., *Diaphorobacter nitroreducens*, *Planomicrobium chinense*, *Promicromonospora* spp., *Mesorhizobium* spp., *Psychrobacillus psychrodurans*, *Pantoea* spp., *Arthrobacter* spp., and *Variovorax* spp. have been found as plant growth–promoting rhizobacteria. These PGPR have been found to bioremediate the polluted soil by using various types of mechanisms such as through siderophore production, phosphate solubilization, biological nitrogen fixation, production of 1-aminocyclopropane-1-carboxylate deaminase (ACC), quorum sensing, signal interference and phytohormone production, exhibiting antifungal activity, production of volatile organic compounds, and induction of systemic resistance, promoting beneficial plant-–microbe symbioses. Thus, there are immense possibilities for identifying other growth-promoting rhizobacteria that could help in bioremediation of polluted soil as well as promote sustainable agriculture.

Keywords Bioremediation · Soil pollution · Plant growth–promoting rhizobacteria · Siderophore production · Sustainable agriculture

8.1 Introduction

Soil is the most wondrous gift of nature to human society, it is a part of an ecosystem, it is the substance existing on the earth's surface, which grows and develops plant life (Terzaghi and Peck 1996), it performs a wide range of functions (Jury and Roth 1990) and renders a number of environmental services that connect it with the human society or in another word soil is essentially a natural body of mineral and organic constituents produced by solid material recycling, during a myriad of complex processes of solid crust modifications, which are closely related to the hydrological cycle (Mirsal 2008). The soil is contaminated by several pollutants which are also known as soil pollutants, and this phenomenon are called as soil pollution, i.e., the occurrence of the chemical or other substances in the soil at a concentration higher than normal causes adverse effects on non-targeted organism. Soil pollution often cannot be directly evaluated, constructing it a hidden hazard (Rodríguez-Eugenio et al. 2018). The status of the World's Soil Resources Report (SWSR) identified soil pollution as one of the main soil threats affecting global soils and the ecosystems services provided by them. The main anthropogenic or manmade (Brookes 1995) sources of soil pollution are the chemicals used in or produced as byproducts of industrial activities (Vorobeichik et al. 2012), domestic (Nyenje et al. 2013), livestock (Zhang et al. 2012a, b), municipal wastes (Ali et al. 2014), agrochemicals (Wimalawansa and Wimalawansa 2014), and petroleum-derived products (Pinedo et al. 2013). These chemicals are released to the environment accidentally (Kim et al. 2018; Awad et al. 2011), for instance, from oil spills or leaching from landfills, or deliberately, as is the case with the use of fertilizers and pesticides, irrigation with untreated wastewater, or land application of sewage sludge. Soil pollution also results from atmospheric deposition from smelting (Zhang et al. 2012a, b; Gunawardena et al. 2013), transportation (Wiłkomirski et al. 2011), spray drift from pesticide applications, and incomplete combustion of many substances as well as radionuclide deposition from atmospheric weapons testing and nuclear accidents. Recently, new types of pollutants are developed such as pharmaceuticals, endocrine disruptors, hormones and toxins, among others, and biological pollutants, which include bacteria and viruses (Rodríguez-Eugenio et al. 2018) called micropollutants in soil. All these types of soil pollution need to be remediated by the development of a novel and science-based method, which includes a newly emerging method, i.e., bioremediation.

Bioremediation is an ecofriendly and an efficient method, in which live microorganism and its products can be utilized for the alleviation of environment contamination (Ojuederie and Babalola 2017). These processes facilitate to crop reestablishment on treated soil. Microorganisms such as plant growth–promoting rhizobacteria (PGPR) and plants employ various mechanisms for the bioremediation of polluted soils (Chibuike and Obiora 2014), and it has been suggested to play a significant and vital role in alleviating the toxicity in different contaminated soils (Khan et al. 2009; Jayabarath et al. 2009; Cardón et al. 2010; Cetin et al. 2011). Use of PGPR strains with many properties, like metal resistance/reduction ability (Joseph et al. 2007; Kumar et al. 2008; Wani and Khan 2010) and capacity to facilitate plant growth through variable mechanisms in contaminated soils (Khan et al. 2009), is considered enormously important for the attainment of the bioremediation program.

8.2 Soil Pollution

Soil pollution includes disturbance of major ecosystem services provided by soil. It can also adversely affect the yield of plants due to toxic levels of contaminants. It can be defined as a chemical or a substance out of place and/or present at a higher than the normal concentration that has adverse effects on any non-targeted organism (Rodríguez-Eugenio et al. 2018). The main anthropogenic sources of soil pollution are the excessive use of chemicals in agricultural (S. Savci 2012), domestic waste (Nyenje et al. 2013), livestock and municipal wastes (Ali et al. 2014), agrochemicals (Wimalawansa and Wimalawansa 2014), and petroleum-derived products (Pinedo et al. 2013). Soil pollution also results from atmospheric deposition from smelting (Gunawardena et al. 2013) and transportation (Begum et al. 2011). Generally, there are two types of soil pollution, which is natural and manmade soil pollution, which includes former factory sites, inadequate waste and wastewater disposal, uncontrolled landfills, excessive application of agrochemicals, spills of many types, etc. Soil pollution can be divided into six types based on the source of pollutant (Fig. 8.1).

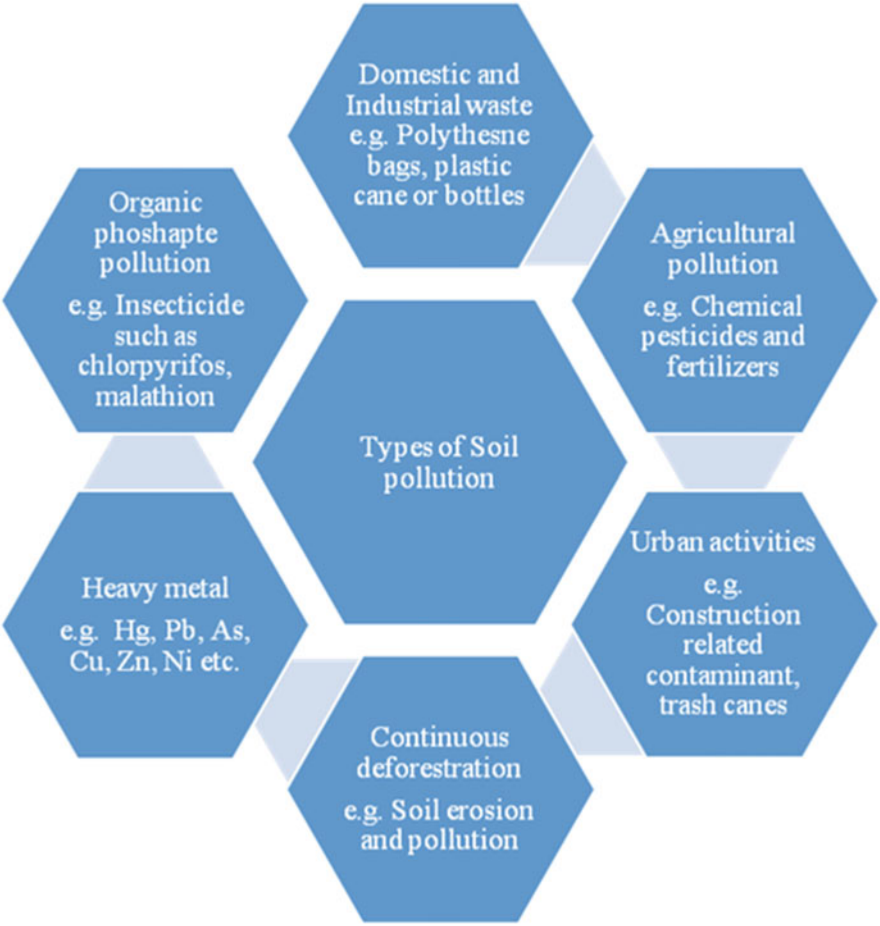

Fig. 8.1 Types of soil pollution based on sources of soil pollution

8.3 Impact of Soil Pollution

Soil pollution adversely affects the plants, animals, and humans health (Lu et al. 2015). Those persons who directly or indirectly inhaled or ingested the soil pollutant may lose the general health or face health problem in the form of diseases such as high lead blood levels in children, arthralgia, osteomalacia, and excessive cadmium in urine (Zhang et al. 2012a, b). However, children are very sensitive to exposure to soil pollutants or contaminants, whenever they come in close contact with the contaminated soil by playing in the ground; then the pollutant may affect those children, and due to this, they suffer from asthma or allergenic-related problems (Heinzerling et al. 2016) as well as adults also affected. Humans living near the polluted soil are facing health-related problems such as migraines, nausea, fatigue, skin disorders, and even miscarriages, and those people who are exposed to soil contamination for a longer period of time are suffering from cancer, leukemia, reproductive disorders, kidney and liver damage, and central nervous system failure (Mishra et al. 2015). Soil pollution is considered a big problem globally with respect to decreasing soil fertility and productivity, so the microbial activity including PGPR helps to cope up with such kind of situation; for example some PGPR have the ability to grow in the polluted soil by utilizing various kinds of pollutants or form the energy through the degradation of the pollutants present in the soil, so the application of such kind of PGPR in a timely manner in the soil helps to alleviate soil pollution by the process of bioremediation (Pilon-Smits 2005).

8.4 Bioremediation

Bioremediation includes the use of living organisms and their products, to remove contaminants from soil (USEPA 2012; Leung 2004) or to transform high toxic into less toxic forms (Memon and Schröder 2009). Certain microorganisms are involved in bioremediation of polluted environment. Maximum bioremediation processes utilize native microbial species including plant growth–promoting rhizobacteria (PGPR) (Khan et al. 2009), fungi (Zaidi et al. 2011), actinomycetes (El-Syed et al. 2011), algae (Huq et al. 2007), or plants (Marchand et al. 2010) which can be helpful in reclamation of the soil at optimum level.

According to Zaidi et al. (2012), bioremediation can be divided into two categories, which is in situ and ex situ bioremediation. In situ bioremediation includes the utilization of microorganism for the treatment of the hazardous chemicals in the soil and surface or subsurface waters while ex situ bioremediation requires diggings of contaminated soil or pumping of groundwater to facilitate microbial degradation; it has some disadvantages. So, in situ bioremediation method is considered more superior than ex situ bioremediation because it does not need digging of the contaminated soil as well as low-cost technology of contaminated soil bioremediation.

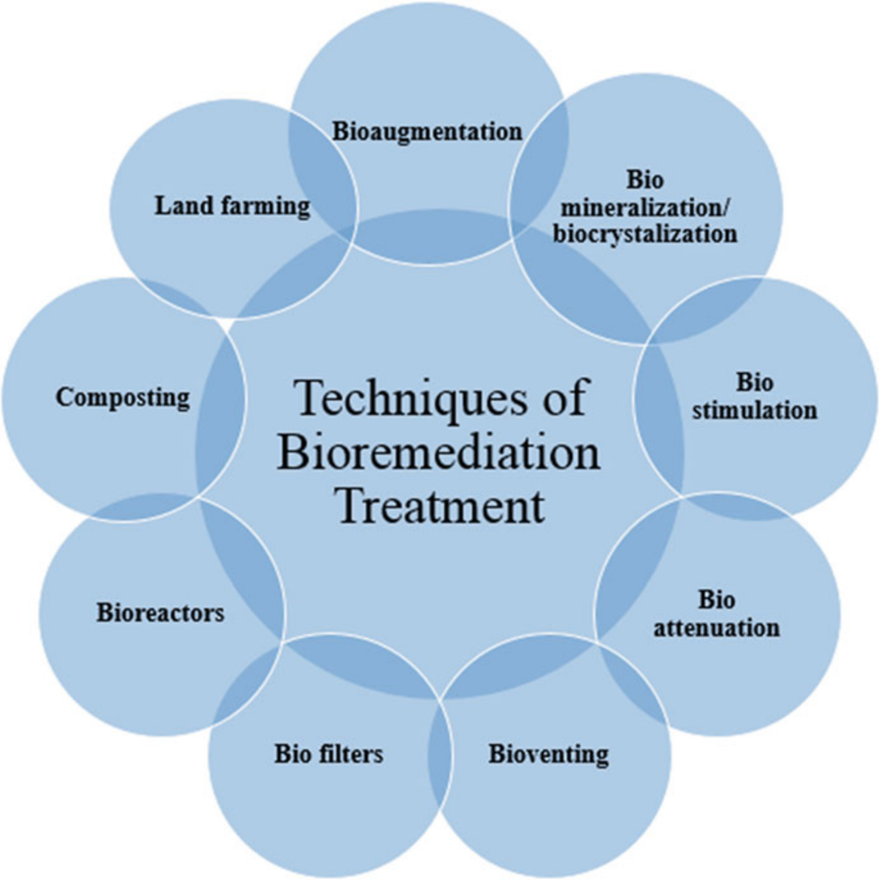

Fig. 8.2 Techniques of bioremediation for treatment of contaminated soil environment

8.5 Techniques of Bioremediation Treatment

Rajendran and Gunasekaran (2019) described eight categories of bioremediation treatment of contaminated soil environment (Fig. 8.2).

8.5.1 Bioaugmentation

Bioaugmentation technique is an in situ process of bioremediation of contaminated soil. In this process, the contaminated soil is treated with the microbial culture, which has immense properties of remediation of the soil through the various biological mechanisms. The microbial activity totally depends on the congenial environmental condition (Zaidi et al. 2012; Vidali 2001).

8.5.2 Biomineralization/Biocrystallization

In this technique, microbes generate the ligands which cause the precipitation of heavy metals as biomass-bound crystalline deposits.

8.5.3 Biostimulation

Biostimulation technique includes the stimulation of the indigenous microbes present in the contaminated soil by employing the necessary nutrients required. Necessary nutrients may supply through the mineral application as well in the form of manure, compost, etc.

8.5.4 Bioattenuation

This technique includes monitoring the process of natural degradation to ensure the decrease of the contaminant with time at the relevant sampling point is done.

8.5.5 Bioventing

It is an in situ bioremediation technique which is a relatively passive technique. In this method oxygen is supllied to the soil in order to stimulate aerobic soil microbial growth and degradation activity. It works for simple hydrocarbons and can be used where the contamination is deep under the surface (Vidali 2001). The monitoring difficulty is there (Zaidi et al. 2012).

8.5.6 Biofilters

Biofilters technique includes the use of microbial stripping columns to treat air emissions. The microbes generally break the toxic substances into a non-toxic compound e.g., carbon dioxide (CO_2), water (H_2O), and salts.

8.5.7 Bioreactors

This process involves the use of a container/reactor for the treatment of the liquid or slurries. The advantage of the bioreactors is rapid degradation kinetics, optimized environmental parameters, enhanced mass transfer, and effective use of inoculants and surfactants. It is a relatively expensive technique that limits its use in bioremediation program, e.g., slurry reactor and aqueous reactor (Zaidi et al. 2012; Vidali 2001).

8.5.8 Composting

It is a type of ex situ and cost-efficient bioremediation program. It is the process of the aerobic and thermophilic treatment in which contaminated soil is mixed with a bulking agent. The development of a rich microbial population and the elevated temperature are a characteristic of composting (Vidali 2001). The extended treatment time is the limitation of the composting (Zaidi et al. 2012).

8.5.9 Land Farming

It is a simple type of ex situ and cost-efficient bioremediation technique in which contaminated soil is excavated and spread over a prepared bed and intermittently plowed until contaminants are degraded (Vidali 2001) or it is a solid-phase treatment system for contaminated soil or maybe in constructed soil treatment cell. The space requirement is the limitation of land farming (Zaidi et al. 2012).

8.6 Plant Growth-Promoting Rhizobacteria

Plant growth-promoting rhizobacteria (PGPR) are a group of bacteria living in the soil in association with plant roots and are known to enhance the plant growth through a variety of direct and indirect mechanisms (Asad 2017) (Fig. 8.3). Direct mechanisms include nitrogen fixation, phosphate solubilization, potassium solubilization, phytostimulation, siderophore production which limits the Fe activity (Bhattacharyya and Jha 2012), heavy mineral uptake by plants (Ma et al. 2011), etc. while indirect mechanisms include antibiotics production, chitinase and glucanase activity, induced systemic resistance against plant diseases which is termed as systemic resistance, exopolysaccharide production, phytoremediation (Nadeem et al. 2014), etc. The PGPR facilitate plant growth under stressful environmental conditions by producing some key enzymes such as ACC-deaminase, chitinase, and rhizobitoxine exopolysaccharides.

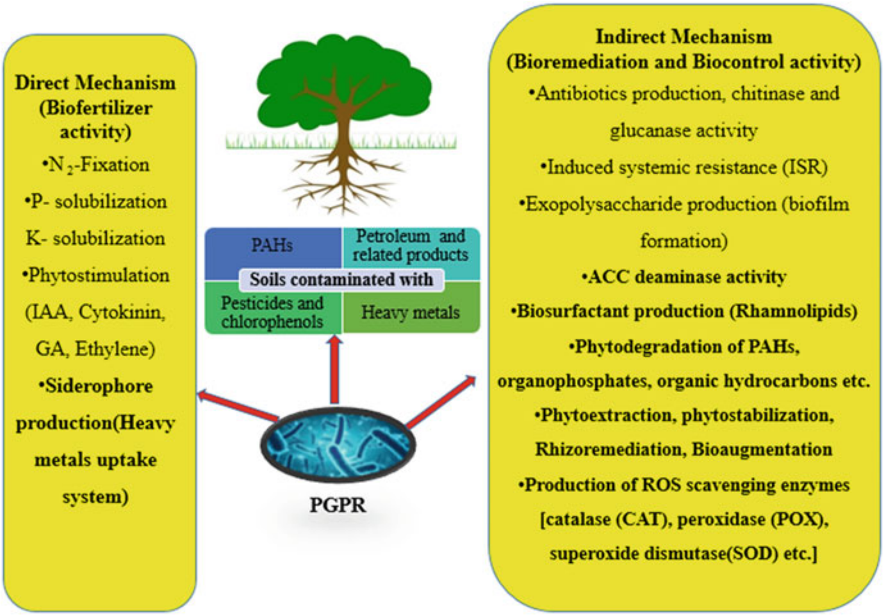

Fig. 8.3 Mechanism of action of plant growth-promoting rhizobacteria (PGPR) in bioremediation of polluted soil

8.7 Role of Plant Growth–Promoting Rhizobacteria (PGPR) in Bioremediation of Polluted Soil

Plant growth–promoting rhizobacteria (PGPR) are the rhizosphere bacteria that can facilitate the plant growth under polluted environment by various mechanisms or they can help in bioremediation of polluted soil (Patel et al. 2016) which can improve the plant growth by siderophore production (Sayyed et al. 2013), phosphate solubilization (Ahemad and Khan 2010), biological nitrogen fixation (Yadegari et al. 2010), production of 1-aminocyclopropane-1-carboxylate deaminase (ACC) (Gontia-Mishra et al. 2017), quorum sensing (Podile et al. 2014) signal interference and phytohormone production (Cassán et al. 2014), exhibiting antifungal activity (Ingle and Deshmukh 2010; Shobha and Kumudini 2012), production of volatile organic compounds (Santoro et al. 2015), induction of systemic resistance (Annapurna et al. 2013), promoting beneficial plant–microbe symbioses (Bhattacharyya and Jha 2012), it could detoxify the contaminated environment sequestration of the metal ions inside the cell (Antony et al. 2011), biotransformation—transformation of toxic metal to less toxic forms (Cheung and Gu 2007; Shukla et al. 2009), adsorption/desorption of metals, etc. (Mamaril et al. 1997;

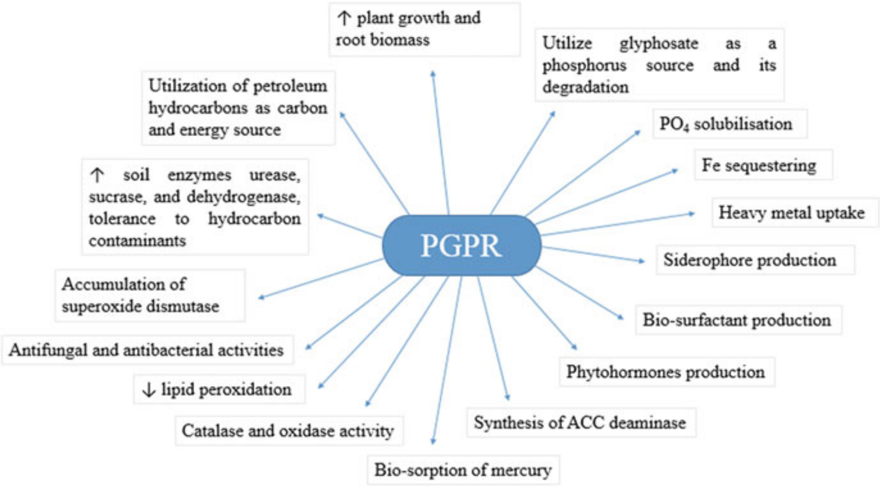

Fig. 8.4 Schematic representation of different mechanism followed by plant growth-promoting rhizobacteria (PGPR) during bioremediation of polluted soil

Johnson et al. 2007) (Fig. 8.4). It is considered extremely important for the success of the bioremediation program. Some examples of plant growth–promoting rhizobacteria and target pollutant with their mechanism to improve plant growth under polluted environment are listed in Table 8.1.

8.8 New Emerging Technologies of Bioremediation

In recent years, there are several new technologies that gained much attention to overcome the negative impact of the contaminants in the soil, leading to improvement in reliability, cost efficiency, and speed of bioremediation (Rayu et al. 2012). The old method of bioremediation which is microbes based is considered slower due to environmental conditions such as soil structure and moisture. New emerging tools based on advanced engineering technology of bioremediation provide much reliability to improve the performance of the bioremediation process. This new technique ranges from mere monitoring and advancement of inherent bioremediation to novel ideas of genetically engineering the functional genes for bioremediation application. Some of the new important tools are as follows:

Table 8.1 Plant growth–promoting rhizobacteria and target pollution with their mechanism to improve plant growth under a polluted environment

PGPR	Target pollutant/pollution	Crops/plants used	Mechanism involved	References
Azotobacter spp.	Cadmium Cd(II), chromium Cr (VI)	*Lepidium sativum*	Phosphorous solubilization and iron sequestering	Sobariu et al. (2017)
Alcaligenes faecalis RZS2 and *Pseudomonas aeruginosa* RZS3	$MnCl_2 \cdot 4H_2O$, $NiCl_2 \cdot 6H_2O$, $ZnCl_2$, $CuCl_2$, $CoCl_2$.	Wheat and peanut	Heavy metal uptake system via microbial siderophore and ions chelation	Patel et al. (2016)
Glomus, Acaulospora, Scutellospora, Streptomyces, *Azotobacter*, *Pseudomonas,* and *Paenibacillus*	Fe^{3+}-contaminated soil	*Pennisetum glaucum*, *Sorghum bicolor*	Filtration barrier against heavy metal transfer, increase iron absorption, siderophore production, and phosphate solubilization	Mishra et al. (2016)
Pseudomonas sp. AJ15	Petroleum oil	*Withania somnifera*	Biosurfactant production, degrade and utilized petroleum as a carbon source	Das and Kumar (2016)
Pseudomonas rhizophila S211	Pesticides	Artichoke	Synthesis of ACC deaminase, putative dioxygenases, auxin, pyroverdin, exopolysaccharidelevan and rhamnolipidbiosurfactant.	Hassen et al. (2018)
Klebsiella sp. D5A, *Pseudomonas* sp. SB, *Lysobacter*, *Pseudoxanthomonas*, *Planctomyces*	Petroleum hydrocarbons	*Testucaarundinacea*	Biosurfactant production, increase root biomass, phytohormones production, and mineral solubilization	Hou et al. (2015)
Pseudomonas sp., *Pseudomonas fluorescence*, and *Bacillus cereus*	Pb, Cd, and Ni remediation	Maize	Catalase and oxidase activity, solubilize bound phosphate, antifungal and antibacterial activities, encountered oxidative stress, enhanced Pb and Ni accumulation in rhizosphere soil and plants	Khan and Bano (2016a, b)

(continued)

Table 8.1 (continued)

PGPR	Target pollutant/pollution	Crops/plants used	Mechanism involved	References
Glomus intraradices, *Acinetobacter* sp.	Petroleum contaminants	Oat	Accumulation of superoxide dismutase, catalase and peroxidase, decreased malondialdehyde (MDA) and free proline contents, increasing soil enzymes urease, sucrase, and dehydrogenase, tolerance to hydrocarbon contaminants	Xun et al. (2015)
Pseudomonas aeruginosa (JX100389), *P. plecoglossicida* (JX149549)	Petrol engine oil	Wheat	Solubilizing and iron sequestering, biosurfactant production, utilization of petroleum hydrocarbons as carbon and energy source	Gangola et al. (2017)
Pseudomonas brassicacearum, *Rhizobium leguminosarum*	Zn	*Brassica juncea*	Increased plant growth, root exudation of Zn chelates, histidine, and cysteine	Adediran et al. (2016)
Sphingomonas, *Pseudomonas*, *Sphingobium*, *Dokdonella*, and *Luteimonas*	Polycyclic aromatic hydrocarbons (PAHs)	–	Fluorene, phenanthrene, pyrene degradation	Bacosa and Inoue (2015)
Burkholderia sp. *XTB-5*	Phenol	*Brassica chinensis*, *Ipomoea aquatic*	Solubilize phosphate and produce 1-aminocyclopropane-1-carboxylate (ACC) deaminase and siderophore	Chen et al. (2017a, b)
Rhizobium radiobacter and *Diaphorobacter nitroreducens*	Organic hydrocarbons	*Armoracia rusticana*	Carbamazepine degradation	Sauvêtre et al. (2018)
Flavobacterium (B7), *Serratia* (B8), *Pasteurella* (B1), and *Azotobacter* (B6)	1,4-Dichlorobenzene (insecticide)	*Jatropha curcas*	Phosphate solubilization, siderophores production, IAA release, and increased seed germination	Pant et al. (2016)

Pseudomonas sp. and *Bacillus* sp.	Glyphosate (herbicide) in	Rice	Utilize glyphosate as a phosphorus source and its degradation	Wijekoon and Yapa (2018)
Planomicrobium chinense, *Bacillus cereus*, and *Pseudomonas fluorescens*	Untreated municipal wastewater (MW)	Maize	Solubilize phosphate, exhibit antibacterial, antifungal activities, decreased Pb, and Ni accumulation in rhizosphere soil and shoot	Khan and Bano (2016a, b)
Rhizobacteria (RB1, RB2, RB3, and RB4)	Organophosphate pesticides (OPP), methyl parathion	Mung bean (*Vigna radiata*)	Promote plant growth, degrade OPP, utilize OPP as carbon and/or nitrogen source	Pratibha and Krishna (2015)
Enterobacter sp. strain EG16	Metal stress (Cd and Fe)	*Hibiscus cannabinus*	Uptake of Fe, alleviated Cd-induced inhibition of bacterial IAA production, and metal immobilization in rhizosphere	Chen et al. (2017a, b)
Enterobacter ludwigii (HG 2) and *Klebsiella pneumoniae* (HG 3)	Mercury	*Triticum aestivum*	Improves plant growth, ACC deaminase activity, IAA production, P, Zn, and K solubilization, reduced proline accumulation, biosorption of mercury	Gontia-Mishra et al. (2016)
Pseudomonas aeruginosa SLC-2, *Serratia marcescens* BC-3, *Bacillus circulans*, *Enterobacter intermedius* and *Staphylococcus carnosus*	Petroleum hydrocarbons	Oat and maize	1-Aminocyclopropane-1-carboxylate (ACC) deaminase activity, indole acetic acid production, siderophore synthesis, and the degradation of petroleum	Liu et al. (2015); Ajuzieogu et al. (2015)
Burkholderia cepacia SE4, *Promicromonospora* sp. SE188 and *Acinetobacter calcoaceticus* SE370	Salts contamination	*Cucumis sativus*	Reduced activities of catalase, peroxidase, polyphenol oxidase, and total polyphenol, lower permeability of the plasma membrane	Kang et al. (2014)

(continued)

Table 8.1 (continued)

PGPR	Target pollutant/pollution	Crops/plants used	Mechanism involved	References
Pseudomonas pseudoalcaligenes and *Bacillus pumilus*	Salts contamination	Rice	Reduce lipid peroxidation and superoxide dismutase activity, reducing ROS toxicity, cell caspase-like protease activity and PCD	Jha and Subramanian (2014)
Mesorhizobium sp. HN3	Chlorpyrifos (CP)	Ryegrass (*Lollium multiflorum*)	CP degradation, root colonization	Jabeen et al. (2016)
Stenotrophomonas (MTS-2), *Citrobacter* (MTS-3), and *Pseudomonas* (MTS-1)	polycyclic aromatic hydrocarbons (PAHs)	–	P-solubilization, acid and alkali tolerance, PAH degradation	Kuppusamy et al. (2016)
Bacillus cereus SPL-4	PAHs like naphthalene, fluorene, phenanthrene, anthracene, dibenz [a,h]anthracene, etc.	The aged wood treatment plant	Lipopeptide biosurfactant production	Bezza and Chirwa (2017)
Acinetobacter sp. PDB4	Pyrene and benzo(a)pyrene (BaP), anthracene (PAHs)	Rice	Solubilized phosphate, siderophore activity	Kotoky et al. (2017)
Bacillus pumilus	Radiocesium (^{137}Cs)	*Brassica* sp.	Increased root surface area and volume resulting in higher ^{137}Cs uptake by plants	Aung et al. (2015)
Pseudomonas fluorescens ATCC 17400	Radionuclide cesium	Red clover	Increased the translocation factor, resorption of Cs onto biofilms	Hazotte et al. (2018)
Microbial consortia (*Acinetobacter calcoaceticus*, *Streptomyces avidinii UrGr6St2*, *Enterobacter ludwigii UrCAN1-3*, *Citrobacter freundii UrCAN5* and *Psychrobacillus psychrodurans UrPLO1*, *Lysinibacillus fusiformis* etc.)	U, Sr	*Agrostis capillaris, Deschampsia flexuosa, Festuca rubra, Helianthus annuus*	Phytoextraction, plant growth promotion, phytostabilization	Langella et al. (2014)

Bacillus sp., *Pantoea* sp., *Pseudomonas* sp., *Staphylococcus* sp., *Paenibacillus* sp., *Advenella*, *Arthrobacter*, and *Variovorax*	Selenium (Se)	*Stanleya pinnata*, *Astragalus bisulcatus*	Reduce selenite and nitrite, produce siderophores, plant growth promotion	Sura-de Jong et al. (2015)
Microbacterium sp. EIKU5, *Shinella* sp. EIKU6, and *Micrococcus* sp. EIKU8	Arsenic (As) and uranium (U)	–	Resistance and oxidation, U removal	Bhakat et al. (2019)
Pantoea sp. BRM17	Phosphogypsum (PG) (a by-product of the phosphate fertilizer industry)	Canola (*Brassica napus*)	Siderophores, IAA, exopolysaccharides (EPS), ammonia (NH_3), and ACC deaminase activity	Trifi et al. (2020)
Bacillus subtilis	Plasticizer Di-butyl phthalate (DBP)	*Ageratum conyzoides*, *Youngia japonica*	Degradation into mono-butyl phthalate and phthalic acid, use as C source	Huang et al. (2018)

8.8.1 Metagenomics

Metagenomics include phylogenetic analysis of soil microbial flora (Daniel 2005) for creating soil-based metagenomics library. It promises a continuous source of pollutant-degrading genes for increased efficiency and utility of transgenic (microbes and plants) technologies for direct use in bioremediation program (Daniel 2005). This technology also facilitates the mass production of the degrading enzymes from uncultivable bacteria for improvement of enzymatic remediation technology. By this technique, we can produce a marketable product based on bioremediation gene/enzyme product from uncultivable microbes (Rayu et al. 2012). For example, thermostable pyrethroid hydrolyzing enzyme could be used in the detoxification of pyrethroids (Fan et al. 2012), a novel gene responsible for the degradation of 3,5,6-trichloro-2-pyridinol; a persistent and toxic metabolite of the insecticide chlorpyrifos was isolated (Math et al. 2010) from cow rumen and gene products for remediation including biphenyl-degrading genes (Sul et al. 2009).

8.8.2 Metabolic Engineering

Metabolic engineering includes the improvement of cellular activities by manipulations of enzymatic, transport, and regulatory functions of the cell with the use of recombinant DNA technology (Nielsen 2001). By this technique, we can combine analysis of the metabolic pathway and other pathways that can help to improve cellular properties by designing and implementing rational genetic modifications (Koffas et al. 1999). This type of metabolic pathway analysis is rapidly becoming one of the significant features of bioremediation, e.g., *Pseudomonas putida* degrades chloro- as well as methylo-aromatics; the combination of tod and tol pathways in *P. putida* can increase biodegradation rate of benzene, toluene, and p-xylene (Rayu et al. 2012).

8.8.3 Protein/Enzyme Engineering

Improving the stability, substrate specificity, and kinetic properties of proteins/enzymes can be engineered (Dombkowski et al. 2014). It can be done to fine-tune enzymes for desired substrate specificities and stereo-selectivity. This method helps to modify the active site volume and topology of cytochrome P450cam enhanced the catalytic activity of the enzyme (Kumar 2010; Holloway et al. 1998). Another modification is the incorporation of multiple binding sites within a single peptide, for binding of the co-factors and other small molecules, can enhance the catalytic power of the enzyme; this is found to bioremediate the metal wastes (Pazirandeh et al. 1998).

8.9 Factor Affecting the Bioremediation

The bioremediation of the polluted environment is a complex process which is influenced by certain factors such as microbial factors including growth until critical biomass is reached, mutation and horizontal gene transfer, enzyme induction, enrichment of the capable microbial populations, and production of toxic metabolites; environmental factors include depletion of preferential substrates, lack of nutrients, inhibitory environmental conditions viz soil, temperature (Chitara et al. 2017), pH, O_2 and nutrients; substrate factor includes too low concentration of contaminants, chemical structure of contaminants, toxicity of contaminants, and solubility of contaminants; biological aerobic vs anaerobic process factor includes oxidation/reduction potential, availability of e-accepters, and microbial population present in the site; growth substrate vs co-metabolism factor includes type of contaminants, concentration, alternate carbon source present, and microbial interaction (competition, succession, and predation); physico-chemical bioavailability of pollutants include equilibrium sorption, irreversible sorption, and incorporation into humic matters, and some of the mass transfer limitations are O_2 diffusion and solubility, diffusion of nutrients, and solubility/miscibility in/with water (Boopathy 2000). The microorganisms are cosmopolitan in nature which can be isolated from everywhere such as at subzero temperatures, extreme heat, desert conditions, in water, with an excess of oxygen, and in anaerobic conditions, with the presence of hazardous compounds or on any waste stream (Boopathy 2000). The microbes utilize the energy source and carbon source and other biological systems. These microbes can be used to remediate environmental hazards. Joshi (2018) divided the microbes into two groups viz. aerobic and anaerobic groups as follows:

8.9.1 Aerobic

This group includes those microbes which exist in the presence of oxygen (Rayu et al. 2012), e.g., *Pseudomonas*, *Alcaligenes*, *Sphingomonas*, *Rhodococcus*, and *Mycobacterium*. These bacteria are helpful in bioremediation of polluted soil and are reported to degrade pesticide and hydrocarbon both as well as alkenes compounds.

8.9.2 Anaerobic

This group includes those microbes which exist in the absence of oxygen (Rayu et al. 2012); for example ligninolytic fungi such as the white-rot fungus *Phanaerochaete chrysosporium* have the ability to degrade an extremely diverse range of persistent or toxic environmental pollutants, such as *Acromobacter*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Acinetobacter*, *Corneybacterium*, *Flavobacterium*, *Micrococcus*, *Mycobacterium*, *Nocardia*, *Pseudomonas*, *Vibrio*, *Rhodococcus*, and *Sphingomonas* species (Gupta et al. 2001; Kim et al. 2007; Jayashree et al. 2012); these bacteria are helpful to use in the bioremediation of polychlorinated biphenyls (PCBs) in river sediments, dechlorination of the solvent trichloroethylene (TCE) and chloroform.

8.10 Advantages of Bioremediation

According to Vidali (2001), the advantages of bioremediation of the polluted soil are as follows:

- It is a natural process so it is perceived by the public as an acceptable waste treatment process for contaminated material such as soil.
- It conserves the natural properties of soil.
- It utilizes energy from sunlight for performing its activity.
- It helps in increasing microbial biomass in the rhizosphere.
- It is useful for the complete destruction of a wide variety of contaminants.
- The end products of treatment are usually harmless which are usually CO_2, H_2O, and cell biomass.
- It is a low-cost application or less expensive than other technologies.

8.11 Limitations

Plant growth–promoting rhizobacteria play a significant role in bioremediation of polluted soil program. The success of these programs solely depends upon the activity of PGPR and those need optimum environmental conditions for their growth and colonization. But recently, the climate change influences the environment; due to this, the PGPR performance disturbs or gets changed (Compant et al. 2010). Therefore, climate change may also affect the microbial population present in the soil surface, subsurface, and plant-associated communities (Drigo et al. 2009). Climate change affects all the metabolic process, i.e., crop or plant physiology and metabolism are affected; for example, in plants the production of amino acid (tryptophan) decrease, which also results in the decrease in the production of IAA, which disturbs the vegetative growth and root proliferation of the plant (Kravchenko et al. 2004). The high temperature may also hamper the growth of plant and physiology together, they are likely to lead to changes in the configuration, abundance, or activity of plant-associated microbial communities. Consequently, population of microorganisms known for their valuable effects on plant health or growth might also be reduced, in terms of exhibiting their desirable properties and colonization capacity under certain environmental conditions (Compant et al. 2010).

8.12 Future Prospects

For the past few decades, the researchers are giving more attention to the management of soil pollution caused by various chemicals or other substances only. The bioremediation of polluted soil serves as one of the best ways to manage the polluted soil. This approach utilizes the plant growth–promoting rhizobacteria (PGPR) whose activity is influenced by climate change. So, the success rate of PGPR is highly associated with climate, so it is important to understand the plant growth patterns along with its surrounding environment before the application of PGPR especially for a particular given set of conditions. Therefore, it is needed to identify a specific PGPR strain for a particular region for ensuring their better performance and effectively facilitate the bioremediation of polluted soil under changing climate conditions.

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Chapter 9
Utilization of Microbial Biofilm for the Biotransformation and Bioremediation of Heavily Polluted Environment

Charles Oluwaseun Adetunji and Osikemekha Anthony Anani

Contents

Abstract It has been observed that beneficial microorganisms play a crucial role in the biodegradation of waste pollutants and natural organic compounds through numerous catabolic pathways which enable these strains to persist in numerous environments. The application of biofilm has been identified as a sustainable biotechnological approaches that could be applied for effective management of heavily contaminated environment. Biofilms are defined as the self-produced extra polymeric matrices that comprise sessile microbial community where the cells are characterized by their attachment to either biotic or abiotic surfaces. These extra cellular slime natured covers enclose the microbial cells and protect from various external factors. The components of biofilms are very vital as they contribute towards the structural and functional aspects of the biofilms. Microbial biofilms

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C. O. Adetunji (✉)
Applied Microbiology, Biotechnology and Nanotechnology Laboratory, Department of Microbiology, Edo University Iyamho, Iyamho, Edo State, Nigeria

O. A. Anani (✉)
Laboratory of Ecotoxicology and Forensic Biology, Department of Biological Science, Animal and Environmental Unit, Faculty of Science, Edo University Iyamho, Iyamho, Edo State, Nigeria
e-mail: adetunji.charles@edouniversity.edu.ng

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comprise major classes of macromolecules like nucleic acids, polysaccharides, proteins, enzymes, lipids, humic substances as well as ions. Therefore, this chapter intends to provide an overview on the application of biofilm for the biotransformation and bioremediation of heavily polluted environments. The modes of action of these biofilms derived from these beneficial microorganism were also highlighted in detail.

Keywords Biofilm · Environment · Contaminants · Bacteria · Macromolecules · Biotransformation and bioremediation

9.1 Introduction

Bioremediation has been identified as an eco-friendly approach through which toxic substances are rendered harmless substances majorly from the air, water, and soil through the application of microorganisms (Alexander and Loehr 1992; Prasad and Prasad 2012). This has been highlighted as a sustainable environmental approaches because it prevents all the health and environmental hazards associated with the synthetic treatment or some other conventional methodology used for the remediation of polluted environments (Vidali 2001). The typical examples of bioremediation has been highlighted for the treatments of the following such as explosives, xenobiotic compounds, petroleum products, heavy metals, aromatic hydrocarbons, jet fuels, pesticides, crude oil, herbicides and radionuclides (Gaur et al. 2014).

The application of beneficial microorganisms most especially bacterial and the application of modern techniques for their improvement for the generation of genetically modified strains have enabled their wider application for the bioremediation of heavily polluted environments. Moreover, some other microorganisms such as fungal, actinomycetes and yeast are utilized for the removal of pollutant from the environment (Mishra and Malik 2014; Cerniglia 1997; Balaji et al. 2014). It has been observed that beneficial microorganisms play a crucial role in the biodegradation of waste pollutants and natural organic compounds through numerous catabolic pathways which enable these strains to persist in numerous environments (Bouwer and Zehnder 1993; Bruins et al. 2000). Some of these beneficial microorganisms are extremophiles in nature, and they could withstand heavy metal polluted, acidic contaminated soil or environment or radioactive environment. It has been observed that there are several factors or conditions that enhance the process of biodegradation (Prince 2000). The usage of beneficial microorganisms has been highlighted in the maintenance environment (Adetunji et al. 2017, 2018, 2019a, b, 2020; Adetunji and Adejumo 2017, 2018, 2019).

Most of these biodegradation processes involve the application of enzymes for their bioremediation of heavily polluted compounds into non-toxic substances such as water and carbon dioxide (Das and Dash 2014). The stages involved in the metabolic pathways necessitate the movement of electron from electron donors to electron acceptors. It has been observed that the electron donor serves as substrates and food for these microorganisms that could biodegrade but are normally restricted in a non-polluted site. However, it has been stated that in polluted environment the liberation of an organic electron donor may enhance microorganisms to strive for any available acceptors to restore the balance of the system.

There are two types of bioremediation which depend on the location of the contaminant treatment. If the methods to adopt involves in situ bioremediation, the pollutant samples are treated in the original place of pollution but in ex situ remediation, management of pollutants takes place typically off-site (Vogt and Richnow 2014; Jorgensen 2007). It has been observed that in situ bioremediation has several advantages which includes reduction on the cost of transportation and disruption of sites. Moreover, it has been observed that optimization of chemical and physical conditions might hasten the process of biodegradation by bacteria most especially when supplemented by nutrients. Another effective way of biodegradation is to apply genetically engineered microorganisms which can modulate the pathways for enhanced biodegradation of heavily polluted environment (Singh et al. 2011; Hedlund and Staley 2001; Nakajima-Kambe et al. 2009).

Also, indigenous bacterial communities have been recognized to possess the potential to metabolize any available heavy metals, and persistent organic pollutants into a lesser toxic constitutes. The presence of limited nutrient and lack of adequate access to these contaminants prevents these process involved in the reduction of these pollutant available in the environment (de Lipthay et al. 2003; Petrie et al. 2003). It has been observed that biofilm and free-living planktonic bacteria could metabolise toxic and pollutants in the environment. Some factors such as reduction in protection, low bioavailability of the pollutants and reduced metabolic activity might result in improper transformation mainly by planktonic bacteria (von Canstein et al. 2002).

It has been observed that some bacterial community possess the capability to biodegrade, neutralize and play active role in the mineralization of numerous xenobiotic compounds in wastewater-activated sludge (Byrns 2001; Bertin et al. 2007). The application of biofilm has been identified as a sustainable and effective means of detoxification of pollutant in the environment, and they also play a crucial role in the protection of microbial diversity as well as enhance the increase in their population (Boon et al. 2003; Accinelli et al. 2012).

The genus Dehalococcoides have been recognized for their potential to produce biofilm with high application in the biofilm reactor community for the bioremediation of dechlorination of trichloroethene (Chung et al. 2007). Guezennec et al. (2012) reported that the inactive oxidation of arsenic and iron by biofilms was effective at gold-quartz mining sites while Williams et al. (2013) wrote that biofilm in the tube could reduce the level of selenium concentration in the tubes having nutrients. Also, Pool et al. (2013) also highlighted the significance of biofilm enzymes and their application in the coal mine drainage regions when applied as biomarkers for stream water quality.

Therefore, this chapter intends to provide an overview on the application of biofilm for the biotransformation and bioremediation of heavily polluted environments. The modes of action of these biofilms derived from these beneficial microorganism were also highlighted in detail. Moreover, further suggestion and recommendations that could facilitate the application of biofilm derived from beneficial microorganisms are also discussed in detail.

9.2 Application of Microbial Biofilm for Biotransformation of Contaminants

Edwards and Kjellerup (2013) in a review looked at the utilization of biofilms in the biotransformation and bioremediation of some environmental and health priority contaminants, pesticides, heavy metals, special body care products, pharmaceuticals and pesticides. The authors stated that the longest ever industrial pollution, which affect every facet of the environment for several decades, has an unlimited toll in the lives of living organisms therein. These several mitigation approaches have been put into place. However, the use of a more efficient, sustainable approach-microbes (bio-films), is needed for a cleaner environment. These beneficial microbes are well known for the shear stress, chemical detoxification and eco-protection. The authors suggested that biofilms can be used as a bioeco-marker for studying of polluted rivers, streams, lake drainage systems, etc. to ensure water quality and the protection of aquatic biota.

Saba et al. (2018) tested and evaluated the biosorption and biotransformation potentials of Exiguobacterium on As (arsenic). The authors used the biofilm and planktonic methods of growth in the analysis of the As transformation and the HPLC-ICP-MS for the biosorption. The results of the biological controlled experiment revealed that the bacteria in the planktonic media were able reduce about 3.73 m/mol of AS^{5+} into AS^{3+} from a synthetic wastewater effluent after 48 h incubation period. While the results of the biosorption showed that the biomass of the biofilms and planktonic media were 29.4 mg/g and 25.2 mg/g, respectively. The arsenic biosorption process showed that the stress level after 3 days was significantly impacted and as against the control at $P < 0.05$. The authors in conclusion stated that native arsenic resistance microbe *E. profundum* PT2 was established for biosorption and biotransform arsenic in both the biofilms and planktonic media. That it should be considered as a good candidacy for the eco-restoration of pollutants in the ecosystem because it is green and cost-effective for the purpose it is designed for.

Agrawal and Kumar (2015) did a review of the bacterial alteration of xenobiotic composites as a clean-up process in an ecosystem. The authors opined that xenobiotic composites are tough recalcitrant materials set off from various environmental outputs (natural and man-made), found in the ecosystem, which have resulted to global worry because of the attendant health risks (tetragenotoxicity, mutagenotoxicity, and carcinogenotoxicity) they pose. Microbial biofilms have been shown to degrade toxins in the ecosystem. The utilization of microbial biofilm metabolites will exhibit the potential in degrading xenobiotics using different pathways. The mode of interaction is based on specific enzymes found in the microbial biofilm genes that aid in the biotransformation of the xenobiotic components The authors in conclusion recommend bacterial biofilms as a potential biotransformation agent of xenobiotic compounds.

Mitra et al. (2013) evaluated and tested the biotransformation of fluoranthene by an intertidal derived biofilm bacteria (*Cunninghamella elegans*). The results of the biological controlled experiment showed that the transformation of fluoranthene by the microbe was more by 22-fold, the growth of the biofilm was more by threefold, and the genetic expression of the cytochrome-P450 was more by 2.1-fold when grown in 2% PMMA-CCF biofilm media as compared to the planktonic media. The entire biological transformation was improved with 10% of sevenfold inoculum. The total converted metabolites, biofilm and cytochrome-P450 genetic materials were 3.5-fold, 3-fold and 1.9-fold, respectively.

In general, the biofilm production was relatively more which allowed the utilisation of fluoranthene based on the exopolysaccharides formed in the bacterial genome which also lead to improved efficiency.

Murphy and Casey (2013) did a review of the biotransformation of organofluorine by catalyst microbial biofilms. The authors recounted the role of microbial biofilms in terms of its stability and resistance to xenobiotic substances. On the basis of this, they are metabolically vigorous for a longer time. These characteristics make biofilms very difficult in treating under clinical conditions and, however, utilize for the catalytic bioremediation of toxicants.

Yang et al. (2011) evaluated and tested the biological transformation of arsenic (As) and selenium (Se) by aggregation strains of biofilms. They stated that AS and Se are elements of environmental concern when release into the ecosystem, because of the potential ecological and health risks importance. Communities of microorganisms or biofilms can use as to transform these toxic metals to less noxious forms such as arsenite and selenite. The results of their study indicated a biotransformation of As to arsenite and selenium to selenite at the K region of XAS (X-ray absorption spectroscopy). An MXF (micro X-ray fluorescence) united with a confocal laser scanning microscopy (CLSM) showed a highly restricted region of condensed Se strain microbial biofilm. The findings from their study showed that the microbial biofilm was able to sequester as well as detoxify As and Se. In conclusion, the impact and fate of As and Se in an aquatic environment can be determined by the role of microbial biofilms.

9.3 Application of Microbial Biofilm for Bioremediation of Heavily Polluted Environment

Mohapatra et al. (2019) did a review of the probable utilization of microbial bacterial biofilm for the degradation of noxious dye and heavy metal–polluted environment. The authors recounted the environmental degradation associated with the release of noxious dyes and heavy metals in the environment was biota live. The emergence of biofilms, which is green and cost-effective intermediated bioremediation technique can be utilized in the remediation of dye and heavy metals in any media. This green cellular sticky matrix has high forbearance property against antibiotics, organic pollutants and strong chemicals apart from dye and heavy metals. They also have higher resistance ability against certain environmental factors such as nutrient level, water current, temperature, salinity, and varied pH levels. They do this by the possession of parallel inheritable factor and chemo-taxis actions that enable them to accomplish their basic metabolic wants. This is very important for bioremediation purpose and utilization.

Ayangbenro and Babalola (2018) did a review of the schemes used in the bioremediation of metals and metalloids using different microbial polymer methods such as biofilms. The authors stated that the conventional means of remediation of pollutants lead to the generation of a lot of waste which might propound series of health and ecological issues. The use of bioremediation techniques such as biofilms has been chosen as a perfect choice in the mitigation of environmental concern pollutants. The reason is because of its eco-friendly nature and low economic cost attached to its usage. More so, they do not generate extra wastes during the decontamination process. Instead, any waste generated are re-utilized into the degradation chain to generate more energy for the entire bio-process. The metabolite generated by the extracellular microbes aid in the decontamination of the metals and metalloids and lessen the noxious level in any media they are introduced. These metabolites or biopolymers have been known to possess different chemicals that show selective potentials to metals and metalloids decontaminations.

Maksimova (2014) did a review of the biotechnological approaches of bacterial biofilms. The authors pointed out that bacterial biofilms can be utilized as a bio-catalysis in the treatment of waste waters as well as the remediation of contaminants in benthic regions of aquatic bodies. They are able to do this because they are self-regenerating and self-immobilizing and possess a level of tolerance to noxious chemicals based on the enzymatic activities on the substrate. In conclusion, the authors resounded that it is important that bacterial biofilms form numerous spores so that they can bio-transform usable products after biodegradation. They suggested that bacterial biofilms can serve as a promising tool not only for the bioremediation of sewages but also in food productions, pharmaceutical production, and bioenergetics.

Meliani and Bensoltane (2016) tested and evaluated the bioremediation potentials of biofilm *Pseudomonas* strain on heavy metal. The authors recounted that biofilm microbes have been known to be important in managing environmental stress, especially heavy metal pollution. That *Pseudomonas* spp. is well utilized as a bioremediation tool for heavy metal clean-up. The results of the biological controlled experiment as compared to the control, showed a greasy thick film layer structure which indicated the degradation of lead and zinc by the mass of *Pseudomonas* biofilms strains. The results from the antbiogram indicated that the biofilms of *Pseudomonas* were resistant to antibiotics and were significant at $P < 0.05$; $r = 0.73$ and more correlated with each other like the metal resistant, which were not significant at $P > 0.05$; 0.31. The findings from their study revealed that biofilms have the latency to undergo environmental stress and as well able to retain a positive ecological niche even with an upsurge of the heavy metal contents in the biological media. Astonishingly, in the growth phase of the biofilm, the stationary phase was more resilient to the heavy metal impact than the log phase. The authors also noticed that there was no real evidence that connects heavy metal resilient in the biofilms based on the data analysis carried on it.

Meliani and Bensoltane (2014) tested and evaluated the potential of augmentation of *Pseudomonas* biofilms and biosorption strains (*P. aeruginosa*, *P. putida* and *P. fluorescens*) on hydrocarbon degradation. The authors recounted the importance of biofilm and biosorption degrading *Pseudomonas* with the combination of planktonic microbes as special alternative tool for the biodegradation of hydrocarbons blends (cyclohexane, benzene, xylene, and gasoline) as well as their resistance to environmental stress. The results of the evaluation of the production of siderosphore biofilm development showed that all the strains were able to manufacture biosurfactant mixtures that enable them to tolerate the aromatic compounds (xylene and benzene) treated with it. Their results in the degradation of gasoline indicated that *P. aeruginosa* was able to show high resilient to gasoline unlike cyclohexane and benzene. While *P. fluorescens* was able to degrade benzene and xylene unlike *P. putida* that was unable to germinate under the presence of benzene. In all the assessment of biodegradation of hydrocarbon blends by strains of *Pseudomonas*, there was no significant difference as well as positive correlation between the strains and the environmental stressors at $P > 0.01$; $r = -0.94$. However, an undeviating negative correlation was observed between the cell hydrophobicity and the E24 at $r = -86$ and $r = -93$, respectively. The authors in conclusion underscore the importance of the utilization of *Pseudomonas* biofilm strains in the biodegradation of environmental concerned pollutants.

Mangwani et al. (2016) did a review on the conformity in bioremediation using microbial biofilms. The authors stated that eco-restoration is a prerogative of the management of polluted environment. The conventional methods are too expensive in combating pollution, apart from that, they do not do a total cleanup of the contaminant. Residues are still left in the source regions of contamination. The utilization of microbial biofilms is in the increase—a bioremediation tool for the probable cleaning of toxins in the environment. Biofilms microbes provide an environmentally sustainable green ecological niche (microenvironment) for an effective bioremediation process. This is because these native microbes are highly resistant to ecological stress and cost-effective. Conglomeration of biofilms in an ecosystem offers a platform for many water-hating noxious compounds. However, they are controlled by QS (quorum sensing)-α-hydroxyketones, diffusion signalling factors, autoinducer-2, peptides, and acyl homoserine lactones main cell message process, which aid in the signalling of metabolite molecules. The alteration of the genetic materials of the QS can aid in controlling certain characters (chemotaxis, motility, catabolic gene expression, horizontal gene transfer, exopolysaccharide manufacturing, and biosurfactant synthesis) that are important in the utilization of the biofilms in environmental management of pollution. The authors in summary stated that QS can be utilized via the fabrication of the QS signals can be used for the fabrication of assembled biofilms which will improve kinetic degradation of environmental concerned pollutants.

Singh et al. (2006) did a review of the environmental implication of biofilms in the bioremediation of pollutants. The authors recounted that biofilms are known for the treatment of obstinate chemicals because of their aptitude to restrain toxic compound and their high matrix microbial dry mass. This entire process is facilitated by the microbial biofilm genome in the aggregated strains. This also spurs the microbes to be resistant and have high chemotaxis potential towards increase in concentration of the pollutants. In summary, the authors recommend several approaches to be employed in boosting the efficiency of strains of microbial biofilms. An enhanced microbial strain will optimize the population growth and vigour varieties of the community of microbes towards severe environmental stressors.

Turki et al. (2017) tested and evaluated the efficiency of biofilms towards the remediation and purification of contaminants in wastewater as well as the characterization of the microbial community therein. The authors discovered the following strains of microbes: *Pantoea agglomerans*, *Cronobacter sakazakii*, and *Enterobacter agglomerans* in the wastewater samples. A further analysis on the sample revealed that the community of *Salmonella* was not impacted by the RB system. Again, the use of C254-UV is inactivated, which revealed that the community of the bacterial had different resilient results in a secondary treated wastewater chamber. There was no identification of *Salmonella* sp. at 1440 milliwatts per square centimeter (mW/cm^2) dose. The result obtained showed that there was no presence of Salmonella in the sample. The authors recommend the utilization of *Pantoea agglomerans*, *Cronobacter sakazakii*, and *Enterobacter agglomerans* as indicators and microbial biofilms for biodegradation of wastewater pollutants.

Farber et al. (2019) tested and evaluated the bioremediation and bioaugmentation of synthetic diesel polluted soil using aggregations of microbial biofilm combined with wood wastes. The authors recounted that bioaugmentation is an alternative to bioremediation, which assist in boosting the community of microorganisms that have the potential in degrading soil pollutants such as diesel. The aggregation of the soil degrading microbes were cultivated on a wood waste that was pre-treated with plasma that was designed to increase the microbial-diesel degrade levels. The results of the study showed that the biofilm capacity of the wood-plasma got to a level of 0.53 ± 0.02 OD 540 nm on day 7 when compared to the non-treated wood waste (0.34 ± 0.02). A degradation rate of 9.3 mg and 7.8 mg at day 1 respectively were noticed in the plasma untreated and treated biofilms in the synthetic polluted diesel at

0.15% g/g. Though, a degradation rate of 5.7 mg/day was observed in the soil treated with planktonic microbes. The soil samples were subjected to a temperature of 50 °C and varied pH levels to ascertain if they will influence the rate of biofilm degradation, the results showed no significant impact. The findings from this study indicated that the major resistant strains of microbial biofilms families were *Sphingomonadaceae* and the *Xanthomonadaceae*. The authors in conclusion stated that being the first study, the utilization of pretreated plasma wood wastes are the best candidate for the bioremediation of contaminated soil especially diesel polluted soil.

Piacenza et al. (2017) did a review of the bioremediation of tellurium (Te) and selenium (Se), chalcogen metals using consortium microbial biofilms. They recounted that the chalcogens are cosmopolitan natural toxic earth metals which can be made available in the ecosystem via human activities. The upsurge of these chalcogens in the environment may contaminate sediments, soils and water, thereby hindering the life therein. However, those organisms that will survive will bioaccumulate it and transfer it along the food chain. In other cases, they might bioconvert or biomethylate these residue toxicants in them, which is a strategy for sustainability, bringing about an eco-friendly and safer ecosystem. Of recent, many technological breakthroughs have been made with the utilization of chalcogen-oxyanions combined to give rise to valuable nanomaterials that are currently useful in the fields of bio-engineering, optoelectronics and biomedicine.

It has been highlighted that microplastic (MP; <5 mm) is responsible for the high contamination of aquatic environment. Their presence in the aquatic environment has been highlighted as a sources of adverse influence against some biota. Research on microlitre influences is frequently built on spherical and virgin polymer particles as model MP. It has been discovered that benthic and pelagic environment surfaces are usually dominated by microorganisms that developed into biofilms. The role of such biofilm on the microplastic and their fate in the environment. In view of the aforementioned, Rummel et al. (2017) wrote a comprehensive review on the physical relationship of early establishment on plastic surfaces and their reciprocal effect on the weathering processes as vertical movement as well as sorption and their eventual liberation by microplastic. Moreover, probable ecological influence of biofilm development on microplastic such as potential detrimental influence of microplastic, trophic transfer of microplastic are practically unknown. It has been documented that there is an interesting fact that biofilm–plastic relationship has the potential to stimulate the impact and fate of microplastic through alternation of the physical features of the particles. Therefore, it has become a necessity to have a better knowledge on the relationship and enhance the ecological importance of current laboratory evaluation by triggering field conditions in which microbial life constitutes a major driver involved in the driving of biogeochemical processes.

Biofilms are defined as the self-produced extra-polymeric matrices that comprise sessile microbial community where the cells are characterized by their attachment to either biotic or abiotic surfaces. These extracellular slime-natured cover encloses the microbial cells and protects from various external factors. The components of biofilms are very vital as they contribute towards the structural and functional aspects of the biofilms. Microbial biofilms comprise major classes of macromolecules like nucleic acids, polysaccharides, proteins, enzymes, lipids, humic substances as well as ions. The presence of these components indeed makes them resilient and enables them to survive hostile conditions. Different kinds of forces like the hydrogen bonds and electrostatic force of attraction are responsible for holding the microbial cells together in a biofilm, and the interstitial voids and the water channels play a significant role in the circulation of nutrients to every cell in the biofilm. The current review adds a note on bacterial biofilms and attempts to provide an insight on the aspects ranging from their harmful effects on the human community to their useful application. The review also discusses the possible therapeutic strategies to overcome the detrimental effects of biofilms.

Edwards and Kjellerup (2013) wrote a comprehensive review in the application of biofilm together with different nutrient cycling of the microbiome for the ecorestoration of polluted environment. The authors laid special emphasis on some specific pollutant such as toxic minerals, heavy metals, hydrocarbons, personal care products and pharmaceuticals. Moreover, it was highlighted that most industrial process led to the discharge of numerous pollutants which led to the pollution of sediment and other surrounding aquatic environment. There are several efforts that have been put in place for the bioremediation of heavily polluted environment. It has been stated emphatically that the application of local bacterial community possesses the potential to be utilized for the bioremediation of tenacious organic contaminants and oxidizing heavy metal pollutants. One of the major challenges that has been discovered in the bioremediation of the aquatic environment is that most of these pollutants are not easily accessible for easy clean up due to nutrient restrictions in the environment. Therefore, the application of biofilm communities has been highlighted as a biotechnological tool for the supply of necessary genetic exchange, beneficial structure, necessary nutrients as well as the prevention from exposure to environmental stress due to chemical and shear stress as well as prevention from predators. Biofilms have also been applied as a biomarkers for the evaluation of stream water quality derived from mine drainage. The structure and durability of biofilm with numerous arrays of metabolic and structural features make communities attractive actors in biofilm-mediated ecorestoration resolutions and ecosystem observation.

9.4 Modes of Action Involved in the Application of Biofilms Derived from Microorganisms for the Remediation of Contaminated Sites

The application of biofilm for the in situ ecorestortion of polluted environment may be carried out frequently. It has been observed that the process involved in the natural attenuation depends on the natural process without the utilization of genetically modified microorganisms through the application of some beneficial important microorganism for the purpose of ecorestoration. The application of biofilm produced by beneficial microorganism in the soil could be utilized for the biotransformation of some heavy pollutant into a lesser component without any presence of hazardous compounds. The process of natural attenuation incorporation with some favourable conditions could facilitate the biodegradation, transformation and immobilization and their eventual detoxification into lesser compounds without the input of human beings (Sayler et al. 1995). Moreover, the passive remediation process necessitates the availability of microorganism present in the biofilm for continual degradation of contaminant in the environment, and this process might take a longer period of time.

The presence of some essential nutrients such as phosphorus compounds and the presence of oxygen and air might enhance the process of biodegradation of pollutants through the process referred to as biostimulation. The natural attenuations may be evaluated at definite times (Vogt and Richnow 2014; Jorgensen et al. 2010). It has been observed that the application of natural attenuation bioremediation techniques is the best strategies for the ecorestoration of heavily polluted sites such as petroleum hydrocarbon sites (Rittmann 2004). This could also be referred to as monitored natural attenuation. Also, bioenhancement and bioaugmentation depend on the addition of specific application of some beneficial microorganisms or their consortia for bioremediation of heavily contaminated environment (Tyagi et al. 2011). Moreover, this process might require the application of nutrients and substrates which might facilitate this process. The microbial populations merging from highly contaminated sites also play a crucial role in the biodegradation of heavily polluted sites. These microorganisms could be stored in the laboratory environment which could subsequently be applied for the treatment of heavily polluted soil. It has been highlighted that the process of bioaugmentation techniques could be utilized for the bioremediation of heavily polluted environment most especially in the situation where the local population of microorganism did not possess adequate biodegradation efficiency. This might also lead to the development of biofilm which play several crucial role in the biodegradation of polluted environment. The stages involved in the process of bioaugmentation may be evaluated utilizing biomarkers based on *luc* or *gfp* to evaluate and monitor the biodegradation effectiveness of the inoculated microbes (Jansson et al. 2000).

The process of bioaugmentation may also be enhanced through the application of genetically engineered methodology or through the techniques that enhances the concentration of nutrients or the biostimulation techniques, air venting and persistence of microbes.

The process of biostimulation stimulates electron donors and acceptors, nutrients, substrates, in order to support the action of microorganisms that could play a crucial role in the biodegradation of polluted environment (Morgan and Watkinson 1989). The two effective techniques that has been highlighted for the ecorestoration of petroleum hydrocarbon oil and the improvement of nitrification performance were biostimulation and bioaugmentation (Grace Liu et al. 2011; Abeysinghe et al. 2002).

Furthermore, the air venting techniques involve the pumping of air into the heavily contaminated sites that are available below the soil surface to enhance the aerobic microbial community and enhances the development of biofilm. It has been highlighted that polluted soil did not possess necessary endogenous microbial degrading population or the availability of necessary conditions that could stimulate the process of biodegradation that might be subjected to ex situ remediation frequently in a reactor.

It has been highlighted that in engineered systems, biofilms are applied in a bioreactor in an inert support. The biofilm bioreactor is utilized for biochemical conversion and biosorption of contaminants most especially from municipal wastewater, heavy metals, industrial wastewater and petroleum hydrocarbon (Boon et al. 2002). The bioreactor based on the application of biofilms is applied for the commercial bioremediation of industrial wastewaters for decades (Qureshi et al. 2005; Bryers 1993).

Some of the merits of biofilm reactors when compared to conventional treatment processes includes decreased interruption in the bioreactor, enhanced concentration and retention of biomass for long periods of time, better tolerance to harsh pollutants, improved volumetric biodegradation capability, improved metabolic action, large mass transfer area and improved process flow rates. It has been discovered that in industrial set up, the biofilm reactors are utilized in situation where some free-floating microorganisms do not possess the capability to generate adequate biomass or the biomass could not be retained for a longer period for effective volumetric conversion. This happens when the microorganism growth is slower most especially in the suspensions or when diluted feed streams are utilized in bioreactors. Moreover, in a typical biofilm reactor, there is a need for support medium for development and adhesion of microbes.

9.5 Different Types of Biofilm Bioreactors

There are different types of biofilm bioreactors which entails biofilm airlift suspension batch reactors, expanded granular sludge blanket, continuous stirred tank, fluidized bed, trickle bed, air-lift reactors and up flow anaerobic sludge blanket (Qureshi et al. 2005; Bryers 1993; Rosche et al. 2009; Singh et al. 2006). Biofilm reactors can be utilized for the bioremediation of off-site or applied for the nearby contaminated sites. A packed bed reactor is typical designed based on the common biofilm with solid supports that are arranged together with biofilm to give adequate supports between the liquid and the biomass. A packed bed reactor entailing a biofilm mercury-resistant strains has been adequately utilized for the ecorestoration of mercury (Wagner-Dobler 2003).

Trickle-bed biofilm bioreactor is another type of biofilm-based reactor used for the treatment of wastewater. Some of the examples of materials used as media in this type of reactor include ceramics, plastics and rock where the biofilm could develop. In trickle-bed biofilm bioreactor, waste water normally settles down from the top through the distribution system over the biofilm surface held on a fixed media. Normally, the pollutant available in the water will be metabolized as it passes through the biofilms. During this process, oxygen may be supplied downwards or upwards which might eventually diffuse through the water to reach the biofilms. The next generation of suspended solids available in trickle-bed biofilm bioreactor necessitates a liquid–solid separation through a clarifier. It has been observed that the presence of biofilms in dome reactor may not have enough feed in some areas and may led to decrease in productivity.

Moreover, it has been observed that the fluidized-bed reactor works based on the coating of beads inside a column with biofilms in which polluted water is pumped upwards and allows the biofilm beads to be suspended during the ecorestoration of polluted water (Shieh and Keenan 1986). This constitutes the major difference between these types of bioreactor and fixed-bed reactor where the media is not suspended. The solids are suspended by flow of gas or liquid at some certain velocity. The application of fluidization allows biofilms to develop on a very big surface area to generate a larger biomass. Oxygen is normally supplied through the application of oxygenator or through the bottom of the reactor. The fluidized-bed reactor is applied for the treatment of streams polluted with inorganic and organic compounds (Shieh and Keenan 1986; Denac and Dunn 1988; Kumar and Saravanan 2009; Costley and Wallis 2001).

Also, it has been observed that rotating biological contactors or modified types of rotating biological contactors are normally applied for the bioremediation of heavily polluted environment majorly wastewater treatment by decreasing biochemical oxygen demand or chemical oxygen demand as well as their high application during the process of denitrification and nitrification (Costley and Wallis 2001; Eker and Kargi 2008, 2010). Rotating biological contactors applied a thin biofilm produced from aerobic microorganisms grown on a bio-discs or rotating cylinder. This work is based on the principle of lowering the disc into the partially submerged effluents and gradually rotating the disc so that the biofilm microorganisms are slowly exposed to effluents and air present, and this allows the biofilm on the disc to enhance the rate of biodegradation of the pollutants. They are also utilized for the bioremediation of PAH, heavy metals, volatile organic compounds and degradation of dyes (Eker and Kargi 2008, 2010; Abraham et al. 2003; Jeswani and Mukherji 2012).

Membrane biofilm reactor generate oxygen or pressurized air through the gas permeable membranes to the joined biofilms developed on the membrane exterior. This type of bubble-free, enormous movement of oxygen disallows the stripping of volatile organic compounds, greenhouse gasses and foaming when an adjuvant such as surfactant is applied. This is normally utilized for the remediation of high oxygen demanding wastewater. The membrane normally serves as a support for the development of biofilms. It has been observed that hydrogen-based membrane biofilm reactor works basically based on the delivery of hydrogen to the biofilm entailing autotrophic bacteria which possess that capability to oxidize hydrogen and utilize electron donor to numerous pollutants such as nitrate and chlorate (Sarayu and Sandhya 2012; Rittmann 2006; Nerenberg and Rittmann 2004). Some other type of reactor includes methane-fed membrane biofilm reactor which is normally utilized for the removal of pesticides and nitrates from polluted water (Modin et al. 2008). Also, it could be utilized for the biodegradation of polychlorinated hydrocarbons

(Fathepure and Vogel 1991). This type of bioreactor enables the bioconversion processes to take place in spate stages. Concurrent denitrification and nitrification take place due to the availability of anoxic and aerobic biofilms available in the novel air-lift internal loop biofilm bioreactor (Zhang et al. 2013).

The other bioreactor is intensified biofilm-electrode reactor which utilized the application of heterotrophic and autotrophic denitrification for the removal of nitrate from polluted groundwater (Zhao et al. 2011). The biofilm reactors are utilized for precipitation of metals such as zinc, copper at the interface of biofilms using sulphate-reducing bacteria entrap (White and Gadd 1998, 2000; Smith and Gadd 2000). Several studies have been performed through adequate optimization of some special conditions that could enhance the usage of biofilm for effective bioremediation of polluted environment (Hosseini et al. 2013; Lin and Hsien 2009; Moreno-Andrade et al. 2009). The application of simulation and modelling studies have been performed to optimize the best biodegradation condition that could enhance and facilitate the process of ecorestoration (Coelhoso et al. 1992; Masic and Eberl 2014; Martin et al. 2015).

9.6 Conclusion and Further Recommendation for Further Study

This chapter has provided a detailed information on the application of biofilm for the bioremediation and biotransformation of heavily polluted environment. Detailed information of the modes of action and the types of biofilm produced by different microorganism has been highlighted. There is a need for several scientists from interdisciplinary field such as civil engineering, soil science and applied microbiology to collaborate on the best approach that could facilitate the application of biofilm for the bioremediation of contaminated environment. The application of strain improvement for the generation of genetically modified strain should be encouraged for the production of enhanced biofilm with enhanced bioremediation activity. Another improved approach that need to be built on entails the application of DNA embracing catabolic genes that enable biodegradation of particular contaminants. This will facilitate the process of natural transformation and bioremediation. Moreover, the application of genetically modified microorganisms with high potential for biodegradation of numerous pollutants such as genetically modified microorganisms while horizontal movement of genes with high biodegradation especially from genetically modified microorganisms to the members of biofilms pollution should be encouraged to facilitate the process of ecorestoration. Furthermore, the cloning of gene that could improve the synthesis of biosurfactant and chemotactic of genetically modified microorganisms can improve the process of bioremediation. There is a need to also perform more research on the reengineering of secreted proteins in biofilm matrix and their wider application for the bioremediation of recalcitrant pollutants, heavily polluted environments and their synergetic effect with biofilm derived from other microorganisms on their consortium for the phytoremediation and bioremediation of xenobiotic compounds.

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Chapter 10
Microbes: A Novel Source of Bioremediation for Degradation of Hydrocarbons

Mridul Shakya, Poonam Verma, Sunil Kumar, and Sardul Singh Sandhu

Contents

Abstract In our daily life, the demand for liquid petroleum products is increasing day by day. Crude oil-derived hydrocarbons, the largest group of environmental pollutants found worldwide, pollute our environments severely. Oil or hydrocarbons cause drastic impacts on living organisms. The many reports about their toxicity emphasize the ultimate need to remove them from marine and terrestrial environments. For cleaning up pollution by these hydrocarbons, bioremediation seems to be the most acceptable and economically justified method. Bioremediation is considered one of the most sustainable cleanup techniques, but its potential has not been fully expressed in the field because it operates too slowly to meet the immediate demands of a given location. The process of bioremediation is carried out by various microorganisms. Therefore, in this review, we present information about methods of oil degradation by such microorganisms as bacteria, fungi, algae, and actinobacteria. These microbes can help degrade oil or hydrocarbons. This review presents the unique characteristics of oil-degrading microbes. In addition, it is a starting point for

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M. Shakya · P. Verma · S. Kumar · S. S. Sandhu (✉)
Bio-Design Innovation Centre, Ekatm Bhawan, Rani Durgavati University, Jabalpur, Madhya Pradesh, India
e-mail: ssandhu@redifmail.com

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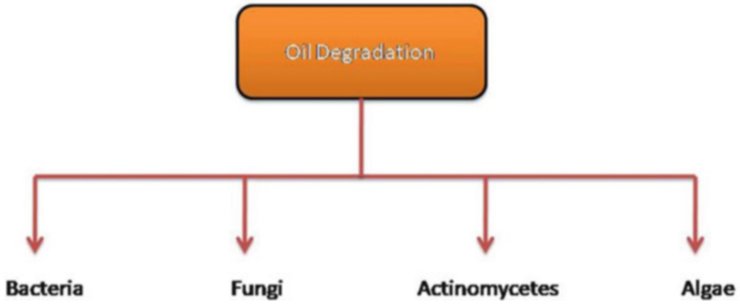

Fig. 10.1 Microbes that degrade oil

wider debate about the limitations and possible improvements of currently employed hydrocarbon bioremediation strategies.

Keywords Hydrocarbons · Degradation · Microorganisms

10.1 Introduction

At the present time, petroleum and its constituent hydrocarbons are widely used as the main energy source in the industrial, transport, and domestic sectors (Varjani and Upasani 2016; Arulazhagan et al. 2010). However, use of these hydrocarbons produces a number of harmful chemical substances that widely affect human beings and the environment. The effectiveness of these substances depends upon the composition, concentration, and biological state of the affected organism at the time of contamination and also on such environmental factors as temperature (Obire and Ayanwu 2009).

In our environment, toxic components of hydrocarbons are released by transport, vehicle factories, thermal plants, oil spills, pipelines, oil well leakages, diesel stations, and contamination by vehicle garages (Costa et al. 2012). The petroleum hydrocarbons are categorized into two broad divisions, aromatic and aliphatic compounds. The simple aliphatic and aromatic compounds are degraded in the environment, but because of their complex structure, the large aliphatic and aromatic constituents of petroleum hydrocarbons are not degraded (Hasanuzzaman et al. 2007). Therefore, different strategies and approaches are used to degrade these hydrocarbons, broadly categorized into three groups: physical, chemical, and thermal approaches (Adnan et al. 2018). All these methods are very costly, and the chemicals required further greatly affect our environment. In the thermal process, large amounts of heat are generated that affect both the flora and fauna of a specific area (Ezeji et al. 2007).

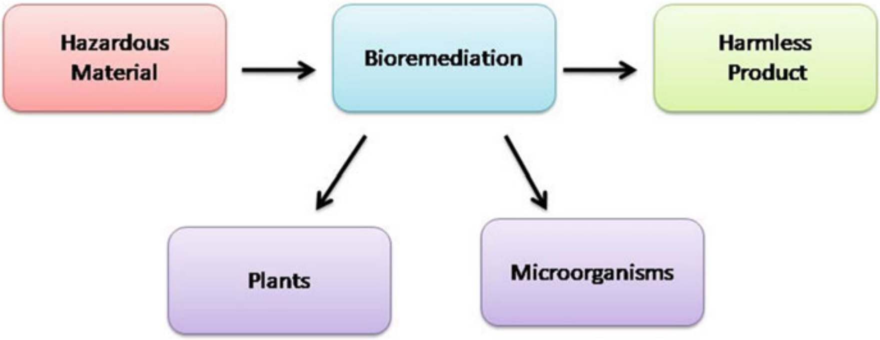

Fig. 10.2 Process of bioremediation

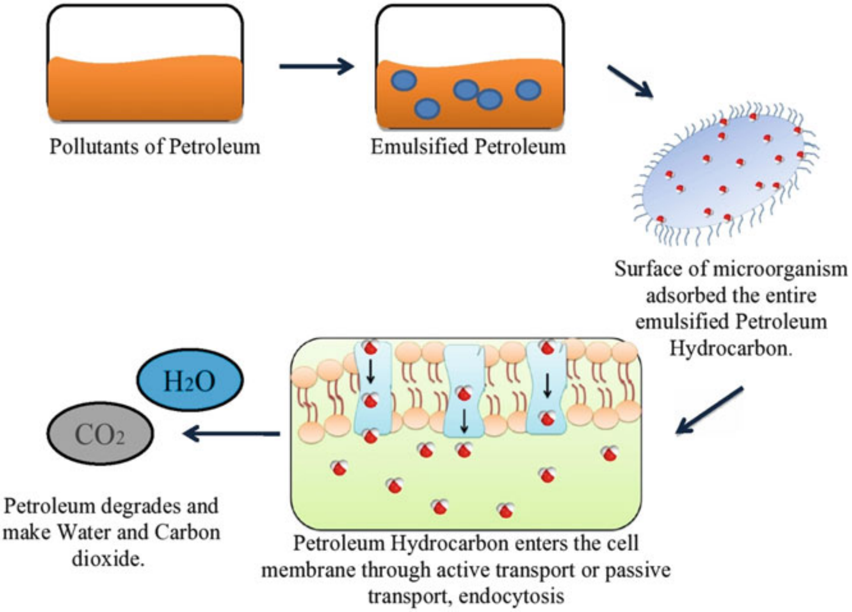

Fig. 10.3 Mechanism of oil degradation

Therefore, the preferred method for degradation of hydrocarbons is biological treatment because of reliability, feasibility, and the high potential for eco-friendly degradation. The biological methods are very simple to use and require low energy for operation. A variety of microorganisms can be used for the process in in vitro as well as in in vivo conditions (Fig. 10.1). Different types of microorganisms—bacteria, fungi, algae, and yeasts—degrade the hydrocarbons in a green revolution for removing hazardous contaminants from the environment (Zhang et al. 2013; Rahman et al. 2003). Native microorganisms have great potential for degradation as compared to others because of the specific metabolic pathways that metabolize the oil content.

Crude oil is composed of several compounds, including aliphatic, aromatic, and polycyclic aromatic hydrocarbons (PAH) and also sulfur-, oxygen-, and nitrogen-containing compounds. PAH compounds are toxic and may be carcinogenic. High concentrations of such pollutants, by their poisonous and carcinogenic nature, can affect cellular metabolism (Tanti et al. 2009). The biodegradation of petroleum hydrocarbons may be contained by considering many factors. An essential limiting factor in the biodegradation of polluted soils is often the low bioavailability and solubility of the hydrocarbons. Crude oil is one of the most significant pollutants in the environment, able to cause extreme damage to human beings and ecosystems. Excessive oil concentration causes serious problems in our body such as liver or kidney disorders, visible harm to bone marrow, and an increased risk of cancer (Mishra et al. 2001). The use of microorganisms in degradation of petroleum and its products has been established as a green, cost-effective, flexible, and environmentally sound remedy. The search for effective and green strategies of oil removal from polluted infected sites has intensified in recent years because the microbial cleanup of untreated oil spills is a slow process (Grangemard et al. 2001). In microbial

Table 10.1 List of hydrocarbon- or oil-degrading bacteria

Sample no.	Bacteria	Degradation	Reference
1	*Pseudomonas*	Hydrocarbons	Leahy and Colwell (1990)
2	*Acinetobacter*	Hydrocarbons	Adebusoye et al. (2007)
3	*Alcaligenes*	Hydrocarbons	Floodgate (1995)
4	*Vibrio*	Hydrocarbons	Leahy and Colwell (1990)
5	*Flavobacterium*	Hydrocarbons	Adebusoye et al. (2007)
6	*Achromobacter*	Hydrocarbons	Floodgate (1995)
7	*Micrococcus*	Hydrocarbons	Leahy and Colwell (1990)
8	*Nocardia*	Hydrocarbons	Adebusoye et al. (2007)
9	*Corynebacterium*	Hydrocarbons	Floodgate (1995)
10	*Pseudomonas stutzeri*	*n*-Tetradecane	Adel et al. (2012)
11	*Bacillus thuringiensis*	*n*-Tetradecane	Abou-Shanab et al. (2016)
12	*Bacillus pumilus*	*n*-Tetradecane	Awad et al. (2011)
13	*Bacillus cereus*	*n*-Tetradecane	Bayoumi et al. (2010)
14	*Pseudomonas* sp.	Hydrocarbons	Brito et al. (2006)
15	*Marinobacter* sp.	Hydrocarbons	Akpoveta et al. (2011)
16	*Alcanivorax* sp.	Hydrocarbons	Juhasz and Naidu (2000)
17	*Microbulbifer* sp.	Hydrocarbons	Bishnoi et al. (2008)
18	*Sphingomonas* sp.	Hydrocarbons	Snape et al. (2001)
19	*Micrococcus* sp.	Hydrocarbons	Lloyd and Cackette (2001)
20	*Cellulomonas* sp.	Hydrocarbons	Chaillan et al. (2004)
21	*Dietzia* sp.	Hydrocarbons	Akpoveta et al. (2011)
22	*Gordonia* sp.	Hydrocarbons	Bishnoi et al. (2008)

remediation, organization of numerous microbes present in the soil can degrade a wide range of oily sludge (Barathi and Vasudevan 2001).

Oil spills affect many species of plants and animals within the surrounding areas as well as humans. The search for green and powerful approaches to defining the rate and overall extent of biodegradation of waste lubricating oil in soils or contaminated sites has intensified in current years (Umar et al. 2013). Microorganisms can metabolize oil much as humans convert their food into energy or power. The soil is the habitat of many organisms, so any changes or variations in soil may further destroy our environment. The impact of an oil spill is enrichment of the soil-degrading microbial populations. No single microorganism has been observed to completely degrade a petroleum hydrocarbon molecule, but particular species or traces of equal species may be capable of degrading concentrations of oil hydrocarbons (Facundo et al. 2001). Species of *Pseudomonas* are known for their capability of hydrocarbon degradation (Jewetz et al. 1999) (Fig. 10.2).

10.2 Mechanism of Oil Degradation by Microorganism

The biodegradation of hydrocarbons by microorganisms in nature has four main steps (Fig. 10.3).

In the first step, pollutants of petroleum are emulsified by surfactant secreted by a microorganism. Then, the surface of the microorganism adsorbs the entire emulsified petroleum hydrocarbon. Now, the petroleum hydrocarbon, which is adsorbed onto the surface of the cell membrane, enters the cell membrane through active transport or passive transport, endocytosis. In the last step, the petroleum hydrocarbon enters into the cell, and undergoes an enzymatic reaction that causes its degradation (Li et al. 2019).

10.2.1 Degradation of Oil and Hydrocarbon by Bacteria

Different species of bacteria are widely used to biologically degrade petroleum hydrocarbons and also to help remove oil spills by degradation (Abou-Shanab et al. 2016). Many studies have shown that bacteria can degrade hydrocarbons such as asphaltenes (phenols, ketones, esters, porphyrins, fatty acids), resins (carbazoles, sulfoxides, pyridines, quinolines, amides) (Steliga 2012), and aliphatics, aromatics, and resins (carbazoles, sulfoxides, pyridines, quinolines, amides) (Table 10.1). The bacterial strains *Pseudomonas fluorescens*, *P. aeruginosa*, *Bacillus subtilis*, *Bacillus* sp., *Alcaligenes* sp., *Acinetobacter lwoffi*, *Flavobacterium* sp., *Micrococcus roseus*, and *Corynebacterium* sp. isolated from polluted areas in Nigeria were observed for degradation of crude oil (Adebusoye et al. 2007).

Petroleum bioremediation is completed by microorganisms that can utilize hydrocarbons as a source of energy (Rosenberg et al. 1998). These bacteria are ubiquitous

Table 10.2 List of oil- or hydrocarbon-degrading fungi

Sample no.	Fungus	Degradation	References
1	*Aspergillus flavus*	Petroleum oil	Adekunle and Oluyode (2002)
2	*A. niger*	Petroleum oil	Bartha and Atlas (1997)
3	*Mucor*	Petroleum oil	Battelle (2000)
4	*Rhizopus*	Petroleum oil	Nwachukwu (2000)
5	*Talaromyces*	Petroleum oil	Ojo (2005)
6	*Penicillium*	Hydrocarbons	Ahmad et al. (2016)
7	*Amorphoteca*	Hydrocarbons	Throne-Holst et al. (2007)
8	*Candida*	Hydrocarbons	Farag and Soliman (2011)
9	*Fusarium*	Hydrocarbons	Al-Nasrawi (2012)
10	*Neosartorya*	Hydrocarbons	Jawhari (2014)
11	*Mycotypha*	Hydrocarbons	Okafor et al. (2009)
12	*Rhizopus*	Hydrocarbons	Mittal and Singh (2009)
13	*Botrytis*	Hydrocarbons	Joshi and Pandey (2011)
14	*Polyporus* sp.	Crude oil	Kristanti et al. (2011)
15	*Amorphoteca* sp.	Hydrocarbons	Jones et al. (2001)
16	*Neosartorya* sp.	Hydrocarbons	Chaillan et al. (2004)
17	*Paecilomyces* sp.	Hydrocarbons	Ramasamy et al. (2014)
18	*Talaromyces* sp.	Hydrocarbons	Wang et al. (1998)
19	*Graphium* sp.	Hydrocarbons	Balaji et al. (2014)
20	*Popularia* sp.	Oil	Sandhu et al. (2016)
21	*Geotrichum* sp.	Oil	Sandhu et al. (2016)

in nature and able to degrade numerous hydrocarbons including short-chain, long-chain, and numerous aromatic compounds, including PAHs. These compounds have low solubility in water. Thus, as the first step in hydrocarbon degradation entails a membrane-bound oxygenase, it is important for microorganisms to be in direct contact with the hydrocarbon substrates. One biological approach to accomplish contact between the microorganisms and water-insoluble hydrocarbons is emulsification of the hydrocarbon. Therefore, it is not unexpected that microorganisms growing on petroleum typically produce emulsifiers. These surfactants assist to disperse the oil and to detach the bacteria from the oil droplets after utilizable hydrocarbon has been depleted (Ron and Rosenberg 2002).

10.2.2 Biodegradation of Oil and Petroleum by Fungi

Crude oil is a primary source of profits for Iraq, which is certainly one of the most important international oil producers and exporters, ranked nearly fourth internationally in terms of oil reserves. Incidental spills of crude oil and frequent illegal disposal of oil wastes lead to serious damage to environments. Cleaning up oil contaminants is a priority project for the restoration of our natural environment.

Chemical, physical, and thermal strategies are available but these methods are very costly and require site recovery. Several physicochemical and biological methods have been assessed for treating oil-contaminated environments (Ezeji et al. 2007). Organic treatment is desired for physicochemical strategies for reasons of its feasibility, reliability, and capability to achieve high elimination efficiency with low price. Other reasons include the simplicity of its low-power layout, creation, operation, and use; biodegradation of hydrocarbons is a cost-effective method compared to chemical methods (Liu et al. 2013). In a biological technique, microorganisms can use hydrocarbons as their sole energy and carbon source and degrade them instead of gathering them at every other level (Zhang et al. 2015). Biological treatment may have an advantage over physicochemical treatment in the removal of spills because it affords crucial biodegradation of oil parts through microorganisms, is a "green" alternative for treating risky contaminants without environmentally degrading effects, and may be cheaper than other strategies (Zhang et al. 2011). Diverse microorganisms, including bacteria, algae, yeasts, and fungi, can degrade hydrocarbons. Indigenous microorganisms with particular metabolic capacities have a considerable role in the biodegradation of crude oil (Rahman et al. 2003). Rahman et al. (2002) suggested that bacterial consortia isolated from crude oil-infected soils have the potential to degrade crude oil fractions. In addition to bacteria, fungi are one of the best oil-degrading organisms. Numerous studies have identified many fungal species able to use crude oil as their sole source of energy, including *Cephalosporium*, *Rhizopus*, *Paecilomyces*, *Torulopsis*, *Pleurotus*, *Alternaria*, *Mucor*, *Talaromyces*, *Gliocladium*, *Fusarium*, *Rhodotorula*, *Cladosporium*, *Geotrichum*, *Aspergillus*, and *Penicillium* (Jawhari 2014). Hanafy et al. (2017) observed that the *Aspergillus* and *Penicillium* isolated from oil-contaminated sites close to the Red Sea within the Yanbu region have been extremely useful in crude oil degradation. Using fungi as a means of bioremediation gives a powerful alternative for cleansing the environment of contaminants (Hanafy et al. 2017). Data are shown in Table 10.2.

10.2.3 Biodegradation of Oil and Petroleum by Algae

Natural contamination has been stated to be the most significant issue affecting the world (Reyes et al. 2016). One of the main causes of environmental pollution is hydrocarbon contamination in soil and water (El-Sheekh et al. 2013). Unrefined petroleum, also called dark gold, is the most significant asset in industrialized nations; however, its handling and transport can cause genuine ecological contamination and interfere with many populations of organisms (Xaaldi et al. 2017). Many recorded data attest to the real genuine harm brought about by oil slicks in ecosystems and to marine creatures, silt, higher-level organisms, fish, coral reefs, avian species, reptiles, and surface water bodies (Afshar-Mohajer et al. 2018). When oil is spilled in the ocean or other waterways, it creates a film that decreases the proportion of daylight reaching the underwater world, which affects the process of

Table 10.3 List of oil- and hydrocarbon-degrading algae

Sample no.	Algae	Degradation	References
1	*Amphora* sp.	Crude oil	Kvenvolden and Cooper (2003)
2	*Prototheca zopfii*	Crude oil and hydrocarbons	Aditi et al. (2015)
3	*Porphyridium* sp.	Petroleum waste	Vidyashankar and Ravishankar (2016)
4	*Microcoleus* sp.	Hydrocarbons	Yakimov et al. (2007)
5	*Agmenellum* sp.	Petroleum waste	Walker et al. (1975)
6	*Anabaena* sp.	Hydrocarbons	Cerniglia et al. (1980)
7	*Coccochloris* sp.	Hydrocarbons	Bibi et al. (2017)
8	*Nostoc* sp.	Hydrocarbons	Lohitesh et al. (2013)
9	*Cylindretheca* sp.	Petroleum waste	Srivastav et al. (2013)
10	*Aphanocapsa* sp.	Hydrocarbons	Shankar and Suneetha (2013)
11	*Chlorella* sp.	Petroleum waste	Rath et al. (2012)
12	*Chlamydomonas* sp.	Crude oil	Venkata Gopichand et al. (2013)
13	*Ulva* sp.	Hydrocarbons	Lohitesh et al. (2013)
14	*Petalonia*	Crude oil and hydrocarbons	Aditi et al. (2015)

photosynthesis. Additionally, total petroleum hydrocarbon (TPH), a natural toxin in the Earth, is poisonous for all human beings and numerous other organisms (Lee et al. 2015). Polycyclic aromatic hydrocarbons (PAHs) are the most lethal components of unrefined petroleum and are related to cancer-causing agents (Duran and Cravo 2016). Bioremediation suggests the utilization of living organisms and their biochemical apparatus to debase or change poisons into less dangerous forms, which has been demonstrated to be a powerful, confined, and more affordable technique (Sharma et al. 2018). In any case, a limitation of the bioremediation procedure with microorganisms is the accessibility of supplements, for example, nitrogen and phosphorus, which influences the speed of oil degradation (Ron and Rosenberg 2014), although advances in atomic innovations on recombinant DNA have permitted the hereditary improvement of numerous organisms and support the speed of remediation. The fundamental segments of raw petroleum are naphthenes, asphaltenes, waxes, pavements, aromatic hydrocarbons, tars, and other unstable mixes, for example, benzene, toluene, ethylbenzene, and xylene. Many mixes, for example, pyrene, benzo(*a*)pyrene and chrysene, are cancer causing, mutagenic, and teratogenic (Sammarco et al. 2013). Numerous microorganisms, including a few types of microalgae (*Monoraphidium braunii*, *Chlamydomonas reinhardtii*, *Chlorella* sp.), parasites (*Trametes versicolor*, *Pleurotus eryngii*, *Phanerochaete chrysosporium*), and bacteria (*Pseudomonas aeruginosa*, *Rhodococcus erythropolis*), have catabolic pathways for the debasement of contaminants (Sharma et al. 2018). Algal growth is fundamental in seagoing biological systems and in light of the fact that they are essential markers, are important in the trophic chain,

Table 10.4 List of oil- and hydrocarbon-degrading actinomycetes

Sample no.	Actinobacteria	Degradation	References
1	*Actinoplanes*	Oil	Cappuccino and Sherman (2002)
2	*Nocardia*	Hydrocarbons	George et al. (2011)
3	*Streptomyces*	Oil	Rahman et al. (2002)
4	*Streptosporangium*	Hydrocarbons	Rifaat and Yosery (2004)
5	*Rhodococcus*	Oil	George et al. (2011)
6	*Nocardia*	Hydrocarbons	Watanabe et al. (2002)
7	*Gordonia*	Oil	Essien and Udosen (2000)
8	*Dietzia*	Oil	Beerka and Steinbuchel (2000)
9	*Micromonospora*	Hydrocarbons	George et al. (2011)
10	*Actinomyces octodloyts*	Petroleum Hydrocarbons	
11	*Saccharomyces cerevisiae* (yeast)	Petroleum Hydrocarbons	

providing oxygen and natural substances to other living things. *Chlorella vulgaris* is a significant species because it adsorb an assortment of natural pollutants (Kong et al. 2010), so the development of microalgae in wastewater treatment is spreading widely for the disposal of supplements, control of physical substance parameters, as feedstock for the generation of biofuel, and expulsion of phenol and polycyclic aromatic compounds, because of its high adsorption limit, bioaccumulation, biotransformation, and biodegradation (He et al. 2016). For this reason, it was proposed here to determine the capability of biodegradation of unrefined petroleum by the microalgae *Chlorella* sp. (Deimer et al. 2018). Data are shown in Table 10.3.

10.2.4 Biodegradation of Oil and Hydrocarbons by Actinomycetes

The tragic history of soil and water pollution by way of oil spillage from the oil industry, tankers, offshore systems, related pipelines, garage tanks and wells, and unlawful oil bunkering has caused essential environmental and fitness defects in oil-structured countries (Ordinioha and Brisibe 2013). Pollution through crude oil, inclusive of oil spills and toxic wastes, is a persistent struggle that has prompted serious threats to human fitness with issues regarding the viability and productiveness of ecosystems (Okoh and Trejo-Hernandez 2006). Mechanical and chemical techniques for the remediation of hydrocarbon-polluted surroundings are frequently costly and technologically complex. Increasing attention has been paid to the growing innovative era for cleaning up this contaminant, with bioremediation being a completely useful method (Vidali 2001). There are many herbal and natural microorganisms that thrive on the decomposition of those toxic compounds. Usage of microorganisms for cleanup efforts, referred to as bioremediation, has been shown to be a successful method for the cleanup of marine regions suffering from oil spills (Coulon et al. 2006). Bioremediation strategies are currently receiving favorable exposure as low-cost and promising environmentally friendly technologies for the remediation of crude oil hydrocarbons without difficulty. Biodegradation of crude oil and derived aromatic hydrocarbons in marine sediments has been reported (Jones et al. 2008). The maximum fast and complete degradation of general organic pollution is introduced under cardiac conditions and the biodegradation system is mediated by unique enzyme structures (Das and Chandran 2011). Extracellular and intracellular assault of organic pollution by microbes through oxidation is catalyzed by peroxidases and oxygenases. The cleanup of toxic natural compounds through numerous microorganisms and fungi takes place through oxidative coupling mediated via oxidoreductases together with peroxidases (Karigar and Rao 2011). Microbes derive power via power-yielding biochemical reactions mediated by these enzymes to cleave chemical bonds and help transfer of electrons from a reduced natural substrate (donor) to some other chemical compound (acceptor). For this reason, it is essential to analyze the function and organization of enzymes for crude oil biodegradation. Actinobacteria have several characteristics that are vital for surviving in extreme situations, including dry environments and nutrient lack, and produce biosurfactants that boost contaminant bioavailability and facilitate the manner of biodegradation (Beilen and Funhoff 2005): these promote the prevalence of Actinobacteria in pristine and hydrocarbon-polluted soil (Quatrini et al. 2008). Consequently, it is important to observe crude oil biodegradation of actinobacterial isolates, particularly from oil-contaminated sites (Table 10.4).

10.3 Conclusion

Bioremediation is the main natural mechanism that can cleanse petroleum and oil pollutants from the environment. This process uses microscopic organisms such as bacteria, fungi, algae, and actinomycetes that live in soil and consume oil or hydrocarbons. A number of factors influencing degradation have been identified to reduce the toxicity of oil contamination in the environment by removing, degrading, or transforming contaminants. Therefore, successful bioremediation treatment requires understanding of those factors.

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Chapter 11
Microbial Bioremediation of Petroleum Hydrocarbons

Sharmila Jayasena and Madushika Perera

Contents

Abstract Petroleum oil, a naturally occurring limited resource, is in high demand globally and has led to the extensive drilling, storage, and international transportation. The past decades have seen several spills and seepage of crude oil resulting from accidents. Petroleum oil, which occurs as crude oil, is a complex and variable

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S. Jayasena (✉) · M. Perera
Department of Biochemistry and Molecular Biology, Faculty of Medicine, University of Colombo, Colombo, Sri Lanka
e-mail: sharmila@bmb.cmb.ac.lk

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mixture of hydrocarbons, comprising saturates, aromatics, resins, and asphaltenes in varying proportions. Due to the toxicity and recalcitrant nature of petroleum hydrocarbons, crude oil pollution is currently considered a global environmental hazard. While a variety of mechanisms (physical and chemical) are used to tackle such accidental exposures, they are costly and have inherent limitations that may affect the ecological balance. Thus, microbial biodegradation of petroleum hydrocarbons has been generating increasing interest as a cost-effective method that causes the least damage to the ecological balance. Hydrocarbonoclastic microbes, both bacteria and fungi have been isolated from contaminated sites and are investigated for their degradation potential. While bacteria have the advantage of fast turn over, they are limited in that most species are able to utilize only a limited and often narrow range of hydrocarbons. Fungi, on the other hand, are intrinsically harder and appear to be more versatile in their utilization of petroleum hydrocarbons. The main challenges in use of microbial biodegradation to tackle pollution are complete removal of all the hydrocarbon components and obtaining adequate efficiency in the process. The need to improve the degradation potential by microbes continues to drive the exploration for new isolates, as well as the more recent interest in investigation of microbial consortia. Use of microbial consortia requires an understanding of their individual requirements as well as the interactions between them.

Keywords Bioremediation · Petroleum hydrocarbon · Crude oil · Microbial consortia · Biofilm · Alkane hydroxylase · Monooxygenase

11.1 Introduction

Petroleum (crude oil) is a naturally occurring raw oil derived from buried biomass over millions of years as a result of natural weathering processes such as thermal decay and the intense pressure at the buried depths. Crude oil occurs in large reservoirs in limited locations as a dark yellowish, brownish, or even greenish viscous liquid, the color depending on the distinct chemical constituents. Petroleum comprises a complex and varying mixture of hydrocarbons that fall into four broad chemical fractions: saturates (alkanes), polyaromatic hydrocarbons (PAH), resins, and asphaltenes, which may occur in various proportions depending on the location, depth, and the age of the oil (Varjani 2017).

Petroleum products represent a primary energy source and an important industrial raw material for people and recent years has seen an increased use of these products. Crude oil is extracted by drilling and then refined by distilling to produce various products. Petroleum products thus include crude oil as well as a variety of crude oil–derived products such as petrol and diesel. They are also incorporated into numerous products such as refrigerator parts.

The increased use of petroleum products has also led to increased environmental pollution by petroleum. Crude oil is transported across the oceans as well as overland in ships or through pipelines. Inadvertent spills and seepage of petroleum products during storage tanks and transportation is a leading cause of environmental pollution of both aqueous and terrestrial environments. Over the years has witnessed several accidental spills; The Prestige oil tanker wrecked northwest of Spain in 2002 released 40,000 tons of oil (Pérez-Cadahía et al. 2007). The Deepwater Horizon (DwH) oil spill in April 2010 in the Gulf of Mexico, which occurred as a result of an explosion, released ~4.9 million barrels of crude oil into the Gulf of Mexico before it was capped in about 3 months. The spilled oil which spread ~450 miles along the coastline of the Gulf of Mexico is considered to be one of the worst environmental disasters in the US history (Bell and Gutierrez 2019).

Petroleum hydrocarbons (PH) are recalcitrant environmental pollutants and are a global hazard, due to its persistent nature and toxicity of certain fractions. It causes widespread damage to all forms of life, both aquatic and terrestrial animals, from microbes to fish as well as whales and birds. Damage may be direct or indirect via the food chain.

While several methods for decontamination including physical and chemical have been used over the years, investigation of microbial biodegradation of crude oil has been an area of interest for several decades. There has been a renewed interest in recent years due to multiple reasons such as increase in oil spills and increased awareness regarding environmental pollution and the general interest toward use of green technologies.

Various species of bacteria, fungi, cyanobacteria, and algae are known to be capable of utilizing petroleum hydrocarbons. Among them, bacteria are considered the most active agents in bioremediation (Varjani 2017). However, no single bacterial species has been reported to date, which has the capacity to degrade all the fractions in crude oil.

The need for increased efficiency in the biodegradation process continues to drive the exploration of novel strains with capacity for PH degradation. The general strategy is based on screening for indigenous microbes from sites of contamination. Recent isolations of individual bacteria as well as mixed consortia have been reported by several investigators in various parts of the world (Al-Dhabaan 2019; Chettri and Singh 2019; Perera et al. 2019; Wang et al. 2019a).

The degradation of petroleum hydrocarbons by microbes can be observed as a three-step process—initially the microorganism needs to have access to the hydrocarbon molecules. Here, the solubility of the molecules or other capacities for gaining access such as secretion of biosurfactants play an important role. The compounds then need to be adsorbed to the cell surface and transported to the cell interior followed by enzymatic degradation and metabolism.

11.2 Chemical Components of Crude Oil

Natural crude oil is a complex and variable mixture of organic chemicals. Generally, four main classes have been identified. They are:

1. Aliphatics (mostly saturates)
2. Aromatics
3. Resins
4. Asphaltenes

The mixtures frequently also contain significant percentages of polar molecules such as nitrogen, oxygen, and sulfur as well as trace metals such as copper, vanadium, iron, and nickel (Hegazi and El-Gayar 2017).

1. Aliphatics/Saturates

   A majority of the aliphatics present in crude oil are saturates, generally comprising linear alkanes. A much smaller percentage of unsaturated aliphatics may also be present.

   Among the alkanes, as mentioned above, the majority are linear alkanes (*n*-alkanes) ranging from <C8 to C30 and above, while some branched alkanes (iso-alkanes) such as pristine and phytane and cycloalkanes (naphthenes) such as cyclohexane and cyclopentane may also be present.

   The linear alkanes can be divided into four groups based on molecular weight (Varjani 2017).

   <C8 gaseous alkanes
   C8–C16 low molecular weight alkanes
   C17–C28 medium molecular weight alkanes
   >C28 high molecular weight alkane

   They are also subdivided into four fractions (F1–F4) based on human and environmental risk (Varjani 2017).

F1	C6–C10	Volatile fraction
F2	C11–C16	Semi-volatile
F3	C17–C34	Non-volatile
F4	C35+	Lowest volatility and solubility

2. Aromatics

   The aromatics frequently present in crude oil are essentially of two types:

   (a) Monocyclic aromatic hydrocarbons:
   Benzene, toluene, ethylbenzene, and xylene (BTEX)
   (b) Polycyclic aromatic hydrocarbons (PAH):

   Naphthalene (two rings)
   Phenanthrene, anthracene (three rings)
   Pyrene, chrysene (four rings)

Fluoranthene, benzo(a)pyrene (five rings)

The four- and five-ringed molecules are considered as high molecular weight polyaromatic hydrocarbons.

3. Resins

   Resins (pyridines, quinolines, carbazoles, sulfoxides, and amides) (Leahy and Colwell 1990) are aromatic compounds with long alkyl chains and are rich in polar functional groups (N, S, O as well as trace metals Ni, V, and Fe). They form an amorphous solid which is soluble in linear alkanes such as *n*-heptane and *n*-pentane.

4. Asphaltenes

   These are high molecular weight compounds having polycyclic clusters, substituted with varied alkyl groups. These are also rich in polar functional groups and are dispersed in saturates and aromatics as colloid. They are soluble in light aromatic hydrocarbons such as benzene and toluene. Phenols, fatty acids, ketones, esters, and porphyrins fall into this category (Leahy and Colwell 1990).

11.3 Petroleum Hydrocarbons as an Environmental Pollutant: Biological Effects of Contamination

Petroleum hydrocarbons are persistent pollutants that cause extensive damage to the ecosystem. Due to their widespread use and accidental release into open systems such as oceans and waterways, they are considered a global environmental hazard.

Pollution of the terrestrial environment, namely soil, occurs during oil drilling and accidental damage to overland pipelines. Transport of petroleum oil is generally through the aquatic medium; oceans, seas, and bays, by either ship or underwater pipelines. Accidental spillage of oil during transport through water conduits occur due to technical failures. Although sporadic, they are increasing in frequency with the increase in global utilization. The impact of such spillage is significant, due to the open mobile nature of the systems. The oil disperses on the water surface and due to its viscosity, forms a slick on the surface causing great damage to aquatic life. It cuts off the exchange of gasses at the air-water surface and depending on the thickness and viscosity, may affect penetration of sunlight into the water (Freitas et al. 2016). This would impede photosynthesis and respiration, with disastrous consequences on the food chain.

The effect of petroleum hydrocarbon contamination, either aquatic or terrestrial, is alteration of the natural dynamic balance of the ecosystem. While some species may be lethally affected, it may also result in enrichment of species with capacity for utilizing hydrocarbons. Either way, efficient mechanisms for restoration of the ecological balance is therefore crucial.

The damage to the ecosystem may be direct, due to toxicity of some petroleum fractions. The BTEX and PAH compounds are known carcinogens and may be teratogenic (Pérez-Cadahía et al. 2007; Costa et al. 2012). Also, they may contaminate the food chain as a result of bioaccumulation, affecting even humans far from the site of contamination (Ite and Ibok 2019).

Petroleum hydrocarbons in the environment undergo “weathering.” This interaction with the environment may be physical (such as dispersion), physicochemical (such as evaporation, dissolution, and sorption), as well as chemical (photo oxidation, auto-oxidation) and biological (such as natural catabolism of polluting hydrocarbons by plants and microbes) and has been reviewed recently (Truskewycz et al. 2019).

Volatilization—The lighter aromatics (BTEX) and other simple ringed aromatics are generally volatile and therefore frequently separate from the more complex fractions, enhanced by increasing environmental temperatures. Alkanes $\leq$C8 are completely evaporated while C9–C12 are partially evaporated in experimental flasks containing 1% crude oil in aqueous medium (Perera et al. pers. comm.)

Dissolution—Solubility in aqueous environment decreases as aliphatic chain length or number of rings in aromatics increase. However, the presence of polar, non-hydrocarbon components will increase the solubility.

Sorption—Sorption of petroleum hydrocarbon (PH) fractions on to oil particles may occur by various mechanisms such as diffusion into nanopores or bond formation with organic matter in the soil. Solubility of the hydrocarbon fractions also affects sorption to soil (Truskewycz et al. 2019).

Dispersion—Oil spills in water spread and tends to form a viscous slick, cutting off oxygen and nutrients to the aquatic microbes and animals, thus affecting the ecosystem. Large masses of oil are generally not easily degraded by microbes due to restricted accessibility, owing to the hydrophobic nature of the oil. However, oil in water may also form emulsions as a result of wind and waves and due to microbial secretion of biosurfactants. This is an important process as it increases the accessible surface area, enhancing uptake of the hydrocarbons by microbes.

Unlike aquatic oil spills where the oil is dispersed horizontally on the surface of the water, in terrestrial oil spills, the movement of oil is vertical into the soil. Such infiltration hinders the evaporation of volatile hydrocarbons, which can be toxic to microorganisms (Leahy and Colwell 1990).

11.4 Microbial Biodegradation of Petroleum Hydrocarbons

Both physical and chemical methods for remediation of petroleum hydrocarbon pollution, particularly oil spills are used. However, they have limitations, both cost-wise as well as toxicity—particularly with the use of chemical emulsifiers. Bioremediation thus provides an alternative “green” mechanism for tackling this issue.

Natural biodegradation of petroleum hydrocarbons is carried out primarily by bacteria and fungi. Numerous species and strains that demonstrate varying capacities for utilization of hydrocarbons have been identified and continue to be identified. Microbes may use these for the production of energy or biomass. Biodegradation is thus the complete or partial mineralization of environmental organic contaminants, largely by microorganisms. Thus, biodegradation represents a natural mechanism through which contaminating petroleum hydrocarbon contaminants can be removed from the environment.

Petroleum hydrocarbons vary in their susceptibility to microbial degradation, generally being the highest for low molecular weight alkanes, lowest for polycyclic aromatics (PAH), and asphaltenes, the latter being the least susceptible (Das and Chandran 2011; Ite and Ibok 2019). However, this may be contradicted, depending on the strain of microbe present in the environment and other factors that affect degradation (Tables 11.1 and 11.2).

While a vast number of microbes, especially bacteria that have the ability to degrade petroleum hydrocarbons to varying degrees have been isolated, each species or strain is capable of utilizing only a specific, and often narrow, range of hydrocarbons. No bacteria that are capable of degrading the entire range of compounds in crude oil have been reported, although a few notable strains with a wide range have been isolated (Wang et al. 2011). A recent article reports the use of a thermophilic, bio-emulsifier-producing strain of *Aeribacillus pallidus* which demonstrated the ability to utilize short chain alkanes as well as some aromatics at 60 °C (Tao et al. 2019). Fungi on the hand appear to be more versatile and demonstrate a wider capacity, although they have a slower turnover compared to bacteria.

The renewed interest in green technology to manage petroleum pollution continues to drive the search for newer, more efficient, and more versatile microbes with a capacity to overcome the main challenges of bioremediation of petroleum hydrocarbons (PH), obtaining high efficiency of degradation and complete degradation of all the components of crude oil.

The tables below summarize a selection of microbes investigated, either singly or as consortia, for the degradation of petroleum hydrocarbons, specifying the substrate used and the efficiencies obtained for biodegradation.

11.5 Uptake of Hydrocarbons by Microbes

11.5.1 Chemotaxis

Some bacteria such as *Pseudomonas* have been shown to use chemotaxis to reach the hydrocarbon molecules, and expression of related proteins has been reported to be upregulated when cultured in crude oil at 500 mg/L. However, under very high concentration of crude oil (20,000 mg/L), it was found that chemotaxis was inhibited, while the secretion of an emulsifier was increased (Wang et al. 2019b).

Table 11.1 Bacterial biodegradation of petroleum hydrocarbons

Micro-organism	Hydrocarbon (initial concentration)	Growth conditions	Rate of degradation	References
Pseudomonas sp. (isolated from soil)	De-asphaltenated heavy oil (12 g/L)	28 °C, with continuous shaking at 200 rpm for 15 or 31 days	1 month: >60% for all *n*-alkanes 15 days: only C13 and C14 are degraded to >50%	Setti et al. (1993)
Pseudomonas fluorescens (strain Texaco)	Wax (aliphatics) (1.5 μg/mL)	20 °C shaking bacteria streaked on nutrient deficient agar plates sprayed with specific *n*-alkane	3 days: Complete removal of *n*-alkanes ≤C20 14 days: 80% of the aliphatic hydrocarbons; complete removal of *n*-alkanes C20–C25 136 days: 14% of the original fraction remained. No degradation of >C45 *n*-alkanes When acclimatized bacteria from 136-day culture was used, utilization of up to C60 *n*-alkanes were observed	Heath et al. (1997)
Pseudomonas otitidis	Crude oil (1%)	At 30 °C, 15-day incubation	50% degradation Degradation efficiency was higher in biofilm-supported cultures vs. planktonic form	Dasgupta et al. (2013)
Pseudomonas aeruginosa SJTD-1	*n*-Alkanes (C12–C30)	At 30 °C with constant shaking (180 rpm) for 7 days	In 36 h; 500 mg/L of tetradecane, hexadecane, and octadecane were transformed completely In 72 h: 2 g/L *n*-hexadecane degraded to undetectable levels	Liu et al. (2014)
Bacillus subtilis A1	Crude oil [1% (v/v)]	37 °C for 7 days at 200 rpm	C10–C14 were completely degraded C15–C19 were degraded up to 97%	Parthipan et al. (2017)

Bacillus thermoleovorans strains B23 and H41	*n*-Alkanes (C9–C23) [0.1% (v/v) filter-sterilized standard gas oil (Tokyo)]	70 °C for B23 65 °C for H41 Without shaking 20 days	>60% degradation	Kato et al. (2001)
Bacillus subtilis. Strain BL-27	Crude oil 0.3% (w/v)	45 °C, 5 days	65% of crude oil (0.3%, w/v) within 5 days; SDS (50–100 mg/L) and Tween 80 (200–500 mg/L), significantly increased the strain's ability to biodegrade, reaching 75–80%	Wang et al. (2019a)
Bacillus sp. PK-12, *Bacillus* sp. PK-13, and *Bacillus* sp. PK-14	Pyrene (50 μg/mL) In the presence of glucose	At 30 °C In 35 days In 4 days	Utilized pyrene as co-metabolite, with glucose With 0.5% (w/v) glucose: PK12-64% PK13-55% PK14-53% With 1% (w/v) glucose: PK12-46% Pk13-19% PK14-37%	Khanna et al. (2012)
Nocardia sp. SoB and *Gordonia* sp. SoCp	*n*-Alkanes C12, C19, C20, C24, C30 (1000 μg/mL each in soil)	28 days, 20 °C 30 rpm	75%	De Pasquale et al. (2012)
Acinetobacter sp., *Pseudomonas* sp., and *Gordonia* sp.	*n*-Tetracosane (*n*-C24)	30 °C, 150 rpm with reciprocal shaker	*Acinetobacter* sp. and *Pseudomonas* sp. degraded >90% *n*-tetracosane in 120 h *Gordonia* sp. degraded nearly 50% in 60 h	Matsui et al. (2014)
Exiguobacterium sp. ASW-1, *Pseudomonas aeruginosa* ASW-2, *Alcaligenes* sp. ASW-3,	Crude oil (1%, w/v)	Consortium immobilized on calcium alginate-activated carbon	Degradation 75.1%	Chen et al. (2017)

(continued)

Table 11.1 (continued)

Micro-organism	Hydrocarbon (initial concentration)	Growth conditions	Rate of degradation	References
Alcaligenes sp. ASS-1, *Bacillus* sp. ASS-2		embedding 150 rpm 25 °C for 7 days		
Geobacillus thermoparaffinivorans IR2, *Geobacillus stearothermophillus* IR4, and *Bacillus licheniformis* MN6 and mixed consortium	C32 and C40 *n*-alkanes (0.1%)	50 °C 120 rpm 20 days	C32 by mixed consortium = 90% C40 by pure strains = 87%	Elumalai et al. (2017)
Colwelliaceae, Alteromonadaceae, Methylococales, Alcanivorax, Bacteriovorax, and *Phaeobacter* in seafloor sediment	Aliphatic hydrocarbons (C11–C35), 17α(H), 21β(H)-hopane (C30-hopane), and PAHs	Corexit 9500A dispersant added at 1:20 dispersant: oil ratio, 4–4.5 °C without shaking	PAHs = 80% Alkanes = 40%; in 20 days Corexit enhanced the degradation of alkanes in 120 days, but did not affect PAH degradation	Bacosa et al. (2018)
Bacillus algicola (003-Phe1), *Rhodococcus soli* (102-Na5), *Isoptericola chiayiensis* (103-Na4), and *Pseudoalteromonas agarivorans* (SDRB-Py1)	Crude oil [1% (v/v)]	28 °C for 14 days at 180 rpm	>85% in 14 days	Lee et al. (2018)

Table 11.2 Fungal biodegradation of petroleum hydrocarbons

Microorganism	Hydrocarbon (initial concentration)	Growth conditions	Rate of degradation	References
Penicillium simplicissimum YK	*n*-Alkanes C30–C40 [0.10% (w/v)]	Two branched alkanes (pristane and squalene) 5% (w/v) and Plysurf A210G 0.001% (w/v) (dispersant) 14 days	26–51%	Yamada-Onodera et al. (2002)
Aspergillus niger	Crude oil (C11–C30) [1% (v/v) = 1.2 g/100 mL]	29 °C, 60 days; flasks shaken manually at regular intervals to allow adequate mixing and homogeneity of the contents	nC17/pristine and nC18/Phytane ratios decreased from the initial value of 2.510 and 7.289 to 0.132 and 0.474	Mittal and Singh (2009)
Fusarium solani and *Rhodotorula glutinis*	Pyrene (40 mg/L)	27 °C for 20 days, in a rotary shaker at 140 rpm, in darkness	*F. solani* 68% *R. glutinis* 63%	Romero et al. (2002)
Aspergillus sp. RFC-1	Crude oil, naphthalene (NAP), phenanthrene (PHE), and pyrene (PYR) (20 mg L)	30 °C and 120 rpm, 7 days	Crude oil = 60.3% NAP = 97.4% PHE = 84.9% PYR = 90.7%	Al-Hawash et al. (2018b)
A. niger, *A. fumigatus* *Fusarium* sp., *P. funiculosum*	Crude oil on agar plates [2% (w/w)]	25 °C 28 days	95% with *A. niger* 90% with community *of A. niger* and *A. fumigatus* 70% with community of *A. niger, A. fumigatus, P. funiculosum,* and *F. solani*	Flayyih Hassan and Flayyih Hasan AI-Jawhari (2014)
Aspergillus niger, A. fumigatus, Penicillium xingjiangense, Mucor racemosus, Rhodotorula sp.	Polycyclic aromatic hydrocarbons (PAHs) degradations in engine oil	Shake-flask culture (180 rpm), 10% (v/v) engine oil, and 0.1% (v/v) Tween 80, at 25 °C for 28 days	*A. niger* (79.3%); *P. xingjiangense* (73.7%); *A. fumigatus* (71.7%) and *M. racemosus* (69.1%)	Chukwura et al. (2016)
Trichoderma viridae, Varicosporium elodeae	Crude oil (1%)	15 days, room temperature	Trichoderma viridae (66.2%) Varicosporium elodeae (40%)	Olukunle and Oyegoke (2016)

(continued)

Table 11.2 (continued)

Microorganism	Hydrocarbon (initial concentration)	Growth conditions	Rate of degradation	References
Aspergillus oryzae, *A. niger*, *Penicillium* commune	Crude oil (1%)	0.1% (v/v) of Tween 80 30 °C 14 days	*A. oryzae* 99% *A. niger* 54% *P. commune* = 48%	El-Hanafy et al. (2017)
(set-up 1), *Aspergillus* sp. (set-up 2), *Rhizopus* sp. (set-up 3), *Aspergillus* sp. + *Rhizopus* sp.	Crude oil (0.5%)	28 days	Control 4.80% Set-up 1 29.10% Set-up 2 26.32% Set-up 3 48%	Wemedo et al. (2018)
Penicillium citrinum NIOSN-M126, *Aspergillus flavus* NIOSN-SK56S22	Crude oil (13.35% w/v)	28 °C on a rotary shaker at 80 rpm for 23 days	*P. citrinum* NIOSN-M126 total crude oil = 77% and the individual *n*-alkane fraction = 95.37%; *A. flavus* NIOSN-SK56S22 = 62%	Barnes et al. (2018)
Aspergillus sp. RFC-1	Hexadecane (1%)	30 °C and 130 rpm for 10 days	86.3%	Al-Hawash et al. (2018b)
Penicillium sp. RMA1 and RMA2	Crude oil 1% (v/v)	14 days of incubation at 30 °C	*Penicillium* sp. RMA1 = 57% *Penicillium* sp. RMA2 = 55%	Al-Hawash et al. (2018a)
Aspergillus terreus, A. sulphureus, Mucor globosus, Fusarium sp., *Penicillium citrinum, Bacillus* sp., *Enterobacteriaceae, Pseudomonas* sp., *Nocardia* sp., *Streptomyces* sp., *Rhodococcus* sp.	Crude oil 0.1 mL spread on the agar plates	30 °C for bacteria	*Rhodococcus* isolates were more active than fungi in *n*-alkane biodegradation In addition to medium chain *n*-alkanes, fungi utilized one or more of the aromatic hydrocarbons studied, while bacteria failed to do so Rhodochrous KUCC 8801 in 3 days—85%; in 5 days—93%	Sorkhoh et al. (1990)
Aspergillus terreus, Fusarium solani, *Pleurotus ostreatus, Trametes villosus, Coriolopsis rigida*	Soil contaminated with 10% crude oil		26–35% in 90 days Higher reduction for *A. terreus* was observed	Colombo et al. (1996)

11.5.1.1 Bioavailability

A key factor that determines the efficiency of utilization of oil pollutants by microbes is the bioavailability. Mainly due to the hydrophobic nature of the hydrocarbons, their aqueous solubility is low, thus limiting their bioavailability. In general, as their molecular weight increases, the bioavailability of hydrocarbons decreases. As the enzymes that metabolize hydrocarbons are present within the cells and are rarely secreted, the molecules need to be taken up and transported to the cell interior. Thus, microbes that have a capacity for biodegradation of petroleum hydrocarbons have invariably developed a mechanism to obtain access to these oils and for their uptake into the cell interior.

Essentially three pathways of uptake have been identified, whereby bacteria are observed to gain access to petroleum hydrocarbons (Hua and Wang 2014): (1) aqueous solubilization of hydrocarbons, (2) pseudo-solubilization through secretion of biosurfactants, and (3) direct contact with large oil droplets.

Water-soluble aromatics and short-chain hydrocarbons which are more soluble in the aqueous phase in comparison to the longer length molecules are the most accessible to microbes and more easily taken up than the less soluble ones.

11.5.2 Biosurfactant Production by Microbes

Many microbes that have the capability of degrading hydrocarbons however have been shown to secrete biosurfactants. Biosurfactants are able to reduce surface tension and increase solubility through emulsification and in essence, pseudo-solubilization, thereby increasing the chance of direct contact between the bacteria and oil droplets.

A variety of microbes have been found to secrete biosurfactants; *Pseudomonas aeruginosa* is among the best-known biosurfactant producing, hydrocarbon-degrading Gram-negative bacteria (Das and Chandran 2011) that produces rhamnolipids (a glycolipid surfactant) (Abdel-Mawgoud et al. 2009). *P. aeruginosa* DS10-129, an indigenous strain isolated from diesel oil and gasoline contaminated sites, has been reported to produce rhamnolipid biosurfactants (Varjani and Upasani 2017). Other species of *Pseudomonas*, namely *P. putida and P. chlororaphis* have also been reported to produce glycolipid type biosurfactants (Das and Chandran 2011).

Bacillus sp. has been reported to produce “surfactins” (Whang et al. 2008), with *B. subtilis* being considered to be the most prominent in surfactin production. Additionally, *B. amyloliquifaciens, B. licheniformis, B. pumilus*, and *B. mojavensis* have also been reported to produce surfactins (Marti et al. 2014; Li et al. 2016; Uttlová et al. 2016). *Acetinetobacter venetianus* RAG has been reported to produce a lipopolysaccharide biosurfactant (Fondi et al. 2016). *Mycobacterium* sp. and *Rhodococcus erythropolis* are known to produce trehalose lipids (White et al.

2013; Kügler et al. 2015). The yeast *Candida* sp. has been reported to produce another group of promising biosurfactants which are sophorolipids (Elshafie et al. 2015).

As apparent from the above, biosurfactants produced by microbes are chemically variable compounds; however, all these emulsifying agents are naturally amphipathic molecules, having both a hydrophobic moiety and a hydrophilic moiety. The hydrophobic component may comprise fatty acid chains of 10–18 carbons or proteins or peptides with hydrophobic side chains. The hydrophilic components are often esters, hydroxyl, phosphate, carboxylate, or carbohydrate groups. These have the ability to emulsify the petroleum oil, producing micro-droplets.

The rhamnolipids produced by *Pseudomonas* sp. are composed of rhamnose sugars attached to one or two beta hydroxy fatty acids (Abdel-Mawgoud et al. 2009). Surfactin is an anionic, cyclic lipopeptide-type biosurfactant, comprising a heptapeptide chain (LLDLLDL), linked to a hydroxyl fatty acid (Peypoux et al. 1999). A *Rodococcus* sp. of marine origin has been reported to produce an extracellular trehalolipid biosurfactant in the presence of a hydrophobic substrate (White et al. 2013).

A recently reported thermophillic strain of *Aeribacillus pallidus* (strain SL-1) was shown to produce a bio-emulsifier which was composed of a mixture of polysaccharides and proteins, with the latter providing the major emulsifying function. Being thermophillic, this strain has applications in bioremediation at temperatures around its optimal temperature of 60 °C (Tao et al. 2019).

It has also been suggested that bacteria may show adaptation to the low bioavailability of hydrophobic carbon sources by changing the hydrophobicity of their cell surface. *Mycobacterium* sp. LB50IT was shown to grow to confluency as a biofilm on solid anthracene, a poorly water-soluble carbon source, when provided as the sole carbon source. However, a similar biofilm/confluent growth was not observed when glucose was provided as an additional carbon source. The anthracene-grown cells were found to be more hydrophobic and more negatively charged compared to glucose grown cells. The authors concluded that biofilm formation and attachment may be an adaptation to optimize substrate bioavailability (Wick et al. 2002).

11.5.3 Transmembrane Transport

After adsorption of the hydrocarbon to the cell surface, its uptake into the cell may be by passive or active methods. Both simple and facilitated diffusion as well as energy utilizing active transport mechanisms have been reported (Hua and Wang 2014).

In Gram-negative bacteria, several outer membrane (OM) proteins have been shown to transport petroleum hydrocarbons into the cell interior. The *E. coli* FadL (fatty acid degradation protein L) (Van Den Berg 2005) and OmpW (Outer membrane protein W) (Hong et al. 2006), as well as the FadL subfamily proteins TodX (Toluene dioxygenation X) from *Pseudomonas putida* and TbuX (Toluene m-monooxygenation X) protein from *Ralstonia bickettli* (Hearn et al. 2008) have been shown to facilitate the diffusion of small hydrocarbons.

Virtuallly all outermembrane proteins involved in such transport are beta barrel proteins having an even number (between 8 and 24) of beta strands and have been classified as porins (Hua and Wang 2014).

Microorganisms have been shown to store the transported hydrocarbon in intracellular inclusion bodies (Mishra and Singh 2012).

11.6 Metabolic Pathways and Molecular Basis of Hydrocarbon Degradation

Several pathways for degradation and utilization of petroleum carbon energy by microbes have been identified, both aerobic and anaerobic. For bacteria, many of the metabolic pathways have been elucidated and commonly involves oxidation, reduction, hydroxylation, and dehydrogenation (Varjani 2017).

Aerobic biodegradation represents the more commonly utilized method for degradation of hydrocarbons by microorganisms and has been widely investigated. The microbes overcome the low reactivity of *n*-alkanes by an initial oxidation reaction using molecular oxygen. Three possible peripheral pathways have been identified; terminal oxidation, which is probably the most commonly used, subterminal oxidation, and ω-oxidation. Oxidation of the *n*-alkane via monooxygenases converts the alkane into its respective fatty alcohol. This is then further oxidized to the corresponding aldehyde using alcohol dehydrogenase and aldehyde dehydrogenase, and then to a fatty acid. The fatty acids are then conjugated to Coenzyme A and then enter the beta oxidation pathway, finally forming acetyl CoA, which is then used for intermediary metabolism by the organism (Wentzel et al. 2007).

Degradation of aromatic pathways require different metabolic pathways. The saturated aromatic ring is cleaved through hydroxylation. As their molecular weight increases, along with loss of aqueous solubility, they become increasingly re-calcitrant. These, when degraded to smaller units, are completely oxidized via the TCA cycle.

Linear alkanes are degraded via several enzyme types, among which the alkane hydroxylases play a prominent role. Several classes of alkane hydroxylases have been found in microorganisms (Wang et al. 2011).

1. Soluble non-heme di-iron monooxygenase (degradation of C1–C5 *n*-alkanes)
2. Membrane-bound particulate copper-containing enzyme (degradation of C1–C5 *n*-alkanes)
3. Membrane-bound *n*-alkane hydroxylases (AlkB) (degradation of C6–C16 *n*-alkanes)
4. Membrane-bound cytochrome P450 enzymes (e.g.: Cyp52, Cyp153) (degradation of C6–C16 *n*-alkanes)

Pathways for degradation of longer chain alkanes are less clear, although a few genes, namely *ladA* and *almA* have been identified. The *ladA* encodes a monooxygenase that is responsible for terminal oxidation of alkanes >C16, first identified in *Geobacillus thermodentrificans* (Feng et al. 2007). The *almA* encodes a flavin-binding soluble monooxygenase that is responsible for degradation of C32 and longer alkanes (Li et al. 2008; Wentzel et al. 2007).

It has been reasonably well established that the alkane hydroxylase encoded by *alkB* gene is a key player in hydrocarbon degradation pathways. AlkB is a rubredoxin-dependent enzyme, and often both genes are found close together when present. Both the *alkB* gene and the alcohol dehydrogenase have been reported to be induced during hexadecane degradation in several bacterial species; *Rodococcus* sp. NJ2 (Mishra and Singh 2012) and *Geobacillu*s sp. (Tourova et al. 2018).

Analysis of the genomes of several *Geobacillus* strains using degenerate PCR primers (Tourova et al. 2018) has shown the presence of multiple *alkB* genes that encode the alkane-1 mono-oxygenase. The *alkB* genes in *Geobacillus* appear to be located on a plasmid and are thought to have been transferred to *Geobacilli* from *Rhodococci* or other related microbe (Tourova et al. 2018).

Another recently reported *Pseudomonas aeruginosa* strain (DN1) was found to contain multiple alkane biodegradation systems, namely two homologs of *alkB* ($alkB_1$ and $alkB_2$), a *cyp153* homolog and two homologs of *alm*-like gene ($almA_1$ and $almA_2$). The strain demonstrated efficient (>85%) degradation of crude oil containing alkanes ranging from C8 to C40. Contrary to current knowledge that the *alkB* system is adapted for degradation of alkanes up to C16, in this strain the *alkB* genes were found to be upregulated in the presence of longer alkanes, C20 and C32 (Li et al. 2019).

The *Dietzia* sp. DQ12-45-1b has both *alkB* (coding for alkane monooxygenase) and *cyp153* genes (coding for P450 alkane hydroxylase of the cytochrome Cyp153 family), and their induction was detected. It was capable of utilizing a wide range of *n*-alkanes (C6–C40), aromatic compounds, and crude oil as the sole carbon source for growth (Wang et al. 2011).

Peroxygenase secreted by *Agrocybe aegerita* has been shown to catalyze with high efficiency, the hydroxylation of linear alkanes at the 2-position and 3-position using H_2O_2 as a co-substrate, as well as the regioselective monooxygenation of branched and cyclic alkanes. However, the peroxygenase appeared to lack activity on long-chain alkanes (>C16) and highly branched alkanes (e.g., tetramethylpentane) (Peter et al. 2011).

Fungi (and some bacteria also) have been reported to use the cytochrome P450 family genes for initiating the degradation of petroleum hydrocarbons. Cytochrome P450 protein isolated from microsomal membrane fractions of *Candida maltosa* has been shown to be involved in the hydroxylation of hexadecane. Analysis of intermediates of *n*-hexadecane oxidation led to the conclusion that mono-terminal attack was predominant, whereas di-terminal oxidation proceeded as a minor reaction (Blasig et al. 1988).

Table 11.3 Genes identified for the degradation of alkanes

Genes	Organism	References
alkB geo-1 to *alkB* geo-8 (8 homologs) alkane-1 mono-oxygenase	*Geobacillus* sp.	Tourova et al. (2018)
alkB-geo1, alkB-geo 4, alkB-geo 6	*Geobacillus stearothermophilus* MH-1	Liu et al. (2009)
Rubredoxin (2 homologs)	*Geobacillus stearothermophilus* MH-1	Liu et al. (2009)
Cytochrome p450 family	*P. aeruginosa*	Wang et al. (2019b)
cyp52-E3, cyp52-M1, cyp52-N1	*Starmerella bombicola* (yeast)	Huang et al. (2014)
cyp52	Trichoderma harzianum (filamentous fungi)	Del Carratore et al. (2011)
Cytochrome C	*P. aeruginosa*	Wang et al. (2019b)
ladA	*Geobacillus thermodentrificans NG80-2*	Feng et al. (2007)
ladA	*Geobacillus toebii 1024* *Geobacillus* sp. *1017*	Tourova et al. (2016)
$almA_1$, $almA_2$	*P. aeruginosa DN1*	Li et al. (2019)

Table 11.4 Genes identified for the degradation of aromatics

Genes	Type of aromatic compound degraded	Organism	References
Tbu gene cluster (*tbuA1, tbuU, tbuB, tbuV, tbuC*)	BTEX, meta cleavage Toluene-3-monooxygenase	*P. pickettii* PKO1	Byrne et al. (1995)
Tmo	BTEX degradation	*P. pickettii* PKO1	Byrne et al. (1995)
Nar gene clusters (*nar*Aa, *nar*Ab naphthalene dioxygenase)	Naphthalene degradation	*Rhodococcus opacus* R7	Di Gennaro et al. (2010)
Phn	PAH degradation	*Burkholderia* sp.	Tittabutr et al. (2011)

The halotolerant yeast, *Debaryomyces hansenii* contains two distinct *cyp450* family alkane hydroxylase genes, which showed 60% amino acid homology to the *cyp52A3* gene of *C. maltosa* (Yadav and Loper 1999) (Table 11.3).

Several genes responsible for degradation of the aromatic fractions of crude oil have also been reported and are listed in Table 11.4. The genes for degradation of toluene and xylene in *P. putida* have been demonstrated to be present in the TOL plasmid (Worsey and Williams 1975), and a gene with similarity to *E. coli fadL* (pWW0 *Xy*lN) was also found to be present in the TOL plasmid, which was involved in xylene uptake (Kasai et al. 2001).

Analysis of global proteomic changes during degradation of petroleum hydrocarbons in *Pseudomonas aeruginosa* P6 cultured in 500 mg/L or 20,000 mg/L crude oil as the carbon and energy source revealed 63 differentially expressed proteins that were associated with cellular pathways related to petroleum biodegradation. This study provides strong support for the concept that microorganisms use different sets of genes for the utilization of the petroleum hydrocarbons depending on its concentration in its environment (Wang et al. 2019b).

The downregulation of several chemotaxis related proteins at high concentrations of crude oil may indicate that, at these concentrations, chemotaxis may be inhibited in *P. aeruginosa*, although at lower concentrations, it uses chemotaxis to locate the hydrocarbon molecules. The concentration of hemolysin (UniProt ID: W1MWQ1), a bio-emulsifier produced by *P. aeruginosa* was also found to increase >3-fold at the higher concentration of crude oil (Wang et al. 2019b).

11.7 Strategies for Bioremediation

Bioremediation is a process whereby biological degradation processes are utilized to eliminate, attenuate, or transform organic contaminant and pollutants to mainly carbon dioxide, water, and biomass, in order to mitigate risks (Ite and Ibok 2019). Microbial bioremediation represents the most "eco-sensible" strategy for the removal of petroleum hydrocarbon contamination, being the most economical mechanism as well as the method which causes the least damage to the ecosystem. It is therefore considered an environmentally sustainable "green" approach for tackling oil pollution.

Bioremediation strategies may be carried out *in situ* (decontamination process is effected at the site of contamination) or *ex situ* (contaminated material is removed from the original position to a treatment plant, on site or at another location).

11.7.1 Use of Microbial Consortia

As individual bacterial species or strains often do not have the required genetic/metabolic diversity to degrade the entire spectrum of components in crude oil, the general strategy is to use microbial consortia comprising several different species, or mixed consortia of bacteria and fungi, to achieve complete degradation. It has been proposed that microbial consortia used in bioremediation efforts should be tailored to suit the particular condition of the contaminated site as well as the polluting hydrocarbon classes. Such a strategy may also require the introduction of different microbial consortia at different stages of the remediation process to ensure complete removal of hydrocarbon contaminants (Truskewycz et al. 2019).

Knowledge of the microbes' capacities for biodegradation and the interaction between the organisms is important for developing optimally functioning bioremediation systems. In microbial communities, individual species may interact with each other in a synergistic relationship that produces a cocktail of bioactive compounds, which may include oxidative and hydrolytic enzymes that have been implicated in processing of various hydrocarbon fractions. Perera et al. (2019) reported a naturally occurring, biofilm-forming *Bacillus-Aspergillus* community which demonstrated synergistic behavior when grown on hexadecane or crude oil (Perera et al. pers. comm.), as the sole source of carbon, where the degradation percentage by the biofilm was higher within the test period than achieved by the sum of the degradation by individual organisms.

Conversely, some other combinations of microbes may interact antagonistically; e.g., *Burkholderia, Paraburkholderia,* and *Thauera* were found to have negative correlations in activated sludge during petroleum hydrocarbon degradation, while *Burkholderia, Paraburkholderia* and *Luteibactor*, as well as *Flavobacerium* and *Aquabacterium* were found to have positive mutual correlations (Cui et al. 2019).

Careful selection of microbial species is therefore warranted in developing a system for biodegradation.

11.7.2 Immobilization of Microbes

Immobilization of microbes is an important technology in bioremediation strategies as it helps to maintain a high biomass. In open mobile systems such as oceans, the hydrocarbanoclastic microorganisms may be lost from the site due to dispersion and the free flow of water. Immobilization techniques are used to retain the microbes at the site of contamination and have many added advantages such as providing a suitable protective microenvironment for the survival of microorganisms as well as allowing cell reuse, thus reducing costs. They have also been shown to provide resistance to toxic chemicals, pH, temperature, etc. and provide genetic stability of the microorganisms (Bayat et al. 2015).

Supportive carriers for immobilization are of two types, namely organic and inorganic. Organic carriers may be natural or synthetic. Examples of natural carriers include agar, agarose, and chitin while acrylamide, polyurethane, polyvinyl, and resins are some synthetic carriers that are used for immobilization. Inorganic carriers may be compounds like clay, activated charcoal, or ceramics (Bayat et al. 2015).

Various techniques are used to immobilize microbes onto the carriers. Recent research (Chen et al. 2017) comparing free bacterial consortia with immobilized consortia has shown that immobilization by embedded techniques improve the crude oil degradation efficiency. A recent study tested the use of cinnamon and peanut shells to embed and immobilize diesel degrading *Pseudomonas* YT strain (Fu et al. 2019). Their study indicated that cinnamon shells were more suitable for immobilization. A sodium alginate-calcium chloride (calcium alginate) biocarrier has been used and performance improved with the addition of activated carbon in the embedding (Chen et al. 2017).

Naturally formed biofilms as previously reported (Perera et al. 2019) may also prove to be an useful alternative where *ex situ* remediation is carried out in a remediation plant.

11.7.3 Biostimulation and Bioaugmentation

Biostimulation is a method to stimulate the metabolic capacity of the indigenous microbial flora of the contaminated site and thus enhance the degradation capabilities by provision of adequate aeration, nutrients, moisture, etc. Bioaugmentation refers to the improvement of the metabolic capacity of the microbial flora at the contaminated site by the introduction of active microbial communities. Either single strains or mixtures of strains may be introduced (Ite and Ibok 2019). Several studies have demonstrated that both biostimulation and bioaugmentation improve the biodegradation capability of weathered contamination sites.

A recent study compared biostimulation with nitrogen and phosphorous verses bioaugmentation with native hydrocarbanoclastic microbes in contaminated soil. The study revealed that improved biodegradation rates were obtained with biostimulation, after 12 weeks test period. Bioaugmentation resulted in changes to microbial composition, with the inoculated microbes quickly becoming predominant with consequent reduction in microbial diversity (Wu et al. 2019). These results indicate that reduction in biodegradation rates in a site that contained native petroleum hydrocarbon (PH) biodegraders may be associated with nutrient insufficiency. Further, that stable maintenance of a diverse microbial composition may be more beneficial, and achievable through adequate maintenance of nutrients.

11.7.4 Use of Dispersants/Surfactants

Use of surfactants during bioremediation of oil spills, especially in marine environment, is a common strategy. Frequently, chemical dispersants such as Tween 80 are used (Tian et al. 2016). These chemicals emulsify the oil and convert them into smaller droplets which are more easily utilized by microbes. However, these chemicals deteriorate the water quality, may perturb the microbial composition in the affected area, and can be fatally toxic to the aquatic fauna (Tian et al. 2016). Additionally, reduction of the droplet size of the oil has been reported to result in increased uptake of PAH by fish (Ramachandran et al. 2004), thus affecting the aquatic life and or entering the food chain.

Recent studies, based on metagenome analysis of microbial clusters experimented in marine microcosms, have shown that the use of biosurfactants alongside microbial biodegradation may prove to be more suitable over chemical surfactants due to their biodegradability, low toxicity, and efficiency imparted in microbial remediation of petroleum hydrocarbons. Their studies indicate that expression of genes related to hydrocarbon degradation was stimulated by the biosurfactant surfactin, while these genes were in fact decreased by the chemical surfactant Ultrasperese II (Rattes de Almeida Couto et al. 2019). The difficulties in the production of biosurfactants in bulk quantities required for the application in field situations, as opposed to chemical dispersants, are currently the limiting factors that prevent their application in the field (Patel et al. 2019).

A recently isolated *B. Subtilis* BL27 (Wang et al. 2019b) was found to be enhanced by SDS and Tween 80 while being indifferent to the addition of biosurfactants rhamnolipid and surfactin. Further, addition of CTAB and TTAB were found to be highly toxic.

Biosurfactants produced by one species of microorganisms may damage the cell membranes of other microbial species or strains. Therefore, when using microbial consortia, this aspect needs to be considered. The use of naturally occurring communities in bioremediation efforts (Perera et al. 2019) may help to overcome this hurdle. In their studies, a natural biofilm-producing bacterial–fungal consortium (comprising *Aspergillus* sp. MM1 and *Bacillus* sp. MM1) demonstrated synergistic degradation of hexadecane (Perera et al. 2019) and crude oil (Perera et al. pers. comm). While both were biosurfactant producers, *Bacillus* sp. MM1 produced comparatively higher biosurfactant than the consortium, indicating it reduces its production in the presence of the fungus. This is in conformity with a previous report (Benoit et al. 2015) which indicated that surfactin production by *B. subtilis* was reduced when co-cultivated with *Aspergillus*. This could be due to the active adaptation of the *Bacillus* to the fungus. Surfactin is not only a powerful biosurfactant, but an antifungal agent (Sarwar et al. 2018).

These reports highlight the need for good understanding of the requirements of the organism or community, prior to their use in a bioremediation system.

11.8 External Factors Affecting Biodegradation

In addition to the presence of suitable microbes, several other factors also affect the biodegradation of petroleum hydrocarbons. The concentration of crude oil in the polluted site greatly affects the biodegradation capacity of the microbes, with increasing concentrations above 2% reported to decrease biodegradation efficiency (Chen et al. 2017). Similarly, environmental factors such as pH and temperature directly affect bacterial survival and growth, consequently affecting biodegradation. The availability of oxygen is also vital in aerobic biodegradation. A knowledge of the factors that influence bioremediation is thus valuable in developing cost-effective bioremediation strategies (Varjani 2017).

11.8.1 Temperature

Cui et al. (2019) in their study observed that while biodegradation rates for PHs increased with increasing temperatures up to ~30 °C, there was no significant difference between 30 and 40 °C in their studies using activated sludge in an airlift loop bioreactor. These results are similar to our own findings, using an *Aspergillus*–

Bacillus consortium to degrade crude oil in aerobic static cultures under laboratory conditions (Perera et al. pers. Comm).

Temperature influences the chemical and physical structures of the PH components; higher temperatures will increase solubility of the hydrocarbons and decrease viscosity, thus increasing bioavailability of the PH to the microbes. Temperature will also affect the growth rate of the microbes as well as the rate of enzyme activity (Bell and Gutierrez 2019). Extremophiles have been reported to degrade hydrocarbons at temperatures of 60 °C (Loginova et al. 1981), while psychrophilic bacteria may degrade PH at temperatures as low as 5–13 °C (Ribicic et al. 2018).

11.8.2 Nutrients

The availability of nutrients plays a crucial role in microbial biodegradation of hydrocarbons, with the growth of the organisms often being affected by low levels of nitrogen and phosphorous. The formation of oil slicks on the water surfaces has been observed to lead to depletion of nutrients on the surface.

11.8.3 pH

The pH of the environment will affect the enzyme activity and cell membrane transport in microorganisms, consequently affecting the rate of biodegradation. Neutral or alkaline pH has been shown to be suitable for PH degradation (Cui et al. 2019).

11.8.4 Oxygen

Molecular oxygen is the optimal electron acceptor for aerobic biodegradation of petroleum hydrocarbons and has been identified as the rate-limiting variable in PH degradation (Varjani and Upasani 2017). It is also frequently the substrate in the initial reaction of PH degradation, in reactions that are catalyzed by monooxygenases. Increasing air flow up to 2.0 L/h was observed to be beneficial for PH degradation in a bioreactor, but above that there was little change in efficiency (Cui et al. 2019).

11.8.5 Salinity

Some oleophilic microorganisms may have certain salinity requirements. *P aeruginosa* NCIM 5541 isolated from a petroleum oil well and demonstrated to efficiently utilize both glycerol and crude oil (Varjani and Upasani 2017) has been shown to grow in PH medium only when supplemented with 5% (w/v) NaCl. This halotolerant nature of this strain will find use in bioremediation of marine oil spills. In general, it has been reported that hydrocarbon degradation increases with increasing salinity (Varjani 2017); however, extreme salinity is expected to be inhibitory to microbes.

11.9 Conclusion

Petroleum or crude oil, as it is naturally found, is derived from buried fossils which have undergone natural weathering processes over millions of years. Today, crude oil is used to produce a multitude of products including diesel and petrol. Its extensive use world over has led to an increase in accidental spillage during storage and transport. Contamination of pristine environments by crude oil or its derivatives has detrimental effects on the ecological balance due to the toxicity and recalcitrant nature of these chemicals. Both aquatic and terrestrial habitats can be affected through accidental exposures, affecting organisms from microbes to larger animals, either causing direct mortality or affecting the food chain through bioaccumulation.

Thus, methods to tackle such accidental spills are continuously being developed and improved. While physical and chemical methods have been used as remedial measures, bioremediation through the use of microbes is being increasingly seen as the most ecologically and economically viable solution. As such, identification of newer and more efficient organisms with the ability to degrade petroleum hydrocarbons and the development of microbial bioremediation techniques are currently extensively researched.

Due to the hydrophobic nature of petroleum hydrocarbons, bioavailability of the carbon source to the microbes is often a limiting factor. The use of surfactants to increase bioavailability is thus a commonly observed practice. Biosurfactant secretion by certain microbes has been found to increase efficiency and proved to be more suitable than the use of chemical dispersants in improving bioavailability of the hydrocarbons. As such, exploration for biosurfactant-secreting organisms is an important area of research. Hydrocarbonoclastic microbes tend to increase at sites of contamination and thus serve as the ideal source for isolating new organisms.

It has been observed that bioremediation rates at contamination sites often reduce with time as a result of limiting inorganic nutrients such as nitrogen and phosphate and/or reduction in microbial population. Biostimulation (introduction of suitable microbes) and bioaugmentation (provision of required nutrients) are strategies that are frequently utilized in bioremediation efforts, to maintain an appropriate and adequate microbe population.

Recent investigations have increasingly focused on the use of microbial consortia as bioremediation agents. The provision of a broader genetic repertoire through the use of consortia has led to the development of more efficient bioremediation systems, by way of both the chemical spectrum remedied and the efficiency of removal. Immobilization of microbes has been found to be both efficient and cost-effective, with recent reports of bacterial–fungal biofilm-based systems proving to be highly efficient. Microbes that naturally coexist have a potential to be adapted for co-habitation and may thus demonstrate a synergistic relationship in utilization of petroleum hydrocarbons. Thus, this is an area of investigation that needs to be further explored in the future.

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Chapter 12
Potential of Extremophiles for Bioremediation

Suresh Kaushik, Aishah Alatawi, Setyowati Retno Djiwanti, Amit Pande, Efstathia Skotti, and Vijay Soni

Contents

S. Kaushik (✉)
Division of Soil Science and Agricultural Chemistry, Indian Agricultural Research Institute,
New Delhi, India

A. Alatawi
Biology Department, College of Science, Faculty of Science, University of Tabuk, Tabuk,
Kingdom of Saudi Arabia

S. R. Djiwanti
Plant Protection Division, Indonesian Spice and Medicinal Crop Research Institute (ISMECRI),
Bogor, West Java, Indonesia

A. Pande
Department of Molecular Biology, Jaypee Hospital, Noida, India

E. Skotti
Department of Food Science and Technology, Ionian University, Argostoli Kefalonia, Greece

V. Soni
Division of Infectious Disease, Weill Department of Medicine, Weill Cornell Medical College,
New York, NY, USA

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Abstract Extremophiles are microorganisms that flourish in habitats of extreme environments, including in high concentration of salts, pollutants, high or low temperature, an acidic or alkaline pH. All extreme environments are dominated by microorganisms belonging to Archaea, the third domain of life, evolutionary distinct from Bacteria and Eucarya. Over the past few years, the molecular biology of extremophilic Archaea has stimulated a lot of interest in the field of bioremediation. Bioremediation is the use of microorganisms for the degradation or removal of contaminants. Contamination of soils, sediments and water due to anthropogenic activities is a matter of concern at global level. Bioremediation has emerged as an effective solution for these problems. Most bioremediation research has focused on the processes performed by the domain Bacteria. Recently, extremophiles are the focus of growing interest for bioremediation because they can tolerate very harsh environmental conditions due to their ability to produce an array of molecules or extremozymes capable of functioning in the environment without denaturing. These extremozymes from extremophilic microorganisms have special characteristics such as stability to elevated temperature, extremes of pH, organic solvents and high ion strength. Due to the stability and persistence of these extremophilic microorganisms under adverse environmental conditions, they can be explored finding new species for using in the bioremediation of environments contaminated with extremely recalcitrant pollutants. Here, we provide an overview of the archaeal extremophilic microorganisms such as thermopiles, acidophiles, halophiles which have potential applications in the field of bioremediation of environmental pollutants, including hydrocarbons, heavy metals, pesticides, petroleum and wastewater treatments.

Keywords Extremophiles · Bioremediation · Extremozymes · Pollutants · Archaea

12.1 Introduction

Microorganisms are the most ubiquitous living entities on our planet and are also the diverse organisms present almost everywhere on the Earth. It is estimated that about 1% of the total microorganisms have been isolated and identified so far and still there are unexplored niches where these microorganisms may be present. A variety of microbes inhabit extreme environments. The extreme environments include high salt concentration, pH, pressure and temperature and low temperature, pH, nutrients concentration and water availability. In addition, high levels of radiations, harmful heavy metals and toxic compounds including organic solvents are the extreme environments. Extremophilic microorganisms are a largely unexplored group that have the abilities to thrive in extreme conditions.

In year 1965, Thomas Brock, a microbiologist, discovered in the thermal vents of Yellowstone National Park a new form of bacteria, *Thermus aquaticus* that can survive at near-boiling temperatures (Fig. 12.1).

Fig. 12.1 Some photographs shown here depicting extremophilic conditions of near-boiling temperature at Grand Prismatic Hot Spring, Yellowstone, National Park, USA. (Source: https://www.national-park.com/welcome-to-yellowstone-national-park/)

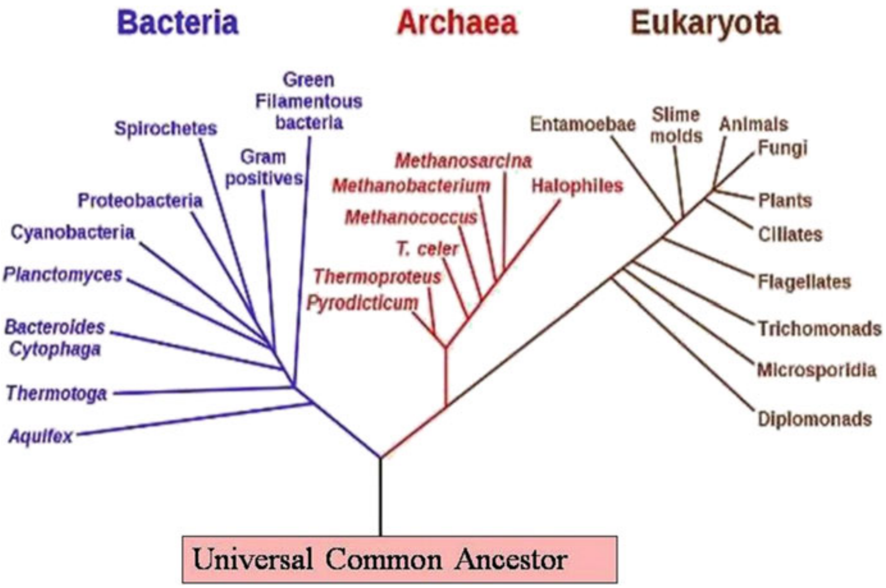

Fig. 12.2 A tree of three domains of life—archaea, bacteria and eukarya, depicting that the most of the extremophiles belong to the domain archaea. (Source: http://www.scienceforthepublic.org/they-didnt-believe-it/archaea-the-third-domain)

The upper temperature for life was thought to be 73 °C at that time. Subsequently, he isolated and collected many microbes from Octopus Spring, a particular geothermal area having large amounts of pink filamentous bacteria at a temperature of 82–88 °C. Taq polymerase, an enzyme used in PCR, was isolated first from *Thermus aquaticus* strain YT-1 and later on, his group showed that *Thermus aquaticus* was widespread in hot-water environments (Brock 1977). Such microorganisms which thrive under extreme conditions are known as extremophiles (from Latin "extremus" meaning "extreme" and Greek "philia" meaning "love"). MacElroy (1974) first coined the term "extremophile" to designate any organism able to support environmental conditions usually fatal to most eukaryotic cells. Most of the extremophilic microorganisms belong to the archaeal species. The word "archaea" means "ancient things" (from Greek), and it refers to a group of prokaryotic single-celled microorganisms characterized for the extreme conditions they need to be alive. The archaea group was classified as a separate group of prokaryotes by Woese and Fox (1977). Most known extremophiles are microorganisms belong to the domain of archaea (Fig. 12.2), bacteria and eukarya (Rothschild and Mancinelli 2001).

Initially, archaea were characterized as a group of single-celled prokaryotic microorganisms living in extremophilic environments with low or high pH (acidophiles, alkophiles), high temperatures (thermophiles), high salinity (halophiles) or anoxia (Najera-Fernandez et al. 2012). Hence, thermophiles, acidophiles, methanogens, halophiles and alkalophilic microorganisms are included in the group of extremophiles. Over the past few decades, studies on these microorganisms have focused on the physiology, enzymology, ecology, taxonomy, molecular biology and genetics. These microorganisms have made adaptations in their genetic and metabolic machinery to flourish in the harsh conditions These microorganisms are good candidates for research in different fields of science including bioremediation and biotechnology due to their ability to grow under a wide range of extreme conditions (Xu and Zhou 2016; Najera-Fernandez et al. 2012; Arora et al. 2014).

Studies on extremophiles have progressed to the extent that there are dedicated scientific journal such as 'Archae' and 'Extremophiles' as well as regular international 'extremophile' symposia and conferences are organized. There are a number of variables that can lead to environments being considered extreme such as temperature, pH, salinity, heavy metals or radiations. Some extremophiles have adapted to a number of factors such as the alkaline pH and high salinity (Tindall et al. 1984), pressure and temperature, i.e. near deep-sea hydrothermal vents (Pettit 2011). Extremophiles have been isolated in diverse zones that possess extreme conditions, and the products obtainable from these extremophiles such as enzymes, proteins and compatible solutes are of great interest to biotechnology, industry and environmental issues.

A worldwide environmental problem has occurred over the past few decades because of the rapid increase in urbanization and industrialization. Environmental pollution is a very big problem today due to hazardous waste leading to scarcity of clean water and disturbance of soil causing decrease in crop production and human health. Bioremediation have many advantages to remediate polluted sites from economic, environmental and practical aspects. The main remediation processes that can be mediated by the action of microorganisms include adsorption and biodegradation of organic contaminants and the immobilization, mobilization and/or transformation of contaminants especially heavy metals. In these processes, microorganisms are stimulated to rapidly degrade hazardous organic pollutants to environmentally safe levels in water and soil. Hence, bioremediation is considered one of the safer, cleaner, cost-effective and eco-friendly technology for decontaminating sites which are contaminated with wide range of pollutants. The most recent research on extremophiles surviving in a wide range of extreme hostile environments has demonstrated the beneficial for bioremediation processes. The remarkable adaptation capabilities of extremophiles convert them into an attractive source of biocatalyst or extremozymes for bioremediation. This review is focussed on the extremophilic microorganisms and their potential applications in bioremediation.

12.2 Extremophilic Microorganisms and Their Diversity

An extremophile is an organism which thrives in or requires "extreme" conditions, i.e. adapted to survive in diverse ecological niches. These conditions can refer to geochemical and physical extremes such as salinity, pH, pressure, temperature, radiation, presence of toxic compounds and water availability. Thus, extremophiles consist of microorganisms that are capable of surviving and thriving in harsh environments and conditions that are detrimental to the majority of life on earth. Extremophiles have been isolated in diverse zones that possess extreme conditions. Life in extreme environments have been studied intensively focussing on molecular mechanisms involved as well as the diversity of organisms. Moderate environments are important to sustain life which means environments with temperature between 20 and 40 °C, air pressure about one atmosphere, pH near neutral and adequate levels of available nutrients water and salts. The presently known upper limit is 50 °C for multicellular eukaryotes, 62 °C for single-celled eukaryotes, 95 °C for bacteria and 121 °C for archaea. Many extreme environments such as saline and/or alkaline lake/ponds, acidic or hot springs, deserts and the ocean beds are found in nature on the earth which are too harsh for normal life to exist. These extremophilic organisms not only tolerate specific extreme conditions but also require these for growth and survival. Many species can survive but are unable to reproduce or grow indefinitely under such conditions. Extreme environments include high pH, temperature, pressure, salt concentration, low temperature, pH, nutrients concentration, water availability, harmful heavy metals, toxic compounds and high levels of radiation.

Extremophiles are categorized according to conditions in which they grow. There are many terms used to describe extremophiles as shown in Table 12.1. Thermophiles/hyperthermophiles grow in habitats with high or very temperatures such as volcanic sites, hydrothermal vents, hot springs; psychrophiles thrive in cold habitats such as on the mountains at high altitude, polar region; barophiles which love high pressure conditions which are mainly found deep inside the oceans and sea; halophiles love very high salt concentrations such as in saline alkaline lakes, sea; alkalophiles thrive at highly alkaline pH such as sodic lakes; acidophiles grow at habitats with pH less than 5, such conditions are found in acid mine drainage sites and acidic lakes; metallophiles can tolerate and grow in the presence of high concentration of heavy metals; xerophiles can grow in conditions with very low water availability which include deserts; anoxiphiles are the organisms having colonized ecosystems deprived of oxygen. Multiple stresses are present in the niche simultaneously and extremophiles which are able to thrive in such habitats are known as polyextremophiles (e.g., thermoacidophiles, haloalkaliphiles). Polyextremophiles organisms are adapted to live in habitats where various physicochemical parameters reach extreme values. For example, many hot springs are acid or alkaline with rich metal content at the same time. Similarly, the deep ocean is generally cold, very low nutrient content (oligotrophic) and exposed to high pressure. Haloalkalophilic *Halomonas campisalis* which can grow at pH up to 12 has been reported from Soap Lake, USA. Recently, the most acidophilic microorganisms such as *Picrophilus oshimae* and *Picrophilus torridus*, which can grow at pH as low as 0.06, have been discovered from hot spring in Noboribetsu, Japan. The most halophilic microbe *Halarsenatibacter silvermanii* has been discovered from a salt lake in USA, which can survive in salt concentration of about 35%. Microorganisms such as *Methanothermococcus thermolithotrophicus* and *Methanocaldococcus jannaschii* are examples of barophilic and thermophilic methanogens, which have been isolated from high pressure niches of deep sea beds.

Table 12.1 Categories, habitat and examples of extremophilic microorganisms

Name of category	Environmental parameter	Habitat	Examples
Hyperthermophile	Temperature	High temperature more than 80 °C	*Pyrococcus* sp. *Pyrolobus fumarii* *Geogemma barossii* *Methanopyrus kandleri strain 116*
Thermophile		Medium temperature 60–80 °C	*Thermotoga neapolitana* *Methanofollis tationis*
Psychrophile		Low temperature less than 10 °C	*Psychrobacter cryopegellain* *Pseudomonas* sp. ATH-43 *Polaromonas vacuolata* *Synechococcus lividus*
Acidophile	pH	pH 3 or below	*Acidithiobacillus ferrooxidans* *Clostridium paradoxum* *Ferroplasma* sp. *Thiobacillus* sp. *Sulfolobus* sp. *Thermoplasma* sp.
Alkaliphile		pH 9 or above	*Halomonas alkaliphila* *Psychrobacter* sp. *Arthrobacter* sp. *Natronobacterium* sp.
Halophile	Salinity	2–5 M NaCl	*Halobacteriaceae* *Haloferax* sp. *Halococcus* sp. *Haloarcula* sp. *Halorubrum* sp. *Hfx. mediterranei* *Methanobrevibacter smithii* *Halarsenatibacter silvermanii* *Natrialba* sp.

(continued)

Table 12.1 (continued)

Name of category	Environmental parameter	Habitat	Examples
Piezophile/barophile	Pressure	High hydrostatic pressure (up to 130 MPa)	Pyrococcus yayanosil *Methanocaldococcus jannaschii* *Methanothermococcus* sp.
Metalophile/ metalotolerant	Metallic concentration	High concentrations of metals	*Ferroplasma* sp. *Cupriavidus metallidurans,* *Halbacterium* sp. *Ralstonia* sp. *Halococcus salifodinae Haloferax* sp.
Toxitolerant	Organic compounds	High concentrations of toxic reagents/organic solvents	*Pseudomonas putida*
Radiophile/ radioresistant	Radiations	High radiation levels	*Deinococcus radiodurans* *D. perariditoris* *Rubrobacter* sp. *Thermococcus gammatolerance*
Xerophile	Desiccation	Anhydrobiotic	*Streptomyces bulli* *Artemia salina*
Osmophile	Osmotic pressure	High osmotic pressure due to high sugar concentration	*Zygosaccharomyces rouxii*
Anaerobe Microaerophile	Oxygen level	Cannot tolerate oxygen Growth in <21% oxygen	*Methanococcus jannaschii* *Clostridium* sp.
Oligotrophic	Nutrition	Limited nutrients	*Pelagibacter ubique*
Polyextremophile	Multiple physicochemical parameters	Multiple stresses such as temperature, pH, salinity simultaneously	*Deinococcus radiodurans* *Halobacterium salinarum* NRC-1 *Halomonas campisalis* *Picrophilus oshimae* *Picrophilus torridus* *Methanothermococcus*

12.3 Extremophiles in Extreme Environments

Extremophiles include members of all three domains of life—bacteria, archaea and eukarya. Most of the extremophilic microorganisms are archaea, but this group also includes eukaryotes such as protists (algae, fungi and protozoa) and multicellular organisms. Culture-dependent and culture-independent (molecular) methods have been employed for understanding the diversity of microbes in extreme environments. Archaea is the main group to thrive in extreme environments. They are quite skilled in adapting to different extreme conditions. Most of acidophilic, halophilic and hyperthermophillic microorganisms belong to the archaea group. These organisms have evolved several structural and chemical adaptations, which allow them to survive and grow in extreme environments (Satyanarayana et al. 2005). Among bacteria, cyanobacteria is the best adapted group to various extreme conditions such as formation of microbial mats with other bacteria from Antarctic ice to continental hot springs. Among eukaryotes, fungi are the most versatile and ecological successful phylogenetic lineage. The phylogenetic diversity of extremophiles is high and very complex to study. Some extremophiles are adapted to the same extreme conditions, even though dispersed broadly in the phylogenetic tree of life. Some genera or orders contain only extremophiles, whereas other genera or orders contain both mesophiles and extremophiles.

Specific biological functions and metabolic processes of these microorganisms are mediated by proteins and enzymes known as extremozymes which are responsible for unusual properties of extremophiles. Extremophiles are capable of surviving in extreme environments due to extremozymes having unique feature because of extreme thermal stability and resistance against chemical denaturants such as detergents, chaotropic agents, organic solvents and extreme of pH (Gaur et al. 2010; Karan et al. 2011). The discovery of new extremophilic microorganisms and their extremozymes has a great impact on the field of biocatalysis and hold tremendous potential as industrial biocatalysts to work under harsh conditions.

The extreme environments are so unique that the organisms are highly specialized with specific protein adaptations such as chaperone systems or enzymes (extremozymes) capable of functioning in the environment without denaturing. These enzymes or proteins are capable of functioning under such conditions in which mesophilic proteins or enzymes may not work. Extremophiles have found use as part of bioremediation of contaminated environments due to their unique metabolic activities and tolerance to certain conditions. A number of proteins or extremozymes sourced from extremophiles have already been utilized in industry for the purpose as diverse as molecular biology reagents or as common place as laundry detergents. The removal and detoxification of contaminants and wastes can be achieved by means of extremozymes such as oxidoreductase (da Fonseca et al. 2015), laccase (Fang et al. 2012), dioxygenase (Saito et al. 2000), alkane hydroxylase (Wang et al. 2010b), haloalkane dehalogenase (Zhang et al. 2013; Nikolaivits et al. 2017). Usefulness of extremophiles in various industrial and other applications such as bioremediation is due to their wide spectrum of unique properties such as stability to elevated temperature, extremes pH, organic solvents and high ionic

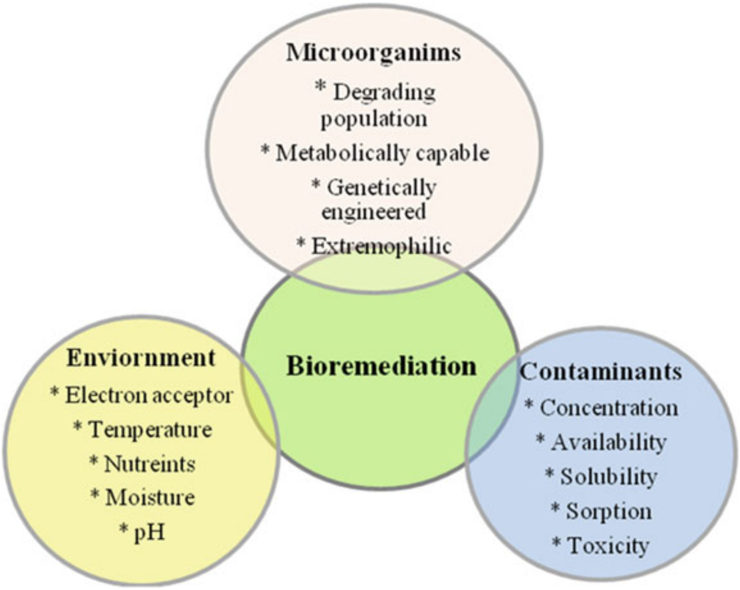

Fig. 12.3 Different components and parameters for bioremediation

strength. Extremozymes being extremophilic origin are robust, long-term storage, resistance to solvents and detergents (Merone et al. 2005) and active over a wide range of temperature. These enzymes are highly convenient for immobilization and could be used in filtration devices of bioremediation processes.

12.4 Bioremediation: A Dynamic Process to Remediate Polluted Sites

Bioremediation is a natural process and is perceived by the public as an acceptable waste treatment process for contaminated material. This method is less expensive, and less energy is required as compared to other methods. This is useful for the complete destruction of wide variety of contaminants. As the biological processes are often highly specific, site factors are important for the success of bioremediation. Bioremediation is limited to those compounds which are biodegradable. An advantage of bioremediation over other methods is that it transforms contaminants instead of simply moving them from one source to another as in the practice of land filing. Bioremediation is a dynamic process, and different components are involved in this process (Fig. 12.3).

Bioremediation is a process by which microorganisms are stimulated to rapidly degrade hazardous organic pollutants to environmentally safe levels in soil, sediments, groundwater and other substances. Microorganisms that are used to clean up contaminated sites use the contaminants as nutrients or energy sources. Stimulation of microbes is achieved by the addition of growth substances, nutrients, terminal electron acceptor or donors resulting in an increase in organic pollutant degradation and biotransformation. Bioremediation may be employed to attack specific contaminants such as chlorinated pesticides that are degraded by microbes or a more general approach such as oil spills that are broken down using multiple techniques.

The basic bioremediation methods include biostimulation, attenuation, augmentation, venting and piles. Biostimulation is focus on stimulation of indigenous or naturally existing bacteria and fungus community at the site (soil/ground water) through the injection of specific nutrients. These nutrients are the basic building blocks of life and allow microorganism to crease the basic requirements such as energy, biomass and enzymes to degrade the pollutants (Madhavi and Mohini 2012). Bioattenuation or natural attenuation is the eradication of pollutant concentrations from surrounding which is carried out using biological processes such as both aerobic and anaerobic biodegradation, chemical reactions such as ion exchange, complexation, abiotic transformation, and physical phenomena such as dispersion, advection, dilution, diffusion, volatilization and sorption/desorption. Many terms such as biotransformation or intrinsic remediation are included within the natural attenuation or bioattenuation (Mulligana and Yong 2004). Bioaugmentation involves the addition of pollutant-degrading microorganism (natural/exotic/engineered) to supplement the biodegradative capacity of indigenous microbial populations on the contaminated site. Natural species are not fast enough to break down certain compounds, so genetically modified or extremophilic microorganisms have potential for bioremediation of soil, groundwater, activate sludge to enhance degrading capabilities of a broad coverage of physical and chemical pollutants (Sayler and Ripp 2000; Thapa et al. 2012). Bioventing involves venting of oxygen via low air flow rate through soil to simulate growth of natural or introduced bacterial and fungus in the soil to existing soil microbes to sustain their microbial activity. Effective bioremediation of petroleum-contaminated soil using bioinventing has been used reported by many researchers (Lee et al. 2006; Agarry and Latinwo 2015). Biopiles (also known as bioheaps, biocells, biomounds, compost piles) is a process to reduce concentrations of aerobically remediable petroleum pollutants in excavated soils during the time of biodegradation. In this process, air is supplied to the biopile system through a system of pumps and piping to enhance microbial activity through microbial respiration (Emami et al. 2012; Kumar et al. 2016).

On the basis of removal of wastes for treatment, there are basically two types of bioremediation—in situ and ex situ bioremediation (Table 12.2). In the former type of bioremediation, application involves in the subsurface and may be applied in the unsaturated zone such as bioventing or in saturated soil and groundwater. This in situ method is a superior method for cleaning contaminated sites as it is cheaper and safer and uses harmless microbes to degrade the harmful chemicals. This in situ bioremediation can be further categorized into intrinsic and engineered in situ bioremediation. In intrinsic in situ bioremediation, the innate capabilities of naturally occurring microbial communities to degrade environmental pollutants are used for bioremediation. In engineered in situ bioremediation, the approach involves the introduction of

Table 12.2 Common bioremediation strategies for considering various factors with merits and demerits

Type	Technology	Factors to consider	Merits	Demerits
In situ	In situ bioremediation	Biodegradative abilities of indigenous microorganisms	Most cost-efficient	Environmental constraints
	Bioventing	Biodegradability and distribution of pollutants	Relatively passive	Extended treatment time
	Bioaugmentation	Chemical solubility	Natural attenuation processes	Monitoring difficulties
	Biosparging	Environmental parameters	Soil and water treatment	
		Presence of metals and other inorganics	Noninvasive	
Ex situ	Landfilling	Biodegradative abilities of indigenous microorganisms	Low cost	Space requirements
	Biopiles	Biodegradability and distribution of pollutants	Cost efficient	Need to control abiotic loss
	Composting	Chemical solubility	Optimized environmental parameters	Extended treatment time
	Aqueous bioreactor	Environmental parameters	Rapid degradation kinetic	Mass transfer problem
	Slurry bioreactors	Presence of metals and other inorganics	Enhance mass transfer	Bioavailability limitation
		Bioaugmentation	Effective use of inoculants and surfactants	Soil requires excavation
		Toxicity concentration of contaminants		High cost capital
		Toxicity of amendments		High operating cost

certain microbes to the site of contamination to accelerate the degradation process by enhancing the physicochemical conditions to encourage the growth of microorganisms. This approach generally costs less than other remediated options and results in complete transformation of organic contaminants to innocuous substance such as carbon dioxide, water. The areal zone can be larger and reach areas that would otherwise be inaccessible. There are some limitations of in situ method of bioremediation. It usually requires an acclimatized population of microorganisms. Toxic concentration of organic compound may inhibit the activity of indigenous microbes. Some contaminants cannot be biodegraded, and intermediate compounds may be more toxic and/or mobile than the parent compound. Over the last several decades, in situ degradation of biologically foreign chemical compounds such as solvents, explosive, polycyclic aromatic hydrocarbons, heavy metals and radionuclides has been used as a cost-effective alternative to incineration or burial in landfills (Alexander 1994).

The ex situ bioremediation is a biological process in which excavated soil is placed in a lined aboveground treatment area and aerated following processing to enhance the degradation of organic contaminants by indigenous microbial population. This process is further divided into slurry-phase bioremediation and solid-phase bioremediation. Slurry-phase process is a controlled treatment that involves the excavation of the contaminants soil, mixing it with water and placing it in a bioreactor to form slurry. Subsequently, soil is removed, dried up, deposited and finally treatment of the resulting fluids. Solid-phase bioremediation is a technology in which the contaminated soil is excavated and placed into piles. Bacterial growth is stimulated through a network of pipes that are distributed throughout the piles. Necessary ventilation is provided for microbial respiration through the pipes by pulling air. This system requires a large amount of space, and cleanup requires more time to complete than with slurry-phase processes. Some solid-phase treatment processes include soil biopile, composting and land farming. The ex situ method is suitable for a wide range of contaminants but is not applicable to heavy metal contaminants or chlorinated hydrocarbons.

12.5 Potential of Extremophiles for Bioremediation

The ability of extremophilic microorganisms to grow under a wide range of extreme conditions makes them good candidates for bioremediation. The biological processes have many advantages from environmental, economic and practical aspects to remediate polluted sites. The immobilization, mobilization and/or transformation of metals/metalloids and adsorption and biodegradation of organic contaminants are the main remediation processes that can be mediated by the action of several microorganisms especially extremophiles surviving in harsh environments with high concentrations of pollutants (Donati et al. 2019). The extremophilic microorganisms have proved to be useful for bioremediation applications. Different kinds of wastes and contaminants are produced from the industrial activities, the mining activities for oils extraction or the accidental oil spills. All these activities release several pollutants in the environments such as hydrocarbons, polycyclic aromatic hydrocarbons, chlorinated hydrocarbons, pesticides and heavy metals (Sivaperumal et al. 2017). Removal and detoxification of these contaminants and wastes can be achieved by means of extremozymes which have unique properties such as high thermostability and resistance to denaturing agents like detergents, organic solvents and extreme pH (Castillo et al. 2005). Hence, there is an increasing interest in the optimization of bioremediation approaches in high salt environments, high temperature and extreme pH ranges (Table 12.3). In this sense, haloarchaea have been successfully tested for biotechnological applications (Arora et al. 2014; Oren 2010; Bonete and Martinez-Espinosa 2011). Recently, Marques (2018) reviewed about the extremophiles as microfactories which are able to provide metabolic or genetic mechanisms as controlled services to cleanup of environmental pollution. A most recent research review on polyextremophilic microorganisms isolated from a wide

Table 12.3 Some examples of extremophilic microorganisms having potential in bioremediation

Contaminants	Extremophiles
Petroleum products (aliphatic and aromatic hydrocarbon compounds)	*Alcanivorax* sp. *Bacillus safensis* *Halobacterium* sp. *Haloferax mediterranei* *Halococcus* sp. *Haloarcula* sp. *Halorubrum* sp. *Methanosaeta* sp. *Methanosarcina* sp. *Nocardioides* sp. *Natrialba* sp. *Nitrosopumilus maritimus* *Paracoccus* sp. *Pseudomonas stutzeri* *Psychromonas ingrahamii* *Streptomyces albaxialis* *Sulfolobus solfataricus*
Heavy metals (e.g. As, Pb, Hg, Cd, Cr, Co)	*Sulfolobus acidocaldarius st*. BC *Sulfolobus solfataricus* *Aeropyrum pernix* st. K1 *Pyrobaculum calidifontis* *Halococcus salifodinae* BK_3 *Haloferax* sp. *Halobacterium noricense* *Halobacterium* sp. *NRC-1* *Halobacterium salinarum* CCM2090
Pesticides (atrazine, carbaryl, carbofuran, coumaphos, diazinon, glycophosphate, parathion)	*Flavobacterium* sp. *Methanosarcina* sp. *Methanococcus mazei* *Methanobacterium congolense* *Methanothrix soehngenii* *Sulfolobus solfataricus*
Waste water (organic compounds, dyes, organic solvents)	*Methanobrevibacter smithii* *Haloferax mediterranei* *Nesterenkonia lacusekhoensis*
Radionuclides (radiations)	*Desulfuromusa ferrireducens* *Rhodanobacter* sp. *Pyrobaculum* sp. *Haloferax* sp. *Sulfolobus solfataricus*

range of environments including deserts, salaras, ice fields, geothermal springs and diverse zones in Chile such as Atacama Desert, Altiplano, Central Chile, Patagonia and Antarctica has discussed the molecular and physiological capabilities of many of these isolates which has great potential for bioremediation processes (Orellana et al. 2018).

12.5.1 Bioremediation of Petroleum Products

Petroleum is composed of hundreds or thousands of aliphatic, branched and aromatic hydrocarbons (Prince 1993) and other organic compounds including organometallic constituents (Butler and Mason 1997). As the petroleum is an important energy source in daily life and industry, its annual consumption has been increasing in the last several decades. Many activities such as municipal and industrial runoff, effluent release, offshore and onshore petroleum industry activities as well as accidental spills cause petroleum hydrocarbon pollution which are toxic to animals, vegetation and humans. These hydrocarbon pollutants which cause adverse impact on human health and environment are classified as priority environmental pollutants by the US Environmental Protection Agency (1986). These hydrocarbon pollutants through spillages and leakage from underground tanks, steamers, abandoned oil refinery sites or unplugging of oil wells cause contamination of surface soil, groundwater and ocean (Souza et al. 2014; Prince et al. 2013).

Hydrocarbon pollutants comprising petroleum and its derivatives (refined products), which are released into the environment by oil spills and polycyclic aromatic hydrocarbons (PAHs) are found in a wide range of habitats and affect the health of many organisms (Giovanlla et al. 2020). Various hydrocarbons have different susceptibilities to microbial attack. Degradation is more difficult in compound with complex chemical structures, e.g. polycyclic hydrocarbons (Fathepure 2014). PAHs and halogenated hydrocarbons can be remediated with microorganisms (Prasad 2016). PAHs are a class of chemical compounds of two or more benzene rings fused in a linear, angular or cluster arrangement. They may be classified as high molecular weight (HMW) or low molecular weight (LMW). Petroleum derivatives such as PAHs having a great affinity for macromolecules such as DNA, RNA and proteins can induce mutations, leading to develop tumours in the skin and other organs (Varjani et al. 2017). As PAHs are known for their toxicity and carcinogenicity, they are recognized globally as priority pollutants (Prasad 2016).

The application of bioremediation for petroleum products is becoming the technique of choice for environmental researchers. Biodegradation of petroleum hydrocarbons varies with the chemical structure and molecular weight of hydrocarbon molecules. The chemical structure of organic pollutants has significant influence on the extent and rate of their biodegradation (Alexander 1981). Presently, a majority of commercial applications of bioremediation depend upon indigenous microorganisms, and most are employed for hydrocarbon-contaminated sites. Bioremediation of extreme environments requires extremophiles that are adapted to these habitats.

Hence, extremophilic microorganisms can play an important role in the bioremediation of these habitats (Khemili-Talbi et al. 2015). Extremophiles have been utilized for the microbial degradation of crude oil and refined petroleum pollutants. The polluting agents can be biodegraded by marine microbes producing extremozymes which are able to catalyse the oxidation of medium-length alkanes. Several microorganisms have been isolated from marine environments as producers of alkane degrading enzymes. Park and Park (2018) described the bioremediation of organic pollutants involving the strategies for alkane degradation under extreme conditions such as low and high temperature, high salt and acidic and anaerobic conditions. Alkane degraders seem to possess exclusive metabolic pathway and survival strategies. Hydrocarbons can be mineralized or transformed through the biodegradation process that occurs in various extreme habitats (Park and Park 2018). Extremophilic microorganisms from Archaea domain from extreme environments have been found as potential resources for the bioremediation of hydrocarbons (Giovanlla et al. 2020). Most bacteria that are capable of degrading petroleum hydrocarbons have been isolated from deep ocean environments. The bacterial species *Bacillus safensis* (CFA-06) isolated from petroleum in Campos Basin in Brazil produces two oxidoreductases, namely a catalase and a new oxidoreductase. Theses enzymes have promising application for petroleum removal because of actively involving in degradation of aromatic hydrocarbons (da Fonseca et al. 2015).

A recent review has focussed on the bioremediation of aromatic compounds such as toluene and xylenes involving the degradation of such pollutants (Blazquez et al. 2018). The degradation of aromatic compounds is another key issue in bioremediation of oil contaminated sites. *Nocardioides* species strain KP7 has been isolated from a Kuwait beach, which produces a dioxygenase enzyme that is able to degrade phenanthrene (Saito et al. 2000). Numerous marine species have been identified as producers of enzymes catalysing the degradation of halogenated compounds. For example, the marine bacteria *Paracoccus* sp. DEH99 (Zhang et al. 2014) and *Pseudomonas stutzeri* DEH130 (Zhang et al. 2013) have been isolated which produce exosomes-haloacid dehalogenases that are able to catalyse the de-halogenation of 2-alanoic acids. The bacterium *Psychromonas ingrahamii* isolated from the sea ice interface, produces a haloacid dehalogenase which degrades chlorinated and brominated short chain (less than C3) haloacids (Nikolaivits et al. 2017; Novak et al. 2013). Yakimov et al. (1999) isolated the *Alcanivorax* group from the North Sea as biosurfactant-producing and alkane-degrading marine bacteria. These bacterial strains were isolated from a variety of marine environments such as oil spill contaminated sites. Genus *Alcanivorax* has been found to play a major role in the first step of crude oil biodegradation in the marine environment and observed that these bacteria are important for the biodegradation of petroleum especially under bioremediation conditions (Harayama et al. 1999). Al-Maghrabi et al. (1999) reported rapid degradation of crude oil using thermophilic bacteria and was found to survive in saline environments. Oil spills have been successfully bioremediated in marine, Arctic and Antarctic environments (Delille et al. 1998; Margesin and Schinner 1999). Kuznetsov et al. (1992) found a halo- and thermotolerant *Streptomyces albaxialis* which degraded crude oil and petroleum products even in the presence of 30% sodium chloride. An extremely halophilic Archaea *Haloferax mediterranei* was isolated and found to grow at 10–25% sodium chloride (Zvyagintseva et al. 1995). Kulichevskaya et al. (1992) isolated some species the bacteria from *Halobacterium* group from salt-rich stratum fluids of an oil deposit which degraded *n*-alkanes with a C10–C30 composition in the presence of 30% (w/v) sodium chloride. The bacterium *Alcanivorax dieselolei* strain B-5, isolated from surface water of the Bohai Sea, produces different alkane hydroxylase extremozymes which degrade either chlorinated or brominated alkanes with different chain lengths, thus displaying potential for biodegradation and other industrial applications (Li and Shao 2014). Others haloarchaeas from the genus *Haloferax* are able to degrade a mixture of PAHs (anthracene, naphthalene, phenanthrene, pyrene) in hypersaline medium (Bonfa et al. 2011).

The archaea *Natrialba* sp. C21 isolated from oil-contaminated saline water in Ain Salah (Algeria) was able to survive under high salt concentrations (25%) solution containing aromatic hydrocarbons (Khemili-Talbi et al. 2015). This strain demonstrated good potential for degrading pyrene (3% v/v) and naphthalene (3% v/v) after 7 days at 40 °C, pH 7.0 and high salinity conditions. Zhao et al. (2017) reported an strain of the haloarchaea 1M1011 isolated from Changlu Tanggu saltern near Da Gang Oil field in Tianjin (China) by enrichment culture in hypersaline medium containing hexadecane was able to degrade 57% of hexadecane (5 g L^{-1}) in the presence of 3.6 M NaCl within 24 days at 37 °C. An extremophilic microorganism *Stenotrophomonas maltophilia* strain AJH1 isolated from a mineral mining site in Saudi Arabia was able to degrade both HMW (pyrene) and LMW (anthracene, naphthalene) in acidophilic mineral salt medium at pH 2 (Arulazhagan et al. 2017). Three haloalkaliphilic *Pseudomonas* strains (HA10, HA12 and HA14) were studied by Hassan and Aly (2018) and reported to degrade BTEX (benzene, toluene, ethylbenzene and xylene) at pH 9 in the presence of NaCl (7% w/v). Three novel catechol 2,3-dioxynease genes, namely C23010, C23012 and C23014 were amplified, cloned and overexpressed from these strains.

In recent past few years, many studies applying extremophilic microorganisms in hydrocarbon degradation were undertaken but toxicity evaluation during this process are not considered. Hence, toxicity assays should be included to evaluate the efficiency of the process in eliminating or reducing toxicity (Giovanlla et al. 2020).

12.5.2 Bioremediation of Chemical Pesticides

Chemical pesticides are any substance or mixture of substances intended for preventing, destroying, repelling or mitigating any insects, weeds and plant pathogens. Pesticides are widely used worldwide to control agricultural and household pests. The most commonly used pesticides belong to the organophosphorus group, and the first organophosphorus insecticide, tetraethyl pyrophosphate, was developed in 1937 (Dragun et al. 1984). Organophosphorus pesticides have been widely developed for agricultural purposes since the 1950s, and these pesticides are highly toxic chemicals (Gupta 2009). The acute toxicity of organophosphorus chemical compounds is due to their capacity to inhibit acetylcholine esterase, a key enzyme involved in the overall regulation of the central and peripheral nervous system. As the organochlorine pesticides such as lindane, dichloro-diphenyl-trichloroethane (DDT) possess longer persistency, tendency towards bioaccumulation, high mammalian toxicity, and potential toxicity towards non-target organism, the use of these has been diminished drastically in developed countries and has been replaced by the less persistent and more effective and efficient other similar organophosphorus compounds such as chlorpyrifos, glyphosate, methyl parathion, parathion, diazinon, coumaphos, fenamiphos, monocrotophos and phorate. The phosphorus is generally present as a phosphonate or a phosphate ester which are normally involved in oxidation, hydrolysis, dealkylation and alkylation. Therefore, the most important step in detoxification by microbial degradation involves through hydrolysis of P-O-aryl and P-O-alkyl bonds. Singh and Walker (2005) have presented a list of microorganisms capable of degrading organophosphorus compounds.

Although pesticides play a key role in the protection of crop yields, their excessive and persistence use resulted in serious soil pollution and deteriorated soil quality. Excessive and continuous use of these compounds has led to the contamination of several ecosystems in different parts of the world (Cisar and Snyder 2000; Tse et al. 2004). Residues of pesticides have been reported in soil, water, milk, food, or fish in numerous countries around the world. As these compounds possess high toxicity and constitutes major health and environments issue (Jaipieam et al. 2009), it is essential to remove them from the environment. Numerous approaches including physical, chemical and biological methods have been considered for developing decontamination strategies against these chemicals, but these methods are not considered for large-scale environmental remediation and also involve harsh conditions (Jacquet et al. 2016). Hence, bioremediation, the treatment that uses living organisms to transform hazardous substances into lesser or non-toxic compounds, is an effective way to clean up the soil polluted with chemical pesticides. The first microbe, *Flavobacterium* sp. that could degrade organophosphorus compounds was isolated and identified in 1973, and subsequently, several bacterial and a few fungal species have been isolated which can degrade a wide range of these compounds in soil systems and liquid cultures. The degradation process of these compounds takes place through the enzymes organophosphate hydrolase or phosphotriesterase catalyse encoded by gene *opd* (organophosphate degrading) which has been isolated, sequenced, cloned in different organisms and altered for better activity and stability (McDaniel et al. 1988; Horne et al. 2002).

In recent years, enzymes from extremophiles have emerged as promising alternative to smoothly and quickly decontaminate these chemical compounds. The phosphotriesterase-like lactonase *Sco*Pox from the archaea *Sulfolobus solfataricus* is an attractive candidate for bioremediation. This enzyme has been engineered and proven to be highly efficient for degrading a number of organophosphorus pesticides (Elias et al. 2008; Hiblot et al. 2012, 2013; Del Giudice et al. 2016). Two degradation products, a phosphodiester and an alcohol, are produced by the hydrolysis process through the phosphotriesterase activity of this enzyme on the organophosphorus pesticides. This *Sco*Pox enzyme being its extremophilic origin is robust, i.e. resistance to detergents and solvents (Merone et al. 2005) and activity over a wide range of temperature and long-term storage (Remy et al. 2016). The enzyme could be used in filtration devices to treat effluent materials with organophosphorus compounds as it can be immobilized easily. Poirier et al. (2017) reported a variant *Sco*Pox-αD6 by engineering *Sco*Pox with enhanced phosphotriesterase activity.

12.5.3 Bioremediation of Heavy Metals

Technological advancement and industrialization have put a mounting burden on the environment by releasing large quantities of perilous waste, heavy metals (e.g., chromium, cadmium, lead) and metalloids (e.g., arsenic and antimony). The build-up of heavy metals and metalloids in soil and waters continues to cause serious health concerns worldwide, as these metals and metalloids cannot be degraded into non-toxic forms, but persist in the ecosystem (Ayangbenro and Babalola 2017). Some metals such as iron, zinc, manganese, copper, cobalt and molybdenum are trace elements necessary for life and required at a certain level. They are functioning as co-factors for some enzymes, regulators of osmotic pressure, micronutrients and stabilization of molecules. They are toxic when generated in excess and depend on the availability and absorbed dose (Rasmussen et al. 2000).

Heavy metals such as arsenic, lead, mercury, aluminium and cadmium are toxic to organisms. The presence of heavy metals in the environment has been a major concern because of their toxicity. The toxicity of heavy metals is related to exposure dose and the metallic chemical species, responsible for bioavailability and mobility in the organism and in the environment. The most soluble and bioavailable metallic species present the highest toxicity, risks to human health and impacts on ecosystems (de Paiva et al. 2015; Ospina-Alvarez et al. 2014). Exposure to heavy metals has been linked with teratogenicity, mutagenicity, cancer, neurological, circulatory, endocrine and immune system disorders (Kim et al. 2015; Korashy et al. 2017). Heavy metal toxicity is demonstrated in their ability to disrupt enzyme structures and functions by binding with thiol and protein groups, or by replacing co-factors in prosthetic groups of enzymes. Exposure to mercury (Hg) and lead (Pb) can cause the development of autoimmunity, leading to joint disease such as rheumatoid arthritis, nervous and circulatory disorders and kidney diseases (Ayangbenro and Babalola 2017). Cadmium (Cd) is known to be mutagenic and carcinogenic. Chromium (Cr) causes nausea, diarrhoea, headaches, hair loss and vomiting in humans. Heavy metals such as Cd, Pb, Hg and Al can exert their toxicity by interacting metabolically with nutritionally essential elements such as Ca and Fe, interfering with vital physiologically functions (Goyer 1997). Arsenic, mercury, lead and chromium may cause oxidative stress due to the production of reactive oxygen species (ROS) (Pinto et al. 2003).

Their elimination from waste water before being released into the environment is important for the maintenance of the ecosystem and from an economic point of view. There are many techniques such as sludge filtration, adsorption processes, chemical oxidation or reduction reactions, chemical precipitation, ion exchange, electrochemical treatment and reverse osmosis which are used to remediate contaminated environments with heavy metals (Siddiquee et al. 2015). However, these techniques are costly, particularly when the metal concentrations are extremely low. As most of the heavy metal salts have high solubility in solution, the separation by chemical and physical techniques is also challenging. Hence, there is a need to evaluate alternative techniques applicable, and it should be appropriate and suitable for the local conditions.

In this perspective, some microorganisms have developed resistance mechanism to adapt to these pollutants and could be promising for bioremediation processes (Giovanlla et al. 2017). Bioremediation is an innovative technique for the removal and recovery of heavy metals ions from contaminated sites. This method involves using living organisms such as bacteria, fungi and algae to reduce and/or recover heavy metal pollutants into less hazardous form. This technique has been used for the removal of heavy metals from polluted soil and wastewater. These microorganisms help to detoxify hazardous components in the environment by the process which occur naturally or can be improved through the addition of nutrients and electron acceptors. Metals whose different valence transformations states vary in toxicity can be detoxify through the valence transformation mechanism. For instance, methyl mercury is converted to less toxic Hg(II) by the enzyme organomercurial lyase produced by mercury-resistant bacteria (Wang et al. 2010a). Similarly, Cr(VI) is reduced to Cr(III) having less mobility and toxicity by microorganisms used in bioremediation. Heavy metals can also be detoxified by other mechanisms such as volatilization, vacuole compartmentalization and metal binding. Metal binding involves chelators such as phytochelatin (e.g. glutathione derived peptides), metal binding peptides and metallothein which bind to heavy metals and facilitate microbial absorption and transportation of metal ions. Volatilization mechanism takes place only in metals which have volatile states such as Hg and Se and involve turning metal ions into a volatile state. The MerA enzyme is utilized by mercury-resistant bacteria to reduce Hg(II) to the volatile form Hg(0) and Se(V) can be reduced to elemental Se(0) to remediate polluted soil and waters (Wu et al. 2010). Thus, bioabsorption, bioaccumulation, biotransformation and biomineralization are some techniques used by microorganisms for their survival in metal-polluted environment. These mechanisms have been exploited for bioremediation technology (Gadd 2000; Lin and Lin 2005).

Various factors influencing the microbial remediation of heavy metals include the concentration of pollutants, bioavailability of metals to the microbe, electron acceptors, pH, oxygen, redox potential, soil structure, temperature, moisture content, nutrient, osmotic pressure and water capacity. Hence, the choice of microorganisms may be native to the contaminated environments or isolated from another environment and brought to the polluted site (Sharma et al. 2000). One such approach is to search for new enzymes from extremophilic microorganisms. Extremophiles are organisms that are able to thrive at extreme environmental conditions (salinity, pH, temperature, pressure, dryness, radiations or concentrations of heavy metals). Most of the extremophilic microorganisms belong to the Achaea domain, and their enzymes known as extremozymes have unique structure-function properties such as stability at high temperature, extreme pH, high ionic strength, in the presence of organic solvents and heavy metals (Cabrera and Blamey 2018; Koga and Moril 2007; Cavicchioli 2011).

Bacteria and archaea that live in extreme conditions have been reported as great microbial resources of heavy metal bioremediation. Sequencing of the genome of extremophilic microorganisms such as *Metallosphaera sedula* (Aurenik et al. 2008), *Leptospirillum ferriphilum* (Mi et al. 2011) and *Sulfolobus solfataricus* (Schelert et al. 2013) has identified clusters containing the Hg-resistance gene merA. Takeuchi et al. (2001) reported the isolate *Acidithiobacillus ferrooxidans* SUG 2-2 to volatilize mercury from acidic soils polluted by this metal. Figueroa et al. (2018) have reviewed about the extremophiles focussing on heavy metal and radionuclide pollution. Some halophilic archaea have developed tolerance to heavy metals. Halophilic microorganisms are often able to absorb heavy metals (Zhuang et al. 2010). Wang et al. (2012) reported that the *Halobacterium* sp. NRC-1 showed high resistance to arsenic due to the presence of genes for arsenite and antimonite extrusion system on plasmid. Kaur et al. (2006) studied the haloarchaeal strategies of adaptation to high metal concentration of iron, zinc, manganese, copper, cobalt, nickel using *Halobacterium* sp. NRC-1 as a model organism. Srivastava et al. (2013) have reported the intracellular synthesis of silver nanoparticles by the haloarchaeal isolated *Halococcus salifodinae* BK_3 when the cells were grown in the medium containing silver nitrate. Similarly, selenium nanoparticles are synthesized when these cells are grown in the presence of sodium selenite. Cadmium tolerance has been reported in haloarchaeal strains from salterns of Ribandar and Siridao in India (Chaudhary et al. 2014). Biosorption of metals by the organism at the surface or by the exopolysaccharides (EPS) secreted to form the biofilms enables organism to tolerate metals (Srivastava and Kowshik 2013). Kawakami et al. (2007) found that *Halobacterium salinarum* CCM 2090 has a Ca(II)-dependent aggregation system. Calcium ion is adsorbed on the surface of the cells and induces ionic cross-bridging between the EPS, resulting in aggregation of the haloarchaeal cells. Cations such as Zn^{2+}, Cu^{2+}, Fe^{2+}, Mn^{2+}, Co^{2+} and Ni^{2+} could replace Ca^{2+}, enabling organisms to tolerate these metals. Popescu and Dumitru (2009) reported the two *Haloferax* stains having the capacity to reduce the concentration of Zn, Ni, Cr and Pb ions by biosorption process from the media with high salinity. *Halobacterium* sp. GUSF was reported to be able to absorb Mn at high concentration and high rates (Naik and Furtado 2014). *Halobacterium noricense* was found to adsorb Cd (Showalter et al. 2016) while *Haloferax* st. BBK2 was found to accumulate Cd intracellularly (Das et al. 2014). *Methanobacterium bryantii* was found to produce extracellular proteins to chelate Cu (Kim et al. 1995).

Hence, the extremophiles belonging to the haloarchaea group can be used in the treatment of hypersaline heavy metals polluted sites and wastewaters for heavy metals removals. However, developing technologies for exploring for microbial environments and understanding the mechanisms driving microbial activity and metal metabolic pathways under wide range of extreme climatic conditions need to be further elucidated before successful and better-controlled site-specific treatments can be undertaken.

12.5.4 Bioremediation of Radionuclides

The extensive use of radioactive materials at research laboratories, industrial sites and biomedical institutions has produced a great accumulation of radioactive waste. Fredrickson et al. (2004) reported that about 90 million gallons of high-level radioactive waste are accumulated across the USA during the World War II. The occasional disastrous accidents at nuclear facilities such as Chernobyl disaster of 1986 and the Fukushima Daiichi nuclear disaster in 2011 have also caused damage to the human health and environment issues by generating a large quantity of radioactive materials or radionuclides in the environment. Most radioactive wastes are generated by nuclear power plants contributing about 95% of the radioactively generated from all sources (Ahier and Tracy 1995; Tamponnet and Declerck 2008). The commonly encountered radionuclides include cobalt-60 (^{60}Co), Plutonium-239 (^{239}Pu), Radium-226 (^{226}Ra), Radon-222 (^{222}Rn), Technetium-99 (^{99}Tc), Thorium-226 (^{226}Th) and Uranium-238 (^{238}U). Other radionuclides created through nuclear reactors by means of the splitting of elemental atoms are Thallium-201 (^{201}Tl), Iridium-238 (^{238}Ir), Caesium-137 (^{137}Cs) and Strontium-90 (^{90}Sr) having longer time to decay (Kumraz et al. 2007).

Radionuclides in the environment are a major human and environmental health concern. Even a small concentration of radionuclides in the environment can have an impact for a prolonged period of time due to their long half-life. The impact of these pollutants is growing with time. Exposure to radionuclides or radiation causes acute health effects that begin with vomiting, nausea, headaches, and with increased exposure, fatigue, weakness, fever, dizziness, diarrhoea, fever, blood in stool and low blood pressure and finally death. Mohner et al. (2006) reported that long-term exposure to radionuclides leads to high risk of leukaemia, kidney damage an genetic damage, resulting in lethal problems, even passing to the next generation.

Excavation and shipping to a distant waste disposal location is the most common means of eradicating soil contaminated with radionuclides. Due to high costs of physiochemical approaches, bioremediation has been viewed as the ecological responsible alternative environmentally destructive physical remediation. Microorganisms carry endogenous genetic, biochemical and physiological properties that make them ideal agents for pollutants remediation in soil and groundwater. Attempts have been made to develop native or genetically engineered or extremophilic microbes for the remediation of environmental containments including radionuclides. Extremophiles have been used to remediate radionuclides. Microorganisms such as *Rhodanobacter* sp. and *Desulfuromusa ferrireducens* were observed to be able to interact with these contaminants which initiate solubility of transformed radionuclides by addition or removal of electrons, leading to increase the mobility of the contaminants and thus allowing it to be easily flushed from the environments (Amachi et al. 2010; Green et al. 2012). This microbial-mediated biotransformation presents opportunities for bioremediation of radionuclides in the environments, either to immobilize them in place or to accelerate their removal.

Bioremediation of environmental niches (soil, sediments and water contaminated with radionuclides) can be achieved by changing in the oxidation state through biologically encoded biomolecules. Similarly, alternation in solubility, transport properties and toxicity of radionuclides can take place by changing in speciation, e.g. detoxification of mercury by methylation (Wang et al. 2012). Enzymatic reduction through oxidation-reduction, changes in pH, biodegradation of radionuclides, biosorption by mass or biomass can bring about changes in solubility of radionuclides (Holker et al. 2002; Law et al. 2010; Hegazy and Emam 2011). Microbial activity is mostly influenced by acceptors and electron donors, nutrients and other environmental factors during the biotransformation of radionuclides.

As the reduced species are greatly insoluble and occur as precipitate, the oxidized forms of radionuclides being soluble in aqueous medium are mobile in ground water. Enzymatic reduction of soluble U(VI) by a c-type cytochrome protein in the periplasm to insoluble species on the surface of the microorganism *Shewanella putrefaciens* is reported by Wildung et al. (2000). A homologous cytochrome (PpcA), a trihaem periplasmic cytochrome c7 of the Fe(III)-reducing bacterium *Geobacter sulfurreducens* that may also play a role in U(VI) reduction in vitro was reported by Lloyd et al. (2003). ^{99}Tc is long-lived radionuclide with half-life 2.13×10^5 years and occurs in nuclear wastes. Tc(VII) is very difficult to remove from solution using conventional chemical methods due to poor ligand-complexing capabilities. The studies on the microorganisms which can reduce Tc(VII) and precipitate the radionuclide into low-valency oxide Tc(IV) was demonstrated by Pignolet et al. (1989). Lloyd and Macaskie (1996) observed the direct microbial enzymatic reduction of Tc(VII) using *Shewanella putrefaciens* and *Geobacter metallireducens*. The use of immobilized cells of sulphate-reducing bacteria such as *Desulfovibrio fructosovorans*, which are capable of treating low concentration of nitrate ions commonly occurring in nuclear waste, was demonstrated on the development of a process to decontaminate water with Tc(VII) species (Lloyd et al. 1999). Tc and U are normally the highest-priority radionuclide contaminants in most radioactive wastes, but other actinides including Th, Np, Pu and Am are also present at the polluted sited (Lloyd and Macaskie 2000; Tamponnet and Declerck 2008). These pollutants can be enzymatically reduced by iron-reducing bacteria such as *Rhodoferax ferrireducens* and *Geobacter* sp. (Kim et al. 2012). The enzymatic reduction of radionuclides can be triggered through indirect reduction of soluble pollutants in soil or sedimentary environments by sulphate or iron-reducing microorganisms. For instance, Fe(III) can be bioreduced into Fe(II) and sulphur S(IV) into S(II) in the form of hydrogen sulphide. *Microbacterium flavescens* grown in the presence of nuclides such as U, Th, Am and Pu produced compounds such as siderophores, organic acids and extracellular metabolites which are capable of dissolving and mobilizing radionuclides with the cells (John et al. 2001).

Biosorption involves the sequestration of positively charges metal ions to the negatively charged cell membranes and polysaccharides secreted on the outer surfaces of bacteria through capsule and slime formation (Praksh et al. 2013). Several microorganisms such as *Citrobacter freundii* and *Firmicutes* have been reported radionuclide biosorbents (Haferburg et al. 2007; Xie et al. 2008). Biosorption alone may not be sufficient to remove radionuclides unless the ground biomass content is enhanced. Biostimulation using specific communities of microorganism can also enhance the bioremediation of radionuclides. Nitrate serves as an energetically favourable electron acceptor for metal-reducing bacterial in nitric acid co-contaminated sediments (DiChristina 1992). Finneran et al. (2002) reported that the lack of microbial reduction in U(VI) due to presence of nitrate as a co-contaminant in sediment. Wu et al. (2006) reported that this issue can be resolved by the ex situ treatment and removal of nitrate and heavy metals before in situ biostimulation to reduce the U(VI). A number of microorganisms such as *Desulfovibrio* sp., *Geobacter* sp. and *Shewanella* sp. have been shown to carry out reductive precipitation of radionuclides. Some microorganisms such as *Citrobacter* sp. can interact with metals ions and immobilize for transformation or generate biofilms to bind metallic ions, hence serving as a platform for the precipitation of insoluble minerals (Keasling et al. 2000). Fredrickson et al. (2000) have shown that the microorganism *Deinococcus radiodurans* can detoxify Cr(VI), Tc(VII) and U (VI) from soil. Brim et al. (2003) reported that the microorganisms such as *Deinococcus geothermalis*, *Deinococcus murrayl* have high resistance against chronic irradiation (50 Gy h^{-1}) and are able to grow at higher temperature (55 °C). Lloyd et al. (2003) has shown that microbial family *Geobacteraceae* has potential for radioactive metal reduction.

Thus, the study of the molecular mechanisms behind the extremophilic microbial transformation of radionuclides and exploiting them in bioremediation would help in tracking the responsible microbial metabolic products towards cell-free bioremediation and further assist in efficient removal of radionuclides from the contaminant environments.

12.5.5 Bioremediation of Wastewater Treatment

The main substances found in wastewater are organic and inorganic compounds, dyes and salts. The primary objective of a wastewater treatment plants is to reduce the concentrations of pollutants to the level at which the discharge of the effluent will not adversely affect the environment or pose a health threat. The leftover sludge at wastewater treatment plants is treated through anaerobic digestion which is one of the most promising and favourable technology. Breakdown of sewage effluent are normally carried out by microorganisms which are able to live in the sludge of treatment plants. They obtain nutrients by degrading the solids in wastewater to various compounds. Biological treatments of wastewater involve not only carbon removal, but also elimination of other nutrients such as nitrogen and phosphorus.

Sequential and combined actions are required for such treatment successively by several groups of microorganisms such as phosphate-accumulating organism and heterotrophic bacteria or microbes which are able to perform nitrification, denitrification or anammox (Gieseke et al. 2001). The extremophiles which can degrade ammonia are now one of the main candidates for wastewater treatment in addition to other natural various types of microorganisms. Other contaminants such as sulphur, manganese, iron and runoff pollutants (hydrocarbons, fertilizers) can also be removed. As the industrial effluents have high salt environments along with other organic compounds and heavy metals, polyextremophilic microorganisms having a higher resistance to metals, complex dyes along with high salt concentration can be used for industrial and other similar wastewater treatment. Such polyextremophilic microbes can be identified and isolated from industrial effluent or waste sites. Wastewater and industrial effluent is a complex mixture of dyes, metals along with other organic compounds and high salt substances. Some industrial effluent may be highly acidic or highly basic.

Bioremediation using living microorganisms particularly halophiles can offer an efficient and cheap option for decontamination of wastewater. In recent years, haloarchaea have been assessed successfully for bioremediation and biotechnological applications (Arora et al. 2012; Oren 2010; Bonete and Martínez-Espinosa 2011) because of extraordinary properties of their enzymes like high thermostability and resistance to denaturing agents such as detergents, extreme pH and organic solvents (Castillo et al. 2005). Activity and maintenance of the stable conformation of the enzymes at high salt concentrations are due to the presence of acidic amino acids in these proteins (Oren 2008). Most of the species from *Haloferacease* and *Halobacteriaceae* families can grow under anaerobic conditions in diverse conditions of salt concentrations (Torregrosa-Crespo et al. 2016; Valentine 2007). Consequently, these microorganisms might be applied for bioremediation in saline and hypersaline wastewater treatments because of their high tolerance to salt, metals and organic pollutants (Bonete et al. 2015; Najera-Fernandez et al. 2012; Torregrosa-Crespo et al. 2016).

Recently, more efforts have been devoted to effectively utilizing high-strength organic wastes by using extremophilic microorganisms. The utilization of high-strength wastes involves major issues, including sludge foaming, the inhibition of key microorganisms of anaerobic digestion such as methanogens, and slower hydrolysis of complex compounds such as long-chain fatty acids and lignin. Hence, extremophilic microorganisms able to deal with these compounds have become of great interest in designing new strategies to treat wastewater. Recent researches suggest that growth and activity of extremophiles were significant in the treatment of activated sludge and wastewater (DeLong 1998; Casamayor et al. 2000; Schramm et al. 1999). The roles of methanogenic extremophilic archaea within a broad range of activated sludge, submerged biofilters and membrane bioreactors have been studied in recent research (Gomez-Silvan et al. 2010; Gray et al. 2002; Damgaard et al. 2001). Under oxic conditions, no methanogenesis was detected, but once oxygen is depleted, methane production ensued. The results suggest that methanogenic archaea can be activated under anoxic conditions (Gray et al. 2002).

The microbial populations in industrial wastewater (rich in ammonia, phenol and with high salinity) treatments are closely related to *Methanobrevibacter smithii,* the predominant methanogen in human intestines (Gomez-Silvan et al. 2010).

The manufacturing of chemical compounds such as pesticides, herbicides and explosive usually generate effluents containing complex mixtures of salts and nitrate or nitrite leading to development of resistant to very high nitrate and nitrite concentrations in some species of *Haloferax.* Hence, it could be useful for bioremediation applications in sewage plants where high salts, nitrate and nitrite concentrations are detected in wastewaters and brines. Halophilic archaea, *Haloferax mediterranei,* are able to carry out denitrification, thus providing excellent models to explore large-scale bioremediation processes to remove nitrogen compounds from brines and salty water. Similarly, a group of marine bacterial oxidoreductases represented by the laccases have been studied by metagenomic approach from a marine library. Bacterial laccases are the enzymes which are able to catalyse the oxidation of phenolic and non-phenolic aromatic compounds and have unusual properties such as high stability at 40° C, for pHs ranging from 5.5 to 9.0, high activity in the presence of chloride and high decolourization capability towards azo dyes (Fang et al. 2012). Hence, such extremophilic microorganisms producing extremozymes find applications in bioremediation of textile dyes in waste water treatment.

12.6 Further Research for Potential Extremophilic Microorganisms and Their Scale-Up

A primary hurdle in the study of extremophilic microorganisms particularly belonging to *Archaea* domain used in bioremediation process is methodological. Several methodologies have been described to study Archaea with a number of archaeal and universal amplification primer pairs for archaeal diversity (Bonfa et al. 2011; Khemili-Talbi et al. 2017; Siles and Margesin 2018; Salam et al. 2017; de Jesus et al. 2015). As PCR amplifications are prone to biases, they may lead to overrepresent and underrepresent various microbial community members (Pinto and Raskin 2012). In recent years, relative read depth analysis of the high throughput sequencing of a 16S rRNA gene amplification product to provide quantitative measurement of specific Archaea taxonomic groups and metagenomic sequencing of unamplified DNA (Fig. 12.4) and quantitative PCR (qPCR) methods are used for analysis of mixed cultures (Smith and Osborn 2009). Second hurdle in studying *Archaea* in bioremediation systems is again methodological. Dose–growth response analysis is generally used to measure community members that outcompete others at a given physicochemical conditions on a given niche under energy stress (Valentine 2007). In the last several years, this field has made significant advances, but it is still developing methodology to identify and isolate the suitable extremophile for using in a particular bioremediation process.

Extremophiles are not cultivable under conventional laboratory culture conditions, but may offer a wealth of valuable bioproducts, ranging from bioactive small

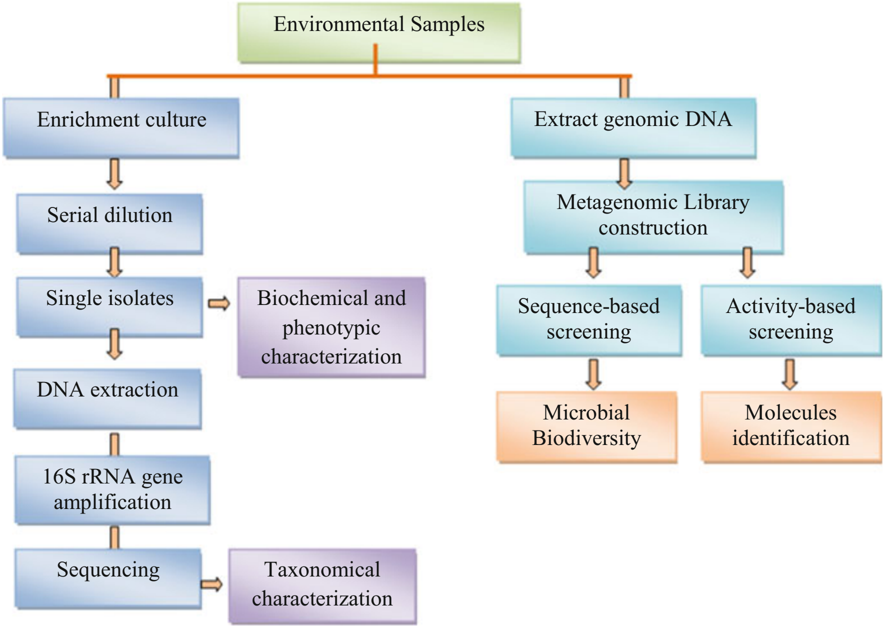

Fig. 12.4 Microbial diversity studies: culture-dependent method involving isolation of strains through serial dilution methods and their genetic, phenotypic and biochemical studies for microbial characterization and culture-independent metagenomic approach involving a library construction with identification of molecules (activity-based screening) and microbial communities (sequence-based screening)

molecules to unique biopolymers and enzymes (Tango and Islam 2002). To overcome the limitation of cultivating extremophiles on a production scale, research work is going on for developing methods and procedures by which extremophiles can be effectively cultivated for increase in the production of extremophilic biomass, enzymes and biomolecules. Culture-dependent and culture-independent molecular methods have been employed for understanding, identification and isolation of extremophilic microorganisms from diversity of microbes in extreme environments (Fig. 12.4). The rigours of culturing these organisms have led to cutting-edge independent molecular techniques such as metagenomics, metatranscriptomics and metaproteomics being employed (Hedlund et al. 2014; Santos et al. 2011). Various techniques such as use of different modes of formation, e.g. fed-batch, cell recycling or continuous cultivation (Schuraldi and Rosa 2002) and optimisation of the medium composition (Gomes and Steiner 2004; Patel et al. 2006), have been adopted to improve biomass production by different research groups. Researchers have developed a unique production-scale bioreactor capable of continuous operations at extreme temperature and pressure. Research work has been attempted to express corresponding genes from extremophiles into mesophilic host (Eichler 2001). Further developmental work in this direction needs to be done because the demand is growing at an exponential rate.

12.7 Conclusions and Future Perspective

Bioremediation provides a technique for cleaning up pollution by enhancing the same biodegradation processes that occur in nature. Bioremediation is considered as one of the best option to treat contaminated environments. Taking into account the amazing metabolic features that define extremophilic microorganisms, these microorganisms may become good candidates to improve bioremediation procedures, or even new bioremediation strategies could be defined using them. Although the potential use of extremophilic microorganisms in bioremediation has been extensively demonstrated, but use of extremophilic microorganisms in bioremediation is still hampered by an incomplete understanding of the genetics and genome-level characteristics of these microorganisms used and metabolic pathways involved and their kinetics. Hence, developing technologies for exploring for microbial microenvironments and understanding the mechanisms driving microbial activity and metabolic pathways (e.g. redistribution, detoxification, mobilization/immobilization, translocation, transformation, biosorption and bioaccumulation) under diverse climatic and extreme conditions need to be further elucidated before successful and better-controlled site-specific treatments can occur. Therefore, more studies from molecular biology and biochemical points of view are required to properly comprehend extremophiles metabolism regulation. Hence, new niches and extreme microecosystems in terms of pH, salt concentration and temperature should be explored to identify and isolate extremophilic microorganisms capable to deal with the pollutants such as heavy metals, hydrocarbons and chlorinated compounds affecting soil and water and have the potential to play key functions for bioremediation. In future, it is predicted that metagenomics tools together with new sequencing technologies will provide the basis for the discovery of new extremozymes from extremophilic microorganisms for bioremediation. Using high-throughput sequencing techniques and advanced bioinformatics tools together with metaproteiomics and metabolomics analyses will allow the identification of genes and metabolites responsible for the production of biomolecules to be used in bioremediation. These multiomics technologies are also filling gaps in the knowledge of gene expression, metabolism and ecology of extremophilic microorganisms which could allow the improvements in knowledge related to their application in the field of bioremediation.

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Chapter 13
Role of Microbes in Bioremediation of Radioactive Waste

Udaya Kumar Vandana, A. B. M. Gulzar, Islamul Houque Laskar, L. Romen Meitei, and P. B. Mazumder

Contents

Abstract Intense release of radionuclides into the environment and their mobility prompted public and research concerned in recent years about the processing of radionuclides. Numerous cases of soil and groundwater are getting contaminated with various radioactive wastes. Currently available technologies are quite cost-effective and technical limitation increased the cost high. Bioremediation, where microorganisms (bacteria, algae, fungi) plays a major role in harnessing the

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U. K. Vandana (✉) · A. B. M. Gulzar · I. H. Laskar · L. R. Meitei · P. B. Mazumder (✉)
Department of Biotechnology, Assam University, Silchar, Assam, India

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biogeochemical cycles of radioactive wastes. In this chapter, we exclusively discuss the role of microbes in decontaminating process of various hazardous radioactive wastes.

Keywords Bioremediation · Radioactive wastes · Actinides · Bacteria · Fungi · Algae · Biotransformation · Biomineralization · Biosorption · Bioaccumulation

13.1 Introduction

The word pollution is one of the global concerns for today's world. Urbanization and industrialization are increasing exponentially to fulfil the demand of people for their modernization. The process of modernization not only improves the living style of human being but also causes severe environmental problems by release of different types of waste material (Fontenelle et al. 2019; Jiang et al. 2008). Radioactive waste material has become a serious environmental problem. In the twenty-first century, every country is trying to increase the power by establishing nuclear power plants, testing the nuclear weapons and reprocessing the nuclear weapons. As a result, the radioactive wastes are generated as a by-product of such power generation (Toth 2008). The release of radioactive wastes into the environment from the atomic power plants or other sources by prosperously or by accidentally contributes to the already present wastes generated (Kumar et al. 2007). The half-life of these radioactive waste ranges from hundreds to thousands years, i.e. more time is required to reduce the radioactivity of that compounds by half. Due to long half-life periods of those waste materials, the disposition of such materials become a challenge for researchers, policy makers and the power generation agencies throughout the world (Marra and Palmer 2011; Sherman 2015). Presently the most common practice to throw out the radioactive waste is direct release in geological storage site. But this process requires high maintenance and day by day the by-product radioactive waste generation is increases therefore, maintenance and storage of such volume of radioactive wastes becomes a big issue (Uzair et al. 2019). There are some physical and chemical methods developed for the remediation of radioactive wastes. Though these technologies showed little impressive results, but due to their high cost, time consuming and some environmentally destructive nature, these techniques fail to gain public acceptance (Valdovinos et al. 2017). In one report, it has been mentioned that the remediation cost of a radioactive contaminated site is minimum trillions of US dollar (Kyne and Bolin 2016). On the other hand, bioremediation refers to the use of biological agents such as microbes, plants or any other living things that help to reduce contamination to a non-toxic level or untraceable level. Due to the low cost, eco-friendly and successful remediation ability, bioremediation gain much attention for cleaning environments (Ite and Ibok 2019). Microbes (bacteria, fungi and algae) have a great influence on radionuclide transformation, speciation and mineralization by various enzymatic or non-enzymatic processes. Microbial interaction with radionuclides has a great potential in detoxification of radionuclides via mineralization, accumulation and transformation (Kumar et al. 2007). A variety of microorganisms such as *Deinococcus radiodurans, Rhodotorula taiwanensis* MD1149, *Mucor mehei, Chlorella vulgaris,* and *Parachlorella* sp. *binos* have been studied for remediation of radioactive wastes (Fredrickson et al. 2000; Shimura et al. 2012; Tkavc et al. 2018; Kumar et al. 2007). Microorganisms adopt various mechanisms like biotransformation, biomineralization, bioaccumulation, etc. to degrade and detoxify radioactive wastes (Singh and Kumar 2020). Microorganisms have the ability to reduce or precipitate the radionuclides in aqueous condition, and this is done by extracting electrons from organic compounds and transferring it to the radionuclides as a final electron acceptor (Kumar et al. 2007). This procedure basically makes the radionuclides stable and prevents spilling from contaminated sites. In this chapter, we will discuss about sources of radioactive wastes, impacts of radioactive wastes on environment and potential role of microbes in remediation of radioactive wastes.

13.1.1 Sources of Radioactive Wastes

Radioactive wastes are the wastes which contain radioactive materials. Radioactive materials are the compounds of unstable atoms which emit ionizing radiations as they decay. Radioactivity is a natural process and any atom which is not in its stable form will give off its extra energy to become stable. This process is known as radioactive decay. The process of decaying is atom specific and no two atoms have similar rate of radioactive decay (Bryant 2019).

Radioactive wastes typically generate from nuclear fuel cycle required for electrical power generation, research, medical, military and industrial applications and also from accidents.

13.1.2 Nuclear Fuel Cycle

Nuclear fuel cycle is a series of processes, resulting in the production of electricity from uranium in nuclear power plant (NPP). Two steps are involved in this process, one when the nuclear fuel arrives at NPP which is regarded as front end and other when the spent nuclear fuel (SNF) leaves the reactor, known as back end. Front-end process is comprised of uranium mining, milling, refining, enrichment and fuel fabrication to be used in nuclear reactor, whereas in contrast back-end process involves storage of used fuel, recycling, reprocessing and ultimately disposal (Rodríguez-Penalonga and Moratilla Soria 2017).

Two main strategies are involved globally to decide the fate of SNF: one is once-through cycle or direct disposal or open cycle and another is twice-through cycle or partially closed cycle. In open cycle, the SNF is considered as high-level waste and disposed in a safe storage facility without going through any chemical processes to mitigate its radiotoxicity. The SNF is supposed to be remained in that situation for millions of years until it gives off its radiotoxicity naturally and transforms itself into safe uranium levels. While in case of closed cycle, much of SNF is reprocessed to extract uranium and plutonium. It is estimated that around 94–96% of uranium and 1–1.5% of plutonium can be recycled from its original SNF quantity to be used as a nuclear fuel, and rests are disposed. Different strategies or technologies are used in different countries to recycle the SNF in closed cycle process.

The final disposal of nuclear wastes from various processes of nuclear fuel cycles should end up in a deep geological repository (DGR), but as of now, there is none operating but under process. The safety of DGR is very much debated but in many international forums it has been accepted as an option for recent time until new strategies arise for better disposal option. For current measures, low and intermediate levels of wastes are buried close to surface but high levels of wastes are disposed of to an underground engineered facility for its radioactivity to decay naturally. The time taken for nuclear wastes in safe storage repositories to reach to its safe levels depends much on its reprocessing technologies.

Radioactive wastes may generate from NFC during or between various stages of characterization, segregation, treatment, transport and disposal. Radioisotopes like 89Sr, 90Y, 95Zr, 103Ru, 105Rh, 129Te, 140Ba, 144Ce, 144Pr and their relevant isotopes are considered as significant hazards at reactor stage and may get released into environment. Apart from that, during fuel element transport and fuel reprocessing state, 90Sr, 129Te, 131I, 137Cs, 95Zr, 95Nb, 106Ru, 144Ce or their other relevant radioisotopes may also get released. Contamination may also happen during solidification of fusion product and final disposal process. The content of final disposal from NFC may get leeched in repository and contaminate the soil mostly with radionuclides like 137Cs, 90Sr and actinides (Smičiklas and Šljivić-Ivanović 2016).

13.1.3 Radioactive Wastes from Medicine

Radioisotopes are increasingly used in health care for therapeutic and diagnostic purposes. The radioisotopes which are mostly used include Technetium 99m (Tc-99m), Iodine 131 (I-131), Iodine 125 (I-125), Iodine 123 (I-123), Tritium 3 (H-3), Carbon 14 (C-14), Yttrium 90 (Y-90), Cobalt 60 (Co-60), Strontium 89 (Sr-89), Iridium 192 (Ir-192), Caesium 137 (Cs-137), Xenon 133 (Xn-133), etc. The department of nuclear medicine in each hospital generates most of the radioactive wastes. The radioactive wastes are mostly in the form of liquid with little solid wastes as used in syringes, needles, vials, contaminated gloves, cotton swabs, clothing, absorbent materials and utensils of patients and minimal amount of gaseous products. Strategies used in disposal of radioactive wastes in hospitals involves safe storage until its radioactivity is reduced to safe levels naturally through decaying and discharge of low activity hazards into sewage system. The discharge is ensured to achieve the community safety standpoint, so that no negative consequences occur in case of sludge formation in nearby area of human population (Khan et al. 2010).

13.1.4 Radioactive Wastes from Research Institutes

Research institutes and universities are often using radionuclides for tracing the metabolic or environmental pathways necessary for monitoring the activities of materials such as drugs, minerals, pesticides and biomolecules. The radionuclides mostly used are C-14, H-3, I-125, etc. Many radionuclides which are used are short lived with few long-lived radionuclides like C-14. Transuranic elements which also have longer half-lives may also be present in the radioactive wastes from research institutes.

13.1.5 Radioactive Wastes from Industry

Sealed radioactive sources (SRS) are mostly found in industrial application of radionuclides. They are contained in specialized devices for testing in a non-destructive manner and for quality control measures and also in luminous display and as a tracer. In industrial setting, spent or unused SRS are great sources of hazard and found in several serious accidents. Tritium 3 (3H), Phosphorus 32 (32P), Nickel 63 (63Ni), Americium 241 (241Am) and Strontium 90 (90 Sr) are some of the radionuclides used in industries for measuring the thickness of the product. Tritium 3 is also used in case of water movement, luminous and electronic valves and Americium 241 can be used in smoke detectors. Cobalt 60 (60 Co) is another radionuclide applied in sterilization and irradiation. For gauging, eye applicators and radiography Krypton 85 (85 Kr), Strontium 90 (90 Sr) and Caesium 137 (137 Cs) radioactive materials are used, respectively.

13.1.6 Radioactive Wastes from Naturally Occurring Radioactive Material (NORM)

Radioisotopes or radioactive materials which are present naturally in the Earth's crust and due to anthropogenic activity their ionizing radiation gets exposed to public domain are commonly referred to as NORM. NORM originates from burning of fossil fuels as well as mining, using of fertilizers and gas production. Uranium mining is the major source of NORM exposure. Key sources are U-238 and Th-232 decay series. Another source of NORM includes radon gas which is itself a decay product from radium, but it is also found in the intermediate step of radioactive decay of many short lived radioactive materials (Nazaroff 1992). Radon exposure occurs to humans directly from their homes only if it is built in granitic ground, and it is the second cause of lung cancer after smoking (Pacheco-Torgal 2012). On the other hand, technologically enhanced naturally occurring radioactive materials (TENORM) involve specifically the natural radioactive materials whose physical, chemical and radiological properties have increased in concentration due to man-made activities and as a result are now more exposed to its radioactive exposure (Abdel Rahman et al. 2013).

13.1.7 Radioactive Wastes or Radioactivity Due to Accidents

Radioactive wastes can arise from nuclear accidents and that is more detrimental compared to other source of wastes. The radioactive wastes that arise from accidents are uncontrolled mass of emission or discharge directly into environment. Some of the notable radioactive material exposures through accidents will be discussed here. Los Alamos criticality accident 1946 which took lives of two persons was due to the anomaly in plutonium assembly (McLaughlin et al. 2000). 1961 Nuclear meltdown at Idaho National laboratory, USA took the lives of three persons due to the malfunction and overheating of the nuclear reactor. Stationary Low Power Reactor (SL 1), an experimental prototype meant for nuclear power generation, was the one that got flawed in Idaho incident (Peplow 2014). Another incident happened the same year in USSR known as 1961 atom accident on submarine. One of the two nuclear reactors powering the K-19 submarine of soviet era got damaged and in a radiation exposure took the life of nine crew members within 2 days after their rescue from the damaged submarine (Erlanger 1992). In 1984, in Casablanca, Morocco, an iridium-192 radiography source was lost from an industry and was taken home by a labourer, and in the subsequent week, the whole family was exposed to its radiation and which took the lives of eight of the family members (Nenot 2009). In 1986, in Prypyat, Ukraine, 15 km from proper populated area of Chernobyl, in the Chernobyl Nuclear Power Plant, four RBMK-1000 reactors were used with the intention of producing 1 MW electrical power generation. The RBMK-1000 reactors were graphite moderated water cooled reactors with a lacking of western style containment vessel. On 26 April, the workers bypassed the safety systems to perform a test which resulted in steam explosion. The steam explosion damaged the upper cover of the reactor, releasing almost all its core water. In a subsequent event, due to the reaction between steam and graphite or zirconium, a possible second hydrogen explosion followed. The explosion immediately took the lives of three persons with 26 others including firefighters who died in the following days due to acute radiation. Another 238 persons survived with acute radiation sickness (Mould 2000). In 1987, In Goinia, Brazil, two individuals from a left out radiotherapy unit of a clinic took two sealed containers home and broke the seal. The sealed containers contained 1375 curies of cesium-137 chloride salt which ultimately got exposed.

They then sold it to another person, and in the following days, a whole area was exposed to it through its unconscious distribution. The incident started 12 September, and by 28 September, many people fell sick. On 29 September, the governmental authority got alerted and began their search of contamination and eradication of threat. The authority set up facilities for injured and contaminated individuals in the city's Olympic stadium. Around 112,800 people were examined, and out of which, 129 people were found to contain radioactive contamination. A total of 5 people died in this incident with 20 seriously injured (Brandao-Mello et al. 2000). In 1996, San Jose, Costa Rica, a Cobalt-60 radiation source in radiotherapy unit was miscalibrated and resulted in 50–60% of overdosages to the patients of San Juan de Dios Hospital. The accident took the lives of 7 patients with 81 injured (Coeytaux et al. 2015). Recent most notable nuclear accident happened in Fukushima, Japan in the year 2011. On 11 March 2011, a major earthquake brought out 15 m of tsunami waves to the land of Japan. Almost 20,000 people died in this natural disaster and prominent damage occurred to the three reactors of Fukushima Daiichi nuclear power plant. The radioactive materials from the three nuclear reactor core evaded to the sea, land and atmosphere. The Japanese authority was managed to shut down the three reactors in mid-December 2011 after the fallout of temperature to 80 °C during October. The exact casualty due to radioactivity is uncertain, but the amount of radioactivity released is supposed to one-tenth of Chernobyl nuclear disaster.

13.1.8 Radioactive Waste or Radioactivity Due to Military Use

Environmental contamination through the release of radioactive wastes or radionuclides by nuclear weapon testing for military use is enormous. After the historic Hiroshima and Nagasaki nuclear bombing during Second World War, the testing of nuclear weapons has reached its peak during the Cold War era. In USA alone, from 1945 to 1980, the atmospheric tests amounted to 428 megatons which is 29,000 times in its size compared to Nagasaki nuclear bomb. Although the adoption of non-proliferation treaty and the end of Cold War era has put a restrain to the ongoing competition for nuclear war heads, it is noteworthy that many other countries apart from then global competitors of West versus Soviets have also achieved their nuclear potential. But it is also noteworthy that many other countries apart from then global competitors of West versus Soviets have also achieved their nuclear potential. The greater concern with nuclear weapon testing is that radioactive debris gets stuck in the atmosphere by partitioning in the troposphere and stratosphere and eventually getting precipitated for shorter or longer periods (Smičiklas and Šljivić-Ivanović 2016). Pu and its relevant isotopes which are released after a nuclear test in particular are of major concern as it has higher biological half-lives approximately 24.3×10^3 to 81×10^6 years (Gabrieli et al. 2011). 241Am, 137Cs, 131I, 90Sr, etc. are the most significant radioisotopes found almost in every nuclear-related incidents or testing which are very much detrimental to human lives (Turner et al. 2003).

13.1.9 Impact of Radioactivity on Environment

Radioactive exposure has varied degrees of impact when it comes to environment and public health. The international community of nuclear experts set up bars to measure the scale of radioactivity and based on its impacts to environment. The international nuclear and radiological event scale (INES) which was brought into force in 1990 by International Atomic Energy Agency (IAEA) and Organization for Economic Co-operation and Development Nuclear Agency (OECD/NEA) had made seven levels of radiological exposures. Levels 1–4 are termed as incidents, whereas levels 5–7 are considered as accidents. The evaluation is on the amount of dosages people received and the number of people involved or the amount released into the environment.

Level 1 is considered as anomaly wherein a member of public community is overexposed to a radioactive source more than its expected statutory annual limit or a radioactive source was picked up or minor defects in safety systems in a facility containing radioactive substances.

Level 2 is overexposure more than 10 mSv (millisievert) to a person or a worker working in a radioactive facility. In case of radioactive facility, radiation level reaching more than 50 mSv per hour or contamination in the facility is also considered as level 2 risk.

Level 3 which is also termed as serious incident involves overexposures to workers more than ten times of its statutory annual limits or non-lethal burns or inflammation from radiation. Exposure more than 1 Sv/h or unexpected severe contaminations in the facility are also this level of threat. Any accidents near a nuclear power plant or any radioactive materials stolen or lost or misdelivered also come under this level.

Level 4 or an accident with consequences involves death of one individual due to radiation. It encompasses minor exposure of radioactive material unlikely to have a need of implementing countermeasures except for food sector which requires control measures. Damage to core structure or fuel melting which if results in release of 0.1% of core material or exposure of significant amount of radioactivity which can have significant impact on public health falls under this level.

Level 5 or an accident with broader consequences involves several deaths due to radiation and requires implementation of countermeasures. This level also deals with greater damage to reactor core which may result in exposure of large amount of radioactivity within the premises of an installation.

Level 6 also known as serious accidents involves release of significant amount of radioactive material from a radioactive source or installation. It requires planned countermeasures to control radiotoxicity of environment.

Level 7 or major accidents can be defined as major release of radioactivity from a source or installation with broader negative consequences on public health and environment. It requires planned and extended countermeasures to mitigate its radiotoxicity (Ojovan et al. 2019).

To better understand the levels set by INES, some instances will be useful here. The radioactive incident of 1987 in Goinia, Brazil where Cs-137 SRS was distributed in an area was a level 5 risk whereas level 7 risks involved Chernobyl nuclear disaster and Fukushima Daiichi nuclear disaster. It is estimated that 6000 mSv of radiation exposure was found within a month of Chernobyl nuclear disaster, and in case of Fukushima, 400 mSv per hour was recorded on 14 March, and it was the maximum recorded value to this date though it fell down later. Apart from the immediate deaths on aftermath of Chernobyl nuclear disaster, till date there are many cases of thyroid cancers reported. According to a UNSCEAR (United Nations Scientific Committee on the Effects of Atomic Radiation) report, around 6000 cases of thyroid cancer were reported related to Chernobyl disaster till 2005. The predicted cause for this sudden increase in incidence of thyroid cancer attributed to overexposure of 131-I due to the fallout of Chernobyl nuclear disaster. In contrast, in Fukushima incidence, the level of radioactive exposure or contamination remains uncertain though the estimated radioiodine exposure is 1% to that of Chernobyl accident (Lee et al. 2013). Nuclear accidents are the sole reason of major contamination of environment. Also Chernobyl nuclear disaster was able to increase the radioactive contamination of soil of Europe 3500 times compared to beforehand of the disaster. Most radionuclides which get dispersed in a nuclear disaster involve 131I, 137Cs, 90Sr, 239Pu and 240Pu (Steinhauser et al. 2014). Major contaminants from Fukushima Daiichi nuclear disaster involved 134Cs and 137Cs which were mostly found in soil samples 32 km from the incident site. Furthermore, in the same soil, other radionuclides like 110mAg, 129Te, 129mTe, 131I and 140La were also detected. The outer cover of leaves of cabbage, bamboo and grasses were also found to have radioactive contamination along with soil (Tazoe et al. 2012).

13.2 Microbes-Assisted Bioremediation of Radioactive Wastes

Enormous volumes of radionuclides and lethal metals containing wastes are generated from atomic fuel cycle and nuclear weapon generation agencies, medical research institutes, mining, etc., and causing adverse effects on earth is a significant concern (International Atomic Energy Agency 2010). As the physical and chemical methods of remediation are much expensive and also generate secondary pollutants, development of new low-cost inventive treatment and remediation advancements, including bioremediation utilizing microorganisms for adjustment or evacuation and recuperation of the contaminants, got much attention (Coelho et al. 2015; Francis 2006).

A wide range of microorganisms including bacteria, fungi and algae showed efficient results in the field of bioremediation of different types of pollutants. Microbial bioremediation of radioactive wastes depends upon the complex interaction of microbes and pollutants (Lloyd and Renshaw 2005). Different types of microbial activity like biotransformation, biomineralization and biosorption and bioaccumulation (Fig. 13.1) can reduce the toxicity of radioactive wastes and also increase the metal transport into the microbial system (Valdovinos et al. 2017; Kumar et al. 2007).

13.2.1 Bacterial Bioremediation of Radioactive Wastes

There are a huge number of bacteria that have the ability to remediate pollutants like metallic compounds and other organic pollutants through detoxification, transformation or immobilization. But, all the bacteria are not able to resist under high ionizing radiation and high acidic conditions (Misra et al. 2012). The waste produced from atomic power plants, nuclear weapon testing sites, mining and medical research industries contains actinides (Marra and Palmer 2011). Radioactivity is one of the most important property of actinides. Actinides and other fission products present in the wastes are able to produce high amount of β-radiation and γ-radiation. Therefore, the use of extremophilic bacteria which are able to resist under high radiations is an essential requirement for bioremediation under such extremophilic conditions (Albrecht-Schmitt 2019; Misra et al. 2012). Several microbial processes are involved in bioremediation of pollutants, but biotransformation, biomineralization, biosorption and bioaccumulation processes are most important bioremediation processes for radioactive wastes (Table 13.1, Kumar et al. 2007).

13.2.1.1 Biotransformation via Bioreduction

One of the most important negative properties of metals or other radioactive metallic waste element is that the elements cannot be destroyed like other organic pollutants, but it can transform or convert one form to another (Ayangbenro and Babalola 2017). Initially, the radioactive wastes are present in either soluble or insoluble form, and after disposal, the microbial process may convert the wastes soluble to insoluble or vice versa. This strategy of microbial process is used in the field of bioremediation (Francis 2006). The presence of electron acceptor like oxygen and electron donor like hydrogen influences the biotransformation. In the absence of oxygen, i.e. under anaerobic condition, bacteria use nitrates, sulphates or carbon dioxide as electron acceptor (Francis 2006; El Mamouni et al. 2002). Bacteria can transform radionuclides by either direct or indirect mechanisms.

1. Direct immobilization of radionuclides

   Direct immobilization of radionuclides includes transformation of radionuclides by enzymatic processes produced by microbes (Kumar et al. 2007). Actinides like Uranium (U), Technetium (Tc), Chromium (Cr), etc. showed efficient enzymatic reduction by microbes. In aqueous condition, oxidized form of actinides like Uranium (U), Technetium (Tc), Chromium (Cr), etc. is present in

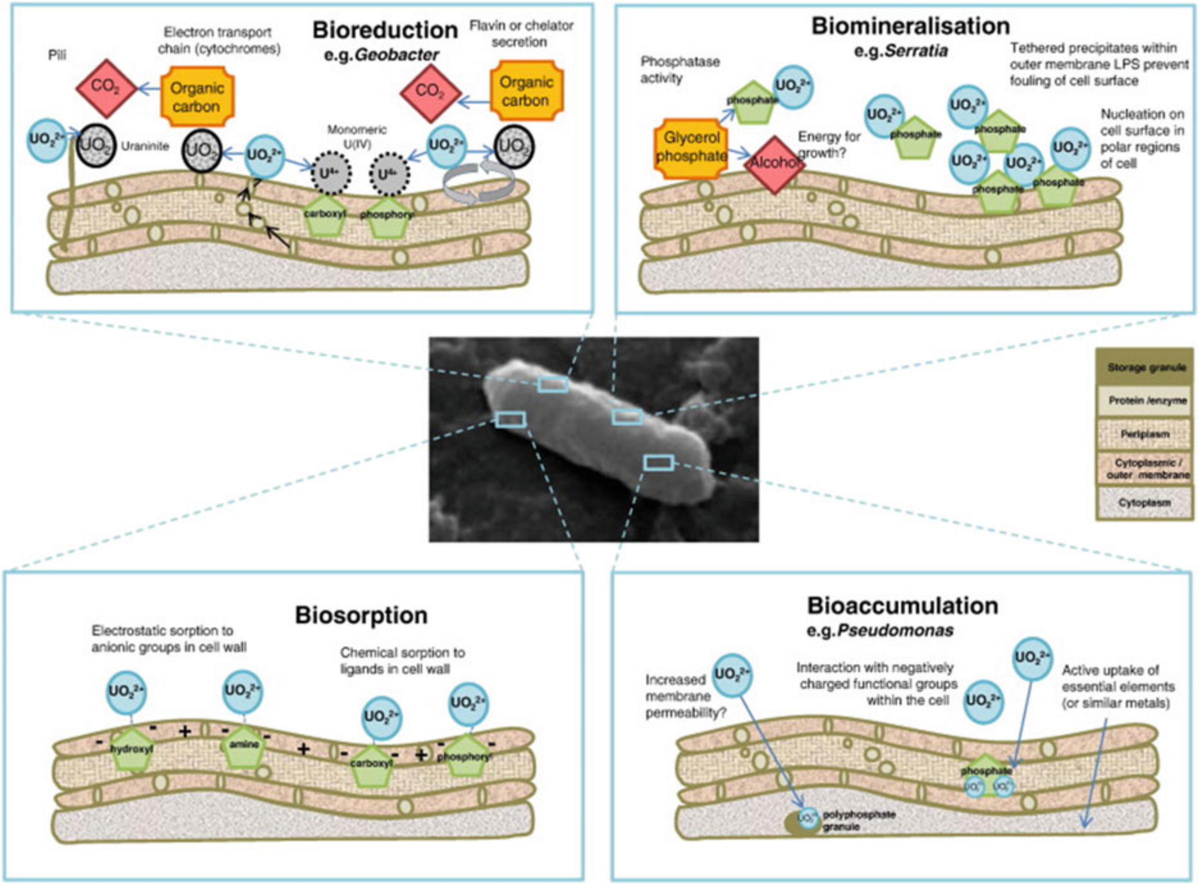

Fig. 13.1 Schematic illustration of microbial bioremediation of radioactive wastes through bioreduction, biomineralization, biosorption and bioaccumulation processes (Newsome et al. 2014)

Table 13.1 List of bacteria involved in bioremediation of radioactive wastes and their basic mechanisms

Sl. no.	Radioactive waste	Microbes	Results	Mechanism	References
1.	Uranium	*Deinococcus radiodurans*	Due to strong DNA repair and antioxidant defence mechanisms *Deinococcus radiodurans* showed tolerance against high ionizing radiations. Expressing *PhoN* gene through rDNA technologies increased shelf life 6 months under room temperature. Bioprecipitation of uranium along with other metal like cobalt also noticed under laboratory condition	Bioprecipitation	Misra et al. (2012)
2.	Uranium	*Desulfovibrio desulfuricans* G20	The cytochrome c_3 mutant *Desulfovibrio desulfuricans* G20 is able to reduce Uranium(VI) to Uranium(IV) where lactate and pyruvate act as electron donor. But the rate of reduction was found to be reduced as compared to the wild-type where hydrogen acts as an electron donor	Bioreduction	Payne et al. (2002)
3.	Cobalt (^{60}Co)	*Deinococcus radiodurans*	Some bacterial strains have the ability to uptake cobalt through *NiCoT* gene. Expressing that gene into high radiation–resistant *Deinococcus radiodurans* through genetic engineering showed increased uptake of radioactive cobalt (^{60}Co) isotope and reduced the total biomass of cobalt	Bioreduction	Gogada et al. (2015)
4.	Neptunium	*Shewanella putrefaciens* and *Citrobacter* sp.	*Citrobacter* sp. has the ability to precipitate tetravalent ions such as Np(IV), Th(IV), Pu(IV) through enzymatic action. While *Shewanella putrefaciens* can reduce the pentavalent Np(V) to tetravalent Np(IV). Therefore, the bacterial consortia treatment showed efficient bioremediation of radioactive ^{237}Np isotope	Bioreduction and bioprecipitation	Lloyd et al. (2000)
5.	Technetium and uranium	*Anaeromyxobacter dehalogenans 2CP-C*	*Anaeromyxobacter dehalogenans 2CP-C* reduces Ur (VI) and Tc(VII) to uraninite and Tc(IV) and H_2 acts as an electron donor. The reduction rate exceeded when the medium is amended with acetate. Fe(II)-mediated reduction mechanism plays indirect mechanism in Tc(VII) reduction	Bioreduction and bioaccumulation	Marshall et al. (2009)

6.	Uranium	*Deinococcus radiodurans*	Introduction a nonspecific acid phosphatase (*PhoN*) gene in *Deinococcus radiodurans* from *Salmonella enterica* enhanced precipitation of uranium from uranyl nitrate solution	Bioprecipitation	Appukuttan et al. (2006)
7.	Technetium	*Desulfovibrio desulfuricans*	The cell of *Desulfovibrio desulfuricans* along with the oxidation of electron donor reduced the Tc(VII). The optimum biotransformation was observed when hydrogen acts as an electron donor. Enhanced biotransformation was noticed when formate or pyruvate was supplied to the cell. And the rate was decreased when lactate and ethanol act as electron donor	Bioreduction	Lloyd et al. (1999)
8.	Plutonium	*Geobacter metallireducens* GS15 and *Shewanella oneidensis*	Both the strains have the ability to reduce Pu(VI/V) to insoluble form of Pu(IV). But in the presence of EDTA as an electron acceptor, rapid reduction of Pu(IV) to Pu(III) was observed. This study suggests that under reducing environment along with ligands may generate reduced form of soluble Pu	Bioreduction	Boukhalfa et al. (2007)
9.	Plutonium	*Bacillus mycoides* and *Serratia marcescens*	*Bacillus mycoides* and *Serratia marcescens* were able to reduce low-level radioactive waste Pu(IV) to Pu(III) under highly acidic aerobic condition	Bioreduction	Lukšienė et al. (2012)
10.	Strontium	*Halomonas* sp.	80% Strontium bioremediation was observed when the radioactive waste treated with strontium-resistant urease-producing bacteria *Halomonas* sp.	Biomineralization	Achal et al. (2012)

highly soluble form, whereas reduced form of actinides is insoluble and immobile under aqueous condition (Humphries and Macaskie 2002; Istok et al. 2004). Therefore, these reduced elements are often found in precipitated form. Under in vitro condition, *Desulfovibrio vulgaris* have the ability to reduce U(VI) and Cr (VI) to U(IV) and Cr(III) where H_2 act as electron donor and cytochrome c_3 as Cr reductase (Lovley and Phillips 1994; Lovley et al. 1993). In 2002, another experiment was performed to understand the involvement of cytochrome c_3 and hydrogenase protein in metal reduction. It was found that the c_3 mutant *D. desulfuricans* strain G20 was able to reduce uranium along with lactate or pyruvate as electron donor, but the rate of reduction was decreased as compared to the wild-type. From this study, it was concluded that cytochrome c_3 is a part of metal reduction along with hydrogenase, and it can be bypassed by additional pathways (Payne et al. 2002).

2. Indirect immobilization of radionuclides

   Indirect immobilization means immobilization of primary molecules via bioreduction of a secondary molecule. For example, iron Fe(III) and sulphur S (VI) can be reduced by microbes into Fe(II) and S(II) form and the oxidation of that bioreduced Fe & S can reduce a primary molecule and transform them into mobile to immobile molecule (Prakash et al. 2013). Technetium-99 [Tc(VII)] is an example of higher risk driving radioactive waste. Indirect mechanism play important role in immobilization of Tc(VII), where bioreduced Fe(II) directly donate electron to Tc(VII). After accepting electron from Fe(II) the reduced Tc (VII) becomes immobile (Kumar et al. 2007). *Geobacter metallireducens* has the ability to reduce Fe(III) as ferrihydrite Fe(II) enzymatically, when the cell is exposed to the highly soluble U(VI), it converts U(VI) to poorly soluble U (IV) (Lloyd 2003). Another indirect immobilization process involves the siderophore production and complexation. For example, *Microbacterium flavescens* developed under radioactive waste condition secretes various organic acids, siderophores, extracellular metabolites which mix with and assemble the radionuclides in the form of dirt (Banerjee et al. 2018; Kumar et al. 2007).

13.2.1.2 Biomineralization

The term biomineralization refers to the process of metal precipitation at the microbial cell surface with the help of ligands such as sulphides, carbonates, phosphates and hydroxides generated by microbes (Jiang et al. 2019). Bacteria like Citrobacter species and Serratia species showed efficient uranium biomineralization (Ding et al. 2019). It was observed that under glycerol phosphate condition, the cell shows phosphatase activity and releases inorganic phosphates which ultimately form complexes with uranium in the form of hydrogen uranyl phosphate at the cell surface (Beazley et al. 2007). Similar uranium biomineralization was noticed earlier when *Pseudomonas* species was supplied with tributylphosphate (Thomas and Macaskie 1996). Bacterial cells covered with uranium phosphates were isolated from uranium contaminated soils, which suggest that biomineralization is a naturally occurring process (Newsome et al. 2014). Rapid precipitation of metal phosphate will form a barrier around the cell surface which may hurdle in cell metabolism (Mondani et al. 2011; Newsome et al. 2014). But in Serratia, the uranium deposition was observed only on one side where lipopolysaccharide prevent fouling of cell surface (Macaskie et al. 2000; Newsome et al. 2014).

13.2.1.3 Biosorption

Biosorption refers to the passive deposition of the soluble substances at the cell surface. The presence of various ionizable groups such as phosphate, carboxyl, hydroxyl, amine, and sulfhydryl at the cell surface generates electronegative attractions for metal cations as results the metal ions get deposited at the cell surface (Lopez-Fernandez et al. 2019). Biosorption is considered as right method for treating low concentration metallic wastes as the process of binding is faster than accumulation process, and also it is easy to remove bound pollutants from the cell surface to regenerate the biosorbant for further use (Newsome et al. 2014; Oyewole et al. 2019). But there are some problems in biosorption like

- Sometimes problems may arise in bioremediation when other non-targeted cations competes and binds with cell surface as a result the rate of bioremediation decreases drastically (Schiewer and Volesky 2000).
- Sometimes the cell surface becomes saturated as a result further binding of cations do not takes place (Newsome et al. 2014).
- If the sorbed cell dies, rapid desorption of cation takes place which may alter in bioremediation process (Knopp et al. 2003).

13.2.1.4 Bioaccumulation

Bioaccumulation of radioactive waste refers to the accumulation of radioactive wastes inside the cell. A wide range of metal accumulation takes place through bioaccumulation (Diep et al. 2018). Certain metal ions show structural similarity with essential elements needed for bacterial growth and developments as a result the adventitious uptake of these ions take place. As uranium has no known biological function, uranium uptake into the cell takes place through membrane permeability caused by uranium toxicity (Newsome et al. 2014). In *Pseudomonas* species, the uranium accumulation takes place in the form of uranyl phosphates. Other microbes like *Arthrobacter nicotianae*, *Micrococcus luteus*, *Citrobacter* sp. N14, and *Bacillus megaterium* showed efficient bioremediation of radioactive wastes through bioaccumulation process (Shukla et al. 2017).

13.2.2 Fungi: Bioremediation of Radioactive Wastes

Fungi play an essential role in soil food web as it decomposes various organic substances. Fungi is able to decompose woods by degrading the key components of wood fibre such as lignin and cellulose (Hildén and Mäkelä 2018). Fungi also showed efficient results in bioremediation of dyes, heavy metals released from textile industries, pharmaceutical industries, etc. (Khan et al. 2019). The environment radioactive waste contaminated sites have low pH, high temperature and extreme radiations, and it seems to be impossible to survive any species at that extreme condition. Therefore, for bioremediation purpose, it is essential to search the microbes which is able to survive under extreme environmental conditions (Fredrickson et al. 2004). Tkavc et al. isolated *Rhodotorula taiwanensis* MD1149, a fungal species which can survive under environmentally harsh condition, i.e. highly acidic condition at pH 2.3, high metal concentration and extreme radiation. The fungi showed bio-film formation under extreme gamma radiation and at low pH (Tkavc et al. 2018).

Fungi *Rhizopus arrhizus* along with the immobilized particles showed biosorption of uranium from bioleaching uranium ore solutions. The amine nitrogen of chitin along with free radicals results uranium biosorption (Gadd and Fomina 2011). The carboxyl and phosphates group of *Saccharomyces cerevisiae* cell wall showed initial uranium deposition (Zhang et al. 2020). pH also plays a major role in biosorption of radionuclides. For examples, at pH 3 *Mucor miehei* sorbs 70–80 mg uranium/g dry weight of fungi, and at pH 4 and 5, the biosorption increases 2–3 times, respectively. While *Rhizopus* sp. showed efficient Cr(VI) adsorption at pH 2.0 (Espinoza-Sánchez et al. 2019; Gadd and Fomina 2011). The crystalline disposition of uranium was observed in *Penicillium digitatum* (Gadd and Fomina 2011).

In the field of bioremediation, mushroom plays a key role. Due to the large fruiting bodies, mushroom gains much attention that it can accumulate large amount of wastes. Mushroom has the ability to degrade, decompose and accumulate different types of organic wastes and agro wastes (Pandey et al. 2018). But in the field of radioactive waste bioremediation, mushroom was less studied. Baeza and Guillén (2006) studied the uranium bioaccumulation in mushrooms, and they determined it in terms of *transfer factor* (TF), i.e. level of radioactivity is detected in mushrooms in comparison to surface soil. They found that *Amanita muscaria* and *Hebeloma cylindrosporum* showed the highest TF values while *Lactarius deliciosus* exhibited the least ranges from 0.043 to 0.49 (Baeza et al. 2004).

13.2.3 Algae: Bioremediation of Radioactive Wastes

Like bacteria and fungi, algae also play crucial role in bioremediation of various pollutants like heavy metals and other organic pollutants. Algal-based bioremediation is known as phycoremediation. Due to autotrophic in nature, algal bioremediation does not require external energy sources for their growth and hence showed enhanced bioremediation (Iwamoto and Minoda 2018). For growth, autotrophic algae need only light, water, carbon dioxide and dissolved minerals. Recently algal bioremediation showed effective role in remediating sites contaminated with radionuclides. *Chlorella vulgaris* showed efficient biosorption of uranium, and the rate of biosorption depends on the availability of carboxylic and phosphate groups. The concentration of uranium, pH and the status of cell is also directly related to the uranium biosorption (Vogel et al. 2010). Different microalgae showed effective results in remediation or radionuclides like radioiodine, caesium, strontium, etc. For example, a green *Parachlorella* sp. binos microalgae when cultured under radioiodine condition. It accumulates radioiodine into the cytosol in light-dependent manner. The microalgae are also able to accumulate strontium and caesium in light-independent manner, and accumulation of strontium was observed into the extracellular matrix of *Parachlorella* sp. (Shimura et al. 2012). *Coccomyxa actinabiotis* sp. *nov.* isolated from nuclear agencies is able to survive under high ionizing radiation doses up to 20,000 Gy, and it is supposed to be 2000 times lethal human dose. The microalgae are also able to accumulate high amount of radionuclides like ^{238}U, ^{137}Cs, ^{110m}Ag, ^{60}Co, ^{54}Mn, ^{65}Zn and ^{14}C (Earis 2009).

13.2.4 Genetic Engineering: Bioremediation of Radioactive Wastes

Due to adverse environmental conditions, it seems impossible for microbes to survive and remediates pollutants. But there are still some microbes which can resist extreme environmental conditions but fail to remediate the contaminants (Katarína et al. 2018). In this case, genetic engineering provides a new insight in the field of bioremediation as many microbes can be designed in such a way that can remediate the contaminants which are not done by normal microbes. In this case by altering gene sequences of desired microbes and enhancing its ability to degrade, digest, and accumulate contaminants or sometimes reconstructing a microbe by inserting a gene which has an extraordinary ability to remediate the specific contamination. Thus, reconstruction of microbes for bioremediation is done specifically (Jaiswal et al. 2019). *Deinococcus radiodurans* is a well-known radio-resistant bacteria that have the ability to reduce radioactive wastes like Cr(VI), U(VI) and Tc(VII) (Fredrickson et al. 2000). Attempts were made to reconstruct *Deinococcus radiodurans* that has the ability to reduce the radionuclides along with other contaminants like other metals and organic pollutants. Incorporation of an E. coli (*merA*) gene provides carbon assimilation property for energy generation generated from toluene and mercury catabolism. Thus, genetically modified *Deinococcus radiodurans* can be a promising tool for bioremediation of radionuclides along with other pollutants (Watanabe 2001). Similarly, expressing the *PhoN* gene in *Deinococcus radiodurans* through rDNA technologies increased 6 months shelf life of the bacteria also increased uranium bioprecipitation along with cobalt (Misra et al. 2012). Expressing *NiCoT* gene into high radiation-resistant *Deinococcus radiodurans* through genetic engineering showed increased uptake of radioactive Cobalt (^{60}Co) isotope and reduced the total biomass of cobalt (Gogada et al. 2015). Most of the bacteria of *Geobacteriaceae* family are able to reduce radionuclides. The *dcuB* of *Geobacter sulfurreducens* coded for fumarate transporter, constitutive expression of *ducB* in *G. metallireducens*, increased respiratory capabilities along with bioremediation of radioactive wastes (Butler et al. 2006). Genetic engineering holds considerable promises, and more studies will require in developing advanced and more efficient technologies for safe and clean environments.

13.3 Factors Affecting Bioremediation of Radioactive Wastes

Microbes have the ability to adapt themselves with the changing environments and showed a promising approach towards radioactive waste bioremediation. But there are some biotic and abiotic factors which alters the biological processes of microbes by altering the behaviour and growth. Lack of knowledge regarding the factors that affect and influence may alter the rate of bioremediation (Boopathy 2000; Varjani and Upasani 2017). Factors that affect the microbial processes are classified into three groups:

1. Physicochemical factors or abiotic factors
2. Biological factors or biotic factors
3. Climatic factors

13.3.1 Physicochemical Factors or Abiotic Factors

The physicochemical factors that affect bioremediation by altering microbial behaviour and growth are mainly pH, solubility, presence and absence of electron donor and acceptor, and the ionic strength. In the process of microbial biosorption, pH plays the key role to absorb pollutants like radionuclides (Srivastava et al. 2014). A slight change in pH may alter the rate of bioremediation. pH value changes cell surface charge by altering the isoelectric points. The ionic strength of various ligands like carboxylic group, phosphate groups, sulphur and amino groups directly depends on pH (Boopathy 2000). Changes in pH value bring changes in ionic strength of such ligands and alter the rate of biosorption. The solubility of metal ions are also pH dependent as with decrease in pH the solubility of the metal ion increases which alter the adsorption by microbial cells (Varjani and Upasani 2017). For examples, at pH 3 *Mucor miehei* sorbs 70–80 mg uranium/g dry weight of fungi, and at pH 4 and 5, the biosorption increases 2–3 times, respectively (Gadd and Fomina 2011).

13.3.2 Biological Factors or Biotic Factors

There are some biological factors that have great influence in bioremediation. The specificity of microbes towards the substrates has a great role in bioremediation, and it has shown that microbes have a wide range of specificity for different types of substrates which may alter the remediation of target pollutants (Boopathy 2000; Abatenh et al. 2017). Complete bioremediation cannot be achieved by single microbial species; therefore, a microbial consortia is required for complete bioremediation. In microbial consortia, the interaction of microbes is the key factor for bioremediation (Abatenh et al. 2014). Individually, maybe all the microbial species are good remediators, but in consortium maybe they are allelopathic in nature. Therefore, proper design of microbial consortia is an important step of bioremediation (Boopathy 2000).

13.3.3 Climatic Factors

Elevated carbon dioxide and temperature are the main factors of global climate change (Boopathy 2000). Though there are no direct evidence of climate change affecting the bioremediation, changes in physicochemical characteristics of microbial niche may disturb various metabolic processes and thereby bioremediation process. The climatic condition greatly influences the microbial extracellular enzyme productions which may help/alter in bioremediation process (Abatenh et al. 2017).

13.4 Conclusion and Future Prospects

Essential research on bioremediation of radionuclides is of fundamental significance to the advancement of new strategies and innovations to secure the earth. Radionuclide bioremediation to a great extent depends upon the capability of the microorganisms to survive under highly radioactive situations. But there are some biotic and abiotic factors which alters the biological processes of microbes by altering the behaviour and growth. Lack of knowledge regarding the factors that affects and influences may alter the rate of bioremediation. Therefore, it is necessary to understand the mechanism how the factors affecting bioremediation will help to find out the permanent solution. In this case, genetic engineering has brought a revolutionary change in the field of bioremediation as it can help to overcome the factors which can affect bioremediation by engineering new pathways or by evaluating regulatory factors that are participating in bioremediation. It is important to comprehend the mechanism that empowers organisms to dispense with the radionuclides from defiled sites. Understanding the molecular network by ‘omic’-based studies such as proteomics and transcriptomics will be helpful for environmental decontamination. Future prospects in the field of bioremediation have a lot of opportunities. Since climate change along with other environmental change will alter microbial communities, as it is predicted that climate change will alter whole earth ecosystem. Therefore, climate change along with microbial processes would be an interesting field of research.

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Chapter 14
Plastic-Eating Microorganisms: Recent Biotechnological Techniques for Recycling of Plastic

Charles Oluwaseun Adetunji and Osikemekha Anthony Anani

Contents

Abstract Plastic has been identified as a recalcitrant polymers which are inexpensive, durable, light weighted, strong, and corrosion-resistant materials. The prolong accumulation of these plastic and most especially the bioplastic has been highlighted to constitute several health and environmental hazards. The movement of these recalcitrant polymers in agricultural soil, water, and sediments has raised several concerns globally. Therefore, there is a need to search for the potential solution that could help in the biodegradation of synthetic polymers. The application of beneficial microorganisms that possess the capability to degrade plastic could be an effective and sustainable approach to all the highlighted challenges. Hence, this chapter intends to write a comprehensive details on the application of probable microorganisms that possess the capability to degrade synthetic plastics. The modes of action utilized by these microorganisms and their biodegradative enzymes are discussed in detail. Further recommendation and suggestions could enhance the practical utilization of plastic biodegrading microorganism most especially for practical or field application.

C. O. Adetunji (✉)
Applied Microbiology, Biotechnology and Nanotechnology Laboratory, Department of Microbiology, Edo University Iyamho, Auchi, Edo State, Nigeria
e-mail: adetunji.charles@edouniversity.edu.ng

O. A. Anani
Laboratory of Ecotoxicology and Forensic Biology, Department of Biological Science, Animal and Environmental Unit, Faculty of Science, Edo University Iyamho, Auchi, Edo State, Nigeria

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14.1 Introduction

The total amount of plastics that are generated every year has been estimated around 140 million tons which are eventually made available to the ecosystem as industrial waste products (Shimao 2001). It has been highlighted that about 30% of most of the plastics that are utilized globally are generated from different sources after utilizing the products which contain cosmetics, detergents, pharmaceuticals, chemicals, as well as packaging materials for water and foods. The trends still continue to rise day-by-day at a very high exorbitant rate of 12% p.a (Sangale 2012). The utilization of plastic has virtually replaced the place of cellulose-based products and paper as a packaging material. This might be linked to the facts that they are resistant to microbial attack, enhanced strength, tensile, lightness, and their durability in nature.

There are numerous types of plastics utilized for packaging purposes which include polypropylene (PP), polyvinyl chloride (PVC), polyethylene (LLDPE, LDPE, HDPE, MDPE), and polystyrene (Khanam and Mariam 2015). Moreover, the low-density polyethylene which could be classified as a thermoplastics class (Pramilla and Vijaya 2015) has been highlighted to be nondegradable in nature which might be linked to its hydrophobic backbone (Myint et al. 2012).

Furthermore, some other merits of plastic polymers with large application in the food packaging agricultural films might be linked to the fact that their durability, cost-effectiveness, and ductility. In view of all the highlighted advantages, plastic has been highlighted as a source of pollution which constitutes several hazards to human and animal health. Moreover, among all the types of plastics that constitute pollution, microplastics have been highlighted as a point of high concern because whenever they are deposited in an aquatic environment, most of the aquatic animals and seabirds normally feed on them. This might lead to high level of mortality as a result of high accumulation of these microplastics in their stomach (Krueger et al. 2015; Acampora et al. 2017). This might eventually affect food chain which might affect human health (Rillig and Bonkowski 2018; Li et al. 2015; Jabeen et al. 2017).

The application of some conventional techniques such as landfilling and incineration might result into environmental pollution, and they might also require several amount of money toward their management (Krueger et al. 2015; Song et al. 1998). Moreover, the recent trends toward the development of biodegradable plastic in recent years have resulted into the minimization of environmental pollution as a result of plastic discharged into the environment (Ioakeimidis et al. 2016; Shimao 2001).

Therefore, there is a need to search for an alternative solution to all these challenges. The application of biodegradation is an environmental approach through which organic materials are broken down into smaller compounds such as H_2O and CO_2 through the action of microorganisms. The process of biodegradation involves the growing of the microbial cell on the solid surface for the production of hydrophilic groups followed by the hydrolysis or oxidation of the long-chain hydrocarbons into short chains through the action of microorganisms mainly through the action of some relevant enzymes while the short-chain polymers are converted into fatty acids after which the fatty acids are later oxidized into humus, H_2O and CO_2 (Shah et al. 2008a, b; Singh and Sharma 2008; Yang et al. 2014, Plastics Europe 2018).

Several scientists have reported numerous microorganisms for their biodegradative potential on plastic. Some of these strains includes *Streptococcus, Aspergillus, Bacillus, Staphylococcus Penicillium, Pseudomonas, Moraxella,* and *Streptomyces* mainly derived from marine, soil, and sludge under natural conditions (Restrepo-Flórez et al. 2014, Pegram and Andrady 1989, Jones et al. 1974). Also, there are several factors that constitute delay in the biodegradation of these plastic within a very short period of time which includes high chemical bond energy, high molecular weight, and strong hydrophobicity(Watanabe et al. 2003). While some strains such as *Nocardia asteroids* and *Penicillium simplicissimum* could take a longer time (Yamada-Onodera et al. 2000).

Hence, this chapter intends to provide a detailed information on the application of beneficial microorganisms for the bioremediation of heavily polluted environment with plastic. The modes of action utilized by these microorganisms were also highlighted. Further recommendation that could enhance more research activity that would promote the process involved in the biodegradation of plastic was also suggested.

14.2 Application of Plastic-Degrading Microorganisms in Environmental Bioremediation

The utilization of plastic polymer in our daily life, agriculture, and industry cannot be overemphasized due to the fact that it might be liked to their cost-effectiveness and their easy use. However, there is an increase in the level of pollution constituted as a result of pollution constituted by plastic polymer most especially polyethylene which constitutes several health and environmental challenges to humans and animals. In view of the aforementioned (Ren et al. 2019), *Enterobacter* sp. D1 was derived from the month of wax moth (*Galleria mellonella*). The colonies growing around the polyethylene film after a period of 14 days of growth containing *Enterobacter* sp. D1. The level of cracks, roughness, and depressions was perceived on the surface of the polyethylene film and was detected by atomic force microscopy and scanning electron microscopy. The presence of various function groups available was detected using Fourier transform infrared spectroscopy which detected the presence of ether and carbonyl group. Moreover, liquid chromatography-tandem mass spectrometry revealed the presence of acids, alcohols, and esters, which indicated the presence of oxidation reaction happening on the surface of the polyethylene film that was inoculated with the *Enterobacter* sp. D1. Their study showed the biodegradative potential of *Enterobacter* sp. D1, most especially the several materials containing polyethylene film.

Patil (2018) evaluated the degradative capability of some microorganisms utilizing opaque techniques for fungi and bacteria. The preliminary evaluation established using opaque showed that two fungal and four bacterial species which were utilized for further investigation. The typical examples of the bacterial strain isolated with biodegradation potential include *Pseudomonas fluorescens, Bacillus amylolyticus, Pseudomonas putida*, and *Bacillus firmus.* These strains were utilized for their biodegradative potential on commercial polythene carry bags of low-density polyethylene for a period of 30 days in a shaker culture when performed in a laboratory condition, utilizing weight determination techniques. It was established that *Bacillus* sp. obtained from garbage soil showed a biodegradability potential of 32%.

Muhonja et al. (2018) utilized fungi and bacteria that possess the capability to degrade low-density polyethylene. The extent of the biodegradation of low-density polyethylene using fungi and bacteria from various sampling sites of dumpsite in Dandora was assessed under laboratory condition. The experiment was carried out using low-density polyethylene under the incubation period of 28 days at 37 °C for fungi, and bacteria for a period of 16 weeks using a rotatory shaker. The level of biodegradation was assessed using GC-MS and Fourier transform infrared spectroscopy. The analysis using Fourier transform infrared spectroscopy showed the presence of new functional group as a result of hydrocarbon degradation from bacterial and fungal. The molecular characterization of the best strain responsible for the biodegradation of low-density polyethylene was carried out using 18SrDNA and 16S rDNA sequences for fungi and bacteria, respectively. The following bacterial strains which entail *Brevibacillus*, *Lysinibacillus, Pseudomonas*, *Cellulosimicrobium*, and *Bacillus* while genus *Aspergillus* was the only fungal strain isolated as polyethylene degraders. The result obtained shows that fungi exhibited a more biodegradative potential of polyethylene when compared to bacteria. The maximum fungal degradation action was obtained in terms of weight reduction of 36.4 ± 5.53% from *Aspergillus oryzae* strain A5 with accession number of MG779508 while 20.28 ± 2.30% was obtained from *Brevibacillus borstelensis* strain B2,2 (MG645267) and *Bacillus cereus* strain A5 with accession number of A5,a (MG645264). The result obtained shows that the following genus which involves *Brevibacillus, Aspergillus*, and *Bacillus* are affirmed to possess a great capability to biodegrade low-density polyethene. The Fourier transform infrared spectroscopy analysis showed the presence of the following functional groups such as carboxyl, ether, and aldehyde while ketone was detected as a transitional product detected in the culture media. The authors suggested that their need to establish the best optimum condition that favors the best microbial activity that could enhance the biodegradation of plastic through the enzyme activity of microorganisms for their eventual commercial application.

Begum et al. (2015) evaluated the effect of soil bacterial obtained from plastic polluted environment. The result of the biochemical and morphological characterization showed that *Pseudomonas alcaligenes* and *Desulfotom aculumnigrificans* were detected to possess the ability to biodegrade polythene bag. It was detected that *Pseudomonas alcaligenes* showed the effectiveness for plastic biodegradation when compared to *Desulfotom aculumnigrificans* after 30 days. It was detected that rise in the incubation period shows tremendous increase in weight loss of the treated polythene bag. Their study indicated that *Pseudomonas alcaligenes* might be utilized drastic reduction of polythene bags available in the natural environment. This might be linked to the cost-effectiveness, environmental friendly utilizing these plastic degrading microorganisms.

The build-up of plastic wastes in the environment has been discovered to constitute threats to the environment while the significance of plastics that are biodegradable has been recognized as ecofriendly with enhanced application in various sections that utilize plastic in their packaging.

Jumaah (2017) evaluated the potential of some microorganisms to biodegrade some plastic material after incubation for a period of 1 month in a submerged fermentation. The result revealed the presence of two Gram negative and three Gram positive bacteria. The following bacterial were detected which involved *Bacillus subtilis, Bacillus amylolyticus, Pseudomonas fluorescens, Bacillus firmus,* and *Pseudomonas putida.* It was discovered that *Pseudomonas putida* exhibited the highest biodegradative potential of plastic after performing submerged fermentation with average value of 30% weight loss per month when compared to *Bacillus subtilis* of 22% weight loss per month. Their study showed that *Pseudomonas putida* exhibited the highest biodegradation potential among all the tested strains when compared to the others.

The role played by the application of plastic in our economy each year has been estimated to around 350–400 million tons. However, it has been discovered that due to low circular utilization and recycling while millions of tons build up in the marine and terrestrial environments. It has been observed that plastic possess the capability to induce several adverse effect on environment and human health most especially the microplastics. Therefore, the application of microorganism for the biodegradation of plastic has been identified as a sustainable tools. In view of the aforementioned, Danso et al. (2019) wrote a comprehensive review on the ester-based polyurethane and polyethylene terephthalate which were high molecular weight polymers.

Their review also highlighted the significant of microorganisms and enzymes that could biodegrade these polymers. They also suggested that the application of dark matter proteins and global metagenomes of non-cultivated microorganisms will help in the biodegradation of plastic. It was also suggested that the application of new biocatalysts and microorganisms could enhance rapid biodegradation and recycling of numerous man-made polymers.

Soud (2019) evaluate the effect of *Streptomyces* spp. for the biodegradation of plastic wastes and some other pollution. This strain was screened for their biodegradation potential of polyethylene low density polyethylene in various assays. The evaluation of their biodegradation potential was based on the dry weight loss of plastic stripes of plastic cup (p) and polyethylene bags (g) after culturing in submerged fermentation using ATCC medium after incubation in the following condition such as 25–30 °C in shaker incubator at 120 rpm. The potential of this strains to produce bioemulsifier and spectrophotometric assay were evaluated after 1 month of incubation which result in the loss in dry weight in polyethylene low-density polyethylene stripes which include 8%, 11%, 19% for (g) stripes and 6%, 9%, 15% for (p) stripes by the following strains (SSP2, SSP4, SSP 14), and spectrophotometric assay documented greatest results for polyethylene low-density polyethylene degradation, it was discovered that strains recorded SSP2 (0.08, 0.55), SSP4 (0.09, 0.65), and SSP 14 documented (0.13, 0.70) for p and g, respectively. In conclusion, the bioemulsifier fabrication and evaluation also showed maximum results that play significant role in biodegradation process, the outcome observed indicates that bioemulsifier production yield by the following strains (SSP2, SSP4, SSP14) isolates are (8.44%, 9.84%, 12.94%) for (g) stripes and (5.74%, 7.24%, 11.84%) for (p) stripes. Their study indicated that strain SSP14 is the best isolate for polyethylene low-density polyethylene degradation which shows that *Streptomyces* could be utilized for the bioremediation of polyethylene low-density polyethylene and could be used for many other microbiological environmental science.

The application of plastic has been identified for several purposes. The release of plastic waste has been identified as the second largest solid waste. The high persistence of plastic in the environment has diverted the attention of numerous scientist. In view of this, Munir et al. (2018) isolated a bacteria that could degrade low-density polyethylene plastic. This was carried out in a mineral salt medium broth, entailing a low-density polyethylene powder. It was discovered that 2 out of the 10 isolate possess the capability biodegrade low-density polyethylene in a preliminary trial. The result obtained showed that strains SP4 and SP2 possessed that capability to decrease low-density polyethylene with the following value, respectively, 12.06% and 10.16% after a period of 4 weeks of incubation. The scanning electron microscopy evaluation revealed that the surface of the treated low-density polyethylene was altered when compared to the untreated film. Furthermore, there was presence of cracks, rough outlook, and attachment of bacterial to their surface. The presence of biodegradation of low-density polyethylene was also affirmed by Fourier transform infrared spectroscopy evaluation. Their study indicated that the bacteria isolated from landfill could be utilized for the biodegradation of plastic material.

Vignesh et al. (2016) evaluated the effect of fungal and bacterial strain that could biodegrade plastic which resides in the dumped soil samples obtained from harbor and Pallikaranai at Chennai. The plastic degrading microorganisms were screened using opaque techniques for the fungi and bacteria. The preliminary method revealed the presence of three fungal and bacteria species with high biodegradative potential which was later recognized as *Streptococcus* sp., *Aspergillus* sp., *Bacillus* sp., *Fusarium* sp., and *Pseudomonas* sp. when tested using biochemical test. This was carried out in a submerged fermentation that involves nutrient broth for bacteria while potato dextrose broth was utilized for the fungal isolates. The potential of these strains to biodegrade LDPE was tested for a period of 30 days under a submerged fermentation utilizing weight determination techniques, and it was discovered that *Bacillus* sp. isolated from petroleum soil possess the capability to degrade plastic up to 23% while *Fusrium* spp. could biodegrade plastic up to 44%. It was discovered that it takes 120 days for the bacteria to biodegrade the plastic while it takes 75 days for the fungi to biodegrade plastic during the period of the experiment.

Poly(ethylene terephthalate) has been recognized as one of the greatest synthetic polymers that build up in the environment at overwhelming rate as unwanted packaging and textiles. It has been observed that the utilization of poly(ethylene terephthalate) had several limitations which might be linked to its high resistance to biodegradation. In view of the aforementioned, Austina et al. (2018) isolated a new bacterium *Ideonella sakaiensis* 201-F6 that possess the capability to utilize poly (ethylene terephthalate) as energy and carbon sources. It was discovered that this strain possess that potential to secrete PETase (PET-digesting enzyme). Their study indicated that 0.92 Å resolution X-ray crystal structure of PETase which showed the common features to lipase and cutinase. It was established that PETase preserves the inherited α/β-hydrolase fold but displays a more open active-site cleft when compared to homologous cutinases. The narrowing of the binding cleft through the application of mutation of two active-site residues to preserved amino acids in cutinases, we amazingly perceive enhanced PET degradation, portentous that PETase is not completely improved for crystalline PET degradation, regardless of apparently surfacing in a PET-rich environment. Furthermore, the authors showed that PETase degrades which is another polyethylene-2,5-furandicarboxylate which is another semiaromatic polyester which has been recognized as a new bioderived polyethylene-2,5-furandicarboxylate replacement with enhanced barrier properties. Conversely, PETase does not possess the capability to biodegrade aliphatic polyesters which shows that it is aromatic polyesterase. Their study indicated that incorporation of protein engineering to enhance PETase activity is accurate and acme the requisite for supplementary growths of structure/activity associations for the biodegradation of synthetic polyesters.

Unresponsiveness and the undiscriminating utilization of chemical polymer has been identified as a factor that constitutes water and land pollution. The application of plastic has been identified in various utilization such as household practices, packaging industries, agriculture. It has been recognized that the indiscriminate application of chemical polymers has led to build up of solid waste in natural environment. This has constitutes several hazards to human and environment. This might be linked to the poor biodegradation of plastic. In view of the aforementioned, Pathak and Navneet (2017) wrote a comprehensive review on the application of microorganisms that possess that capability to biodegrade plastic and synthetic polymers. The authors also shed light of the potential of bacterial and fungal isolates for the biodegradation of plastic. Some of the highlighted strain includes *Mucor rouxii, Pseudomonas aeruginosa*, *Pycnoporus cinnabarinus, Pseudomonas stutzeri, Fusarium lini*, *Clostridium thermocellum, Streptomyces badius,* Aspergillus *flavus, Rhodococcus ruber*, *Aspergillus niger, Comamonas acidovorans*, and *Butyrivibrio fibrisolvens.*

Table 14.1 shows some techniques used in the degradation of plastics, types of enzymes, and microorganisms involved.

14.3 Specific Examples of Microorganisms that Could Degrade Plastic

The economic benefits derived from the use of plastics cannot be quantified. About 350–400 million load of it are produced year in year out. Plastics have been proven to be nonbiodegradable. This nature of it can cause serious environmental and health problem especially microplastics. However, of recent, several techniques have been developed and employed in order to break this jinx of nonbiodegradability. Danso et al. (2019) did a review of the viewpoint of environmental and biotechnological prospects of using bacterial in the degradation of plastics. The authors stated that PET (polyethylene terephthalate) and PUR (polyurethane) are active chemicals found in plastics which are very noxious. That many microbial consortia are still been evaluated to ascertain specific enzymes that can degrade plastics. However, several studies have been carried out on the degrading potential of some microbial consortia without specifying any enzymes that can breakdown polymers with high molecular mass such as polyethylene, polyurethane (ether-based), polypropylene, polyvinylchloride, polyamide, and polystyrene. In conclusion, the authors recommend that specific research on the richness of enzymes and microbial consortia action on plastics should be carried out in order to tap into the protein-metagenomes of native and non-cultivated bacterial potentials. This will pave new grounds for bio-catalyst consortia that can degrade plastics to useable products (oligomers and monomers) for human benefits in turn reduce the worldwide issues caused by plastics.

Shah et al. (2008a, b) in a review looked at the remediation potential of bacteria on plastics. The authors recounted the environmental problems causes as a result of the nonbiodegradable nature of plastics. This has necessitated the global awareness of their potential ecological and health threats. The authors stated that bioremediation is very important for water immiscible plastics, the reason that, when they enter the aquatic environment eventually, they cannot be incinerated nor recycled. In conclusion, the authors recommend that it is very important to understand the mechanisms involved in bioremediation via considering the microbial consortia to be used in synthetic or natural plastics. In addition, the biochemical reaction involved in the interaction between the microbial consortia and the plastic materials should also be understood immensely. Again the utilization of in vitro techniques is highly encouraged in the biodegradation of plastics.

Bassi (2017), in a book, reviewed the biological technology for the management of plastics. The author stated that wastes generated from plastics are unending and need urgent management strategies. The utilization of microorganisms in the breakdown of plastics can yield many biomaterials that can be used in the agricultural and

Table 14.1 Biotechnological techniques used for the biodegradation of plastics

S/N	Strain/species	Methods of degradation	Types of enzymes	References
1	Strain TF1 (*Actinomadura* sp.) and strain T12-1 (*Actinomadura keratinilytica*)	Turbidity	Serine hydrolase	Sriyapai et al. (2018) and Sukkhum et al. (2009)
2	Strain 9AHK119 (*Thermobifida alba*) and *Thermomonoaspora*	Not specified	Cutinase	Sukkhum et al. (2009), Hu et al. (2010) and Kitadokoro et al. (2019)
3	Strain T9-1 (*Nonomuraea fastidiosa*) and strain L44-1 (*Nonomuraea terrinata*)	Turbidity	Not specified	Sukkhum et al. (2009)
4	Strain FTPLA (*Thermopolyspora flexuosa*) and *Thermopolyspora*	Not specified	Not specified	Sangwan and Wu (2008) and Husárová et al. (2014)
5	Strain KKU215 (*Streptomyces* sp.) strain APL3 (*Streptomyces* sp.)	PLA-packaging surface change and weight loss	Serine hydrolase	Sriyapai et al. (2018) and Yottakot and Leelavatcharamas (2019)
6	*Streptoalloteichus* sp. and strain RM423 (*Pseudonocardia*)	Residual films in the culture broth TOC (total organic carbon) and film-weight loss; CO_2 content.	Not specified	Jarerat et al. (2002) and Apinya et al. (2015)
7	Strain AS4.1531^T (*Pseudonocardia alni*)	Film-weight loss; monomer production	Not specified	Konkit et al. (2012)
8	*Kibdelosporangium aridum*	Film-weight loss; monomer production	Protease	Jarerat and Tokiwa (2003)
9	Lentzea (*Saccharothrix wayanadensis*)	Film-weight loss; monomer production	Protease	Jarerat and Tokiwa (2003) and Nair et al. (2012)
10	Strain SCM_MK2-4 (*Amycolatopsis oliviviridis*)	Turbidity	Lipase, esterase, and protease	Penkhrue et al. (2018)
11	Strain CMU-PLA07^T (*Amycolatopsis thallandensis*)	Not specified	Not specified	Chomchoei et al. (2011)
12	*Amycolatopsis orientalis*	Film-weight loss	Serine protease	Li et al. (2008)
13	Strain K104-1 (*Amycolatopsis* sp.)	Turbidity	Serine protease	Nakamura et al. (2001)

(continued)

Table 14.1 (continued)

S/N	Strain/species	Methods of degradation	Types of enzymes	References
14	Strain 41 (*Amycolatopsis* sp.)	Film-weight loss; monomer	Protease	Pranamuda et al. (2001)
15	Strain ATCC 27649 (*Amycolatopsis mediterranei*)	Clear zone	Not specified	Pranamuda and Tokiwa (1999)
16	Strain KT-s-9 (*Amycolatopsis* sp.)	Film-weight loss; monomer production	Protease	Tokiwa et al. (1999)
17	Strain 3118 (*Amycolatopsis* sp.)	Film-weight loss; monomer production	Protease	Ikura and Kudo (1999)
18	Strain HT-32 (*Amycolatopsis* sp.)	Film-weight loss; monomer production	Protease	Pranamuda et al. (1997)
19	Strain B7-3 (*Micromonospora viridifaciens*	Turbidity	Not specified	Sukkhum et al. (2009)
20	Strain B12-1 (*Micromonospora echinospora*)	Turbidity	Not specified	Sukkhum et al. (2009)

food industries. In conclusion, the author recommends the utilization of bacterial enzymatic and biocomposites or grafting techniques for the degradation of plastic wastes.

The usefulness of plastics in our current generation is so enormous. The non-biodegradable nature of plastics has led to their long shelf life in the environment. However, this has led to an uncontrolled proliferation of them in the ecosystem and persistent pollution. Wierckx et al. (2018) did a review of the opportunities and challenges faced in the biodegradation of plastic wastes. This is an attempt to reduce pollution. Moreover, studies have shown the emergence of engineered microorganisms which can degrade or decontaminate recalcitrant high polymers molecular connection via some enzymatic reactions. In conclusion, the authors recommend a better viewpoint on plastic remediation by the utilization of pre-treatment-thermo-chemical and substrates of microbial enzymes as a future panacea.

Philp et al. (2013), in a review, looked at the possibility for a bio-economy using a bio-based plastic technique in recycling plastic wastes from biodegradation. The specificity for a bio-economy is derived from the utilization of chemicals and oil-based materials from the biodegradation of useful materials from biorefineries by microbes and biomass-derived substances from the process (biocomposting). In conclusion, the authors recommend more improvement, awareness, and attention in their shared market values, while anticipating a sustainable contributions toward climate change alleviation.

Zheng et al. (2005) did a review of the biotechnological improvement in the degradation of plastics and associated wastes. The authors recounted the importance of plastics to every facets of human lives. The need of the breakdown of plastics by microbes is very important because of the ecological and health risks they portend.

Extracts such as starch and pro-oxidants employed as artificial materials to make and change plastics degradation potentials are recent studies techniques used in plastic degradation. However, thermoplastics gotten from polyolefins, are native recalcitrant material which is known to be resistance to bioremediation process. However, several methods such as chemical and photo/light degradation are used in the process. Nonetheless, polymers of plastics (thermoset plastics) such as polyurethane and aliphatic polyester are easily broken down or eaten by microbes because of the probable urethane or hydrolytic cleavage molecular bonds they possess as well as a major source of nitrogen and carbon for the microbes. The authors in conclusion suggest the utilization of co-polyesters from aliphatic aromatic hydrocarbons for commercialization because of their biodegradability and mechanical characterization. More so, probable novel methods should be looked into in order to reduce the influence of plastics wastes on the ecosystem.

PLA (polylactic acid) has been recognized as one of the environmental concerned plastics derived from bioplastics, a renewable and biodegradable polymer which is used to supplant petroleum-established plastic materials. Butbunchu and Pathom-Aree (2019) did a mini review of the potentials of Actinobacteria in the biodegradation of polylactic acid for bioplastic. The authors stated that Actinobacteria enzymatic action, as in degradation of the bioplastic, is a function of economic value and environmental safety for waste control. Specific examples of such bacterial found in this phylum Actinobacteria is the family *Thermomonosporaceae, Streptosporangiaceae, Streptomycetaceae, Micromonosporaceae and Psuedonocardiaceae*. The authors stated that the cultivation of the degrading species of the phylum Actinobacteria in the laboratory settings has been shown to be a serious trial procedure. They resounded that a well-sounded taxonomic understanding on data of specific taxa of importance will pave a way to enhance cultivation and isolation for polylactic acid degrading microbes. More so, information on novel quality of the genome of the polylactic acid bacteria will improve their degrading potentials. In conclusion, the authors recommend the utilization of two important viable and highly vigor Actinobacteria; *Actinomadura* and *Amycolatopsis* to be the best candidates for degrading bioplastic. More so, their economic worth in the market have gone higher. In addition, high consideration should be placed on these strains when sampled, cultured, and isolated for remediation purposes.

Gaytán et al. (2020) tested and evaluated the degrading potentials of bacterial consortia on xenobiotic residues and polyurethanes recalcitrant from different landfill. The authors elucidate the mode of action of bacterial consortia play when they feed on polyurethanes plastics. That degrading polyurethanes plastics bacteria can grow in water polyurethanes dispersion (WPUD) media as the solitary model and carbon base for the BP8 landfill bacterial consortia. The composition of the WPUD are mainly glycol ethers, isopropanol and *N*-methyl 2-pyrrolidone-xenobiotic extracts and PE-PU-A (polyether-polyurethane-acrylate). The results of the study showed that the biodegradation process yielded ether groups by oxidative and hydrolytic mechanisms, recalcitrant aromatic urethanes, C–C and BP8 cleaves esters, both in the hard and soft segments of the co-polymer. The results of the metagenomic study, revealed five genomes of which three of them were new strains of microorganisms. More so, the results of the biodegradative process showed that the metabolic pathways, putative enzymes, genes programming enzymes and metagenome were the most identified activities in the bacterial consortia over the PE-PU-A co-polymer and the additives. In conclusion, the authors recommend bacterial consortia gotten from landfill as the base candidate for the biodegradation of xenobiotic residues and polyurethanes recalcitrant in the environment.

The impacts of plastic on benthic marine biogeochemical cycling and distribution of organisms are currently gaining immense environmental attention. Pinnell and Turner (2019) tested and evaluated the response of bacterial consortia to bioplastic and plastic in marine benthic ecosystem using the metagenomic shotgun technique in water–sediment boundary. The results of the study indicated that there was misty comparison between the plastic biofilms and the control (ceramic biofilm). The most dominated and distinct biofilms bioplastic was SRB (sulfur-reducing bacteria). The results of the gene pools of the bioplastic showed that the process was enhanced by many enzymes; dsrAB (dissimilatory sulfite reductases), aprBA (adenylyl sulfate reductases), depolymerases, and esterases. In addition, about twice of 20 enhanced genetically phenotypic different PHB (polyhydroxybutyrate) enzymes (depolymerase) indicated that the bacterial consortia was evenly distributed. The results of the metagenomic of the engineered genome, revealed two new species/strains; Desulfobulbaceae and Desulfobacteraceae amid the SRB with their genome consisting of both sulfur reduction and bioplastic degrading genes. The findings from their study showed that there was a significant enhancement of the gene pools and diversity of the bacterial consortia by the bioplastic. The authors in conclusion stated that if pollution from plastic is transacted for pollution from bioplastic, there will be a large sedimentary contributions, and the bacterial response might unknowingly impact the biogeochemical and benthic activities via the stimulation of the SRB.

Chukwuma et al. (2012) in a review looked at the challenges and prospects in using biotechnological tools for the sustainability of the environment. The authors stated that for a sustainable ecosystem, the best way of controlling wastes is by recycling them into useable forms, so that the living an nonliving factors of the environment can maintain a healthy and esthetic steady state. This natural technique is the best approach in removing harmful substances from the environment-bio-tools. The authors listed some bio-tools (biomass fuel production, bioremediation, biofiltration, biosolvent/biodetergent, biocatalysis, bioleaching, biomonitoring, and aquaculture management/treatment) currently used in mitigating pollutants in water, sediment, and soil. In conclusion, the authors stated that bioenvironmental tools for bioremediation are sustainable and the mechanism are closer to nature, efficient, and faster than other conventional methods.

Roohi et al. (2017) did a review of the potential of enzymatic degrading bacteria on plastics. The authors recounted the benefits (biomedical implants, garbage bags, paper coating, and packaging) derived from the recycling and renewal of plastic wastes. However, the increase in wastes from plastic production is alarming. the breakdown of the bio-polymer is linked to the production of water molecules, methane, carbon dioxide, and low-weight monomers. The authors in conclusion proposed a novel disposal method for the breakdown of polymers as well as new enzymatic degradation of plastic and inexpensive manufacture of decomposable plastic.

Siracusa (2019) in a review looked at the degrading potential of artificial biopolymers by microbial consortia. The authors stated that the demand for polymers that are biodegradable has risen (20–30%) over the last 10 years with a market share of <0.1%. They said that the incentives gotten from natural renewable resources can reduce the total dependency on petroleum resources. The wastes from natural materials such as wood, cellulose-straw, potatoes, cereals-starch, and oilseed crops can be converted into polymers and chemical intermediates. However, the utilization of renewable natural materials for bioplastic production, cannot be vouched for any negligible environmental influence. Moreover, bioplastics are commonly biodegradable, nevertheless the dispersion of the composting technology is a precondition for their advancement. In conclusion the authors suggested that more efforts should be put in place in order to optimize high performance and less expensive products for a sustainable ecosystem.

Of recent, plastic pollution has drawn more attention because of the ecological and health risks it portends. Shovitri et al. (2017) tested and evaluated the degradation of plastics by soil-burial technique with strains PL-01 (*Pseudomonas*) and PL-01 (*Bacillus*) native microbes. The authors recounted their previous study on plastics using similar strains. The strains were able to breakdown about 10% of plastics. However, the results from their current study for 16 weeks revealed positive influence by the two strains on the degradation of the plastics. *Bacillus* sp. had more impact than *Pseudomonas* sp. It was noticed that transparent plastic degraded faster than other colors (white and black) plastics “Kresek” bags. The results of the biodegradation performance of the soil microbes showed that the native mangrove soil microbes performed better in plastic degradation and biofilm formation without *Pseudomonas* and *Bacillus* strains addition. The FTIR (Fourier transform infrared) examination confirmed that there were reduced peaks of diffusion, indicating chemical efficient assemblage changes happening in the plastic compound after the study regime (16 weeks).

Pathak and Navneet (2017) did an extensive review of the current level of polymer degradation using different bacteria and fungi strains. The authors recounted the ecological risks associated with the undiscriminating use of artificial polymers on water and land. The application of plastic is very elaborate. Over use of the artificial polymers can increase the level of pollution in the environment which in turn affect the living and nonliving components therein. This pollutant, plastics, is seen as a potential threat because it is nonbiodegradable. However, microorganisms (bacterial and fungi) are the current bio-tools used in the biodegradation of xenobiotic and recalcitrant pollutants like plastics. Specific examples of such are: bacteria (*Butyrivibrio fibrisolvens*, *Clostridium thermocellum*, *Comamonas acidovorans*, *Rhodococcus ruber*, *Streptomyces setonii*, *Streptomyces badius*, *Pseudomonas stutzeri*, and *Pseudomonas aeruginosa*) and fungi (*Mucor rouxii*, *Pycnoporus cinnabarinus*, *Fusarium lini*, *Aspergillus flavus*, and *Aspergillus niger*). They stated that biofilm development enhances the degradation efficacy of plastic pollutant, then mineralization follows. The most efficient bacteria (*Pseudomonas aeruginosa* CA9) has been recounted to have well-enhanced biodegradation potential with low-density polyethylene (LDPE). While AKS2 strain (*Pseudomonas* sp.) has been recounted to degrade and form biofilm on low-density polyethylene by improving the bacterial development by 31% hydrolytic activity and 26% superficial hydrophobicity. *Psuedomonas stutzeri* was recounted for an increase in molecular mass/weight of polyethylene glycol (PEG) breakdown. Two strains 75Vi2 and 252 (*Streptomyces setonii* and *Streptomyces badius*) were recounted to degrade and colonize polyethylene by making hydrolyzing enzymes and biofilm on it. They stated that the enhancement of the degradation of polyethylene was via introduction of additives of peroxidant during the production process. This made it vulnerable to light and chemical mineralization and in vitro beneficial for polyurethane-polyester breakdown via polyurethane esterase enzymatic hydrolysis and production. The reason for this was because of the chief gene pudA, programming the enzyme polyurethane esterase. *Aspergillus niger* a fungi species produces an enzyme (acetyl xylan esterase) which works in synergy with endo-xylanase for competent breakdown of xylan. *Aspergillus flavus* and *Aspergillus niger* have been recounted to be best for the fast mineralization of average-length monomer chains. While *Aspergillus niger* has been known to be more effective in polythene degradation, *Aspergillus flavus* has been recounted for both polythene and polycaprolactone (PCL) degradation. In the same vein, Streptomyces, *Aspergillus flavus,* and strain NRRL 1835 (*Mucor rouxii*) have been reported to be linked with starch founded polyethylene breakdown. The fungal species *Fusarium lini* has been reported to be associated with the manufacturing of an enzyme dehydratase that is involved in the breakdown of polyvinyl alcohol with water and carbon dioxide formation. The white fungus (*Pycnoporus cinnabarinus*) has been associated with polyvinyl alcohol (PVA) degradation with the manifestation of a chemical agent—Fenton's reagent. In conclusion, the authors recommend more studies on the evaluation of effective and new bacterial species in order to reduce the ecological and health risks associated with plastics in the environment.

Sangale et al. (2019) isolated and tested the biodegradation potential of fungi sourced from mangrove soil in the degradation of polythene. The authors stated the wide utilization of plastics, of which polythene had the largest (64%) share. However, there are many approaches been developed to control and reduce the increasing amount of wastes from plastics of which biodegradation promises to be more effective, eco-friendly, and sustainable. The polythene degrading fungi (109) was sourced isolated from the soil-root (rhizosphere soil) of *Avicennia marina* from 12 zones across the coast of West Indian and screened under pH of 3.5, 7, and 9.5 for 60 days based on the tensile strength and weight of the polythene. The results of their study indicated that strains PNPF15/TS (*Aspergillus sydowii*) and MANGF1/WL (*Aspergillus terreus*) were the most efficient fungi that degraded the polythene plastics out of the 109 isolates of fungi in the following rates: 94.44 ± 2.40% loss in TS, pH 3.5 and 50.00 ± 4% WL, pH 9.5, respectively. The results from the scanning electron microscope (SEM) revealed that the breakdown polythene had cracks such as disturbances (holes, fissures, and scion) which showed weathering. The result of the Fourier transform infrared (FTIR) spectroscopy showed the various formations after the ultraviolet (UV) and chemical treatments in the control as 1630–1840 cm^{-1} (carboxylic group), 2915 cm^{-1} (CH stress), 1630–1840 cm^{-1} (carboxylic group). and 1710–1740 cm^{-1} (carbonyl group). The findings from their study showed reduced peaks after the treatment with the fungi strains. This indicates eating of the polythene plastics by the fungi acids (carboxylic and carbonyl) as well as the polymerization of the plastic polythene unit structures.

Plastics are inexpensive, strong, harsh resilient materials, durable, and light weighted substances, which have been reported to have long lasting adverse effect on the ecosystem. Raziyafathima et al. (2016) in a review, looked at the degradation of plastics wastes by microbes. The authors recounted the ecological and health risks posed by wastes from plastics when heated up by UV light. In the light of this, scientists have developed biodegradable plastics that are eco-friendly and not noxious even at room temperature. The authors, in conclusion, recommend the use of microbes for the effective degradation of wastes from plastics.

Changes brought by anthropogenic activities on the marine ecosystem as a result of plastic influence can impede the health of the coastal environment. Urbanek et al. (2018) did a review of plastic degradation by plastic-eating microorganisms in an icy marine ecosystem. The authors stated that the impact from plastic pollution without permanent remediation can live an indelible ecosystem injury. The artificial plastics are the major debris in the benthic region of the ecosystem that constitute a blockage to the food chain structure occasioned by humans. However, this problem remained unresolved, but several approaches have been used to reduce the impacts on the marine ecosystem. Biodegradation a process using microorganisms to degrade wastes like plastic in the environment. Nonetheless, in a cold region, the authors presented some microbes that can be utilize to degrade plastics in cold environment. Specific examples of are; *Rhodococcus*, *Micrococcus*, *Arthrobacter*, *Corynebacterium*, *Streptomyces*, *Pseudomonas*, *Flavobacterium*, *Cryobacterium*, *Cryobacterium*, *Leifsonia*, *Agreia*, *Subtercola*, *Micrococcus* and *Polaromonas* that are sourced from cold environment. Others are *Shewanella*, *Pseudoalteromonas*, *Marinomonas,* and *Colwellia*. The authors stated that the impact of biofouling bacterial consortia are not well understood as well as the relationship between the microbes and the plastics. However, the microbes inhabiting colder regions of the world have natural potentials differ from others from other marine ecosystems. The reason is that the nature of the environmental condition as well as the increasing rates of wastes from plastics forces them to acclimatize to new-fangled substrates. The authors in conclusion opined that natural acclimatization of microbes might take much time. This will eventually slow the rate of degradation, and pollution from plastics will increase and might be irremediable.

Odusanya et al. (2013) in a preliminary study, isolated, characterized and evaluated the degradation of plastic bottle by microbes in Nigeria. The LLDPE (Linear Low Density Polyethylene) potable plastic bottle was used employing a simple proprietary solvent technique to powderize and solubilize it. Utilizing an enrichment culture techniques, eight bacterial colonies were isolated which were capable of breaking down LLDPE into useable carbon source. The most productive organism observed was *Serratia marcescens*. Results showed that the organisms isolated and characterized were gram-rod bacteria. While the H_2S and indole production was also negative. The test for fermentation was positive for citrate, sorbitol alanine, fructose, glycerol, sucrose, and glucose. However, the factors surrounding the coloration could not be found but the UV zone absorbed some radiation and form ferric chloride precipitate. The results from the scanning electron microscope exposed some depression that were linked to the potential breakdown of the plastic by the *Serratia marcescens*. The plane surface of films in the control without the introduction of *Serratia marcescens* was shown by the micrographs. The results of the glass transition temperature (Tg) of the undegraded and graded plastics as confirmed by the DSC (differential scanning calorimetry) were 63.33 and 52.43 °C, respectively, indicating an increase of the rate of movement small chain length formed after the biodegradation of the plastics. More so, the measurement of the differential scanning calorimetry in addition revealed the crystallization enthalpy (AH) before and after to be 89.936 and 31.945 J/g respectively. A reduction was spotted in the crystallization temperature and enthalpy of crystallization to be 118.980–112.25 °C and (−) 83.241 to (−) 34.776 J/g respectively. The findings from their study showed that the Differential Scanning Calorimetry was able to indicate the relationship of reduction between crystallinity and the biodegradation procedures.

Muhonja et al. (2018) isolated, tested, and evaluated the degradation potential of fungi and bacteria isolates from a specific dumpsite in Kenya in degrading polyethylene. The results of the FTIR (Fourier transform infrared) spectroscopy analysis showed the presence of new-fangled clusters accredited to the degradation of hydrocarbon by the consortia of fungi and after incubation. The results of the evaluation of the 18S rDNA and 16S rDNA sequence of the fungi and bacteria, revealed that the fungi belong to the genus *Aspergillus*, while the bacteria belong to the genus *Lysinibacillus*, *Cellulosimicrobium*, *Brevibacillus*, *Bacillus*, and *Pseudomonas* were connected as degraders of polyethylene. Further analysis of their results showed that the bacteria were poor degrader when compared with their counterparts; fungi which were better degraders. It was observed from their study that the highest mean from the fungi reduction activity was 36.4 ± 5.53% linked to strain A5, 1 MG779508 (*Aspergillus oryzae*). While for the bacteria, strain B2, 2 MG645267 (*Brevibacillus borstelensis*) and strain A5, a MG645264 (*Bacillus cereus*) had the highest mean a values of 20.28 ± 2.30% and 35.72 ± 4.01% respectively. The results of the LDP (low-density polyethene) degradation, established that *Brevibacillus*, *Bacillus*, and *Aspergillus* were good degrader contenders among the other strains. This findings was in advance established by the presence of carboxyl efficient clusters, ether and aldehyde after the Fourier transform infrared (FTIR) spectroscopy analysis of the ketone; and intercessor culture media product and the polythene pieces. In conclusion, the authors suggested the application of the findings from their study into large-scale and commercial purposes.

14.4 Conclusion and Future Recommendations

This chapter has discussed extensively the practical application of beneficial microorganism that could degrade plastic and synthetic polymers. It was established in this chapter that microbial degradation of plastic has several merits when compared to physical and synthetic approaches. Furthermore, the application of engineered biodegradation pathways should be encourage to enhance the biodegradability capability of these potential strains. The modes of action through which these strain break down the surface of the polymer were also discussed in details. The application of techniques such as atomic force microscopy and scanning electron microscopy was also elucidated for the validation of the role of these biodegradative strains most especially their degradative role on the surface of these plastics. The application of Fourier transform infrared spectroscopy for the detection and monitoring of the biodegradation of these plastic was also highlighted in detail. This chapter also established that the application of potential strains isolated from landfill environment could be utilized for the biodegradation of plastic wastes in a controlled environment such as landfill or in dumped soil.

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Chapter 15
Bioaugmentation: A Powerful Biotechnological Techniques for Sustainable Ecorestoration of Soil and Groundwater Contaminants

Charles Oluwaseun Adetunji and Osikemekha Anthony Anani

Contents

Abstract The recent advances in industrialization and constant application of agropesticides have led to increase in the release of hazardous compounds into the environment. Most of these hazardous compounds possess several adverse effects which entail high level of toxicity, accumulate, and persist in the environment, impairment of human health because most of these toxic compounds are anthropogenic and mutagenic in nature. The application of microorganisms possess the capability to remove pollutants available persistently in contaminated soils. Bioaugmentation has been recognized as a sustainable bioremediation technology which involves the application of beneficial microorganism for the ecorestoration of heavily polluted environment. Therefore, this chapter provides a comprehensive detail on the application of bioaugmentation for the ecorestoration of heavily polluted environment. Information on the gene bioaugmentation, rhizosphere bioaugmentation, and their utilization in the bioremediation of polluted soil has been discussed in detail. Special emphasis has been laid on some specific gene

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C. O. Adetunji (✉)
Applied Microbiology, Biotechnology and Nanotechnology Laboratory, Department of Microbiology, Edo University Iyamho, Auchi, Edo State, Nigeria
e-mail: adetunji.charles@edouniversity.edu.ng

O. A. Anani
Laboratory of Ecotoxicology and Forensic Biology, Department of Biological Science, Animal and Environmental Unit, Faculty of Science, Edo University Iyamho, Auchi, Edo State, Nigeria

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responsible for the process of bioaugmentation. Moreover, the movement of horizontal gene transfer during the process of bioaugmentation such as transformation, conjugation, and transduction is also highlighted. Future recommendation and useful suggestion are also highlighted which could boost the application of bioaugmentation for bioremediation of polluted environment.

Keywords Bioaugmentation · Bioremediation · Microorganisms · Environment · Gene · Contaminants

15.1 Introduction

The drastic advancement in the technological development and the dynamic civilization coupled with high rate of industrialization, intensive application of large-scale heavy metals, wars and chemical xenobiotic have led to several environmental and health hazards (Bayat et al. 2015; Akhtar et al. 2003). Typical examples of such pollutants include polycyclic aromatic hydrocarbons, pesticides, petroleum products (Belanger 2010, Chatterjee and Lefcovitch 2014), chloro- and nitrophenols and their derivatives, organic dyes, and heavy metals (Mohamed et al. 2016; Rodgers-Vieira et al. 2015; Smułek et al. 2015; Wasilkowski et al. 2014; Wojcieszyńska et al. 2013, 2014; Greń et al. 2010).

The application of agricultural pesticides for the management of agricultural pest and the consumption of pesticides for agricultural purposes was approximated up to 2.36 million tons (Moreno-Medina et al. 2014). The continuous application of these pesticides may lead to several adverse effects on the beneficial component of the ecosystem such as human, soil structure, soil enzymes, and soil microorganisms because pesticides also have a detrimental effect to non-target organisms (Moreno-Medina et al. 2014; Mesnage et al. 2014; Roberts and Karr 2012).

Furthermore, it has been highlighted that some metabolites of some pesticides are also toxic and may be a major source of soil contamination. For example, 2,4-dichlorophenol and *p*-nitrophenol are the significant metabolite derived from 2,4-dichloropenoxy acetic acid and *p*-nitrophenol degradation (Herrera et al. 2008; Wojcieszyńska et al. 2008; Liu et al. 2007; Gallizia et al. 2003).

Therefore, there is a need to search for a sustainable and biological solution that could lead to bioremediation of these heavily contaminated environment. The application of some beneficial microorganism have been highlighted as a sustainable biotechnological solution that could mitigate all the highlighted environmental hazards and contamination (Adetunji et al. 2017, 2018, 2019a, b, 2020; Adetunji and Adejumo 2017, 2018, 2019). This might be linked to the fact that they possess the capability to biodegrade all the hazardous and synthetic pollutants (Lade et al. 2015; Kaczorek et al. 2013).

Bioremediation has been recognized as the application of beneficial microorganism for the sanitization of heavily polluted environment. This might be linked to the fact that these microorganisms could use most of these pollutants as a substrate, then degrade, metabolize, or then chelate various toxic compounds (Tausz and Donath 1930; Mosa et al. 2016). Microorganisms possess the capability to biodegrade contaminants by cometabolism or the utilization of these pollutants as a carbon source (Mosa et al. 2016; Garbisu and Alkorta 2003). Bioremediation has been identified as a sustainable biotechnological solution that could mitigate all the highlighted environmental challenges, This might be linked to the following attributes such cost-effectiveness, noninvasive, eco-friendly, and sustainable without any form of contamination (Garbisu and Alkorta 2003; Perelo 2010; Kulik et al. 2006; Xu and Lu 2010). Bioremediation of heavily contaminated soil can be performed ex situ which might be at a certain place or in situ which might be at the place of contamination. (Xu and Lu 2010; Angelucci and Tomei 2016; Tomei and Daugulis 2013). The process of in situ bioremediation involves three major processes such as natural attenuation, bioaugmentation, and biostimulation (Suja et al. 2014; Pimmata et al. 2013).

The process of bioaugmentation involves the introduction of certain microorganisms that possess some special potential to break down some certain contaminants which some indigenous microorganisms might not be able to break down, or when these indigenous microflora are not available in sufficient amount (Pimmata et al. 2013; Simarro et al. 2013). Therefore, in order for these microorganisms to perform the process of bioaugmentation effectively, they must possess certain features such as capability to degrade certain pollutants whether in immobilized or mobilized inoculum state, and they must be able to survive in an adverse environment move through pore available in the soil. The process of bioaugmentation involves the application of indigenous microorganisms or genetically modified microorganisms could be utilized for the bioremediation purposes. The process of bioaugmentation depends on the level of relationship between indigenous and exogenous populations of microorganisms due to the fact that they all depend and compete for the availability of nutrients (Simarro et al. 2013; Hamdi et al. 2007; Alisi et al. 2009; Ueno et al. 2007).

Therefore, this chapter intends to provide a comprehensive detail on the application of bioaugmentation as biotechnological tool for the bioremediation of heavily contaminated environment. The modes of action by which bioaugmentation were analyzed in detail. Various microorganisms that play crucial role in various bioaugmentation processes were highlighted. Future recommendations that will promote the sustainability of bioaugmentation approaches were also highlighted.

15.2 Techniques Used for Bioaugmentation of Soil and Water with Specific Examples

Microorganisms naturally degrade waste materials or hazardous materials into usable form(s). In some cases, the process (bioremediation/biodegradation) might be less efficient and very slow. The addition of microbial or archaea cultures which are needed to enhance the rate of degradation of pollutants in a bioremediation process is called bioaugmentation (biological remediation). Bioaugmentation is usually employed in waste management to resurrect the activated slurry bio-reactor machine (ASBM). Microbes (fungi, rotifers, nematodes, protozoans, and bacteria) which are proficient in the ASBM degradation of wastes aid in the degradation of wastes to a non-toxic usable forms. These organisms are usually under studied in order to ascertain if they have the potential to catalyze a bioremediation reaction. If the native strains do not have the potential to speed/breakdown/bioaugment a bioremediation process effectively, an exogenous assortment with a more enhance capability is introduced in order to biostimulate the entire process.

Bioredegradation has been identified as a cheaper, ecofriendly solution for the bioremediation of polluted environment using microorganisms. Maruthi et al. (2013) perform an experiment to establish the role of fungal isolates in the biodegradation of organic compounds present in polluted soil with diesel and petrol. The result of the preliminary screening led to the isolation of two fungal strains that possess the capability to biodegrade total organic carbons from the oil-polluted sites. The experiment was performed inside an Erlenmeyer flasks under aerobic conditions. It was discovered that the total organic carbons vary from 0.7 to 32% depending on the concentration and the strain types. It was discovered that *Aspergillus niger* and *Phanerochaete chrysosporium* had the highest total organic carbons with 21% and 32% respectively before amending with nutrient. The level of total organic carbons was decreased after the media was amended through the addition of sulfur, nitrogen, and phosphorus most especially by *Phanerochaete chrysosporium* strains. The study showed that *Aspergillus niger* and *Phanerochaete chrysosporium* possess the capability to liberate more CO_2 and biodegrade the substrate hydrocarbon present in the polluted oil sites, and they could be used in the waste recycling process.

Ferraro et al. (2019) evaluated the application of an anaerobic bioremediation treatment for the recuperation of polycyclic aromatic hydrocarbons (PAHs) of polluted soil. The PAH-polluted sol was artificially primed, and seven various pollution conditions were evaluated. The soils were polluted with benzo[a]pyrene (D), naphthalene (A), pyrene (C), and anthracene (B) while the other treatments contained other experimented such as PAHs (i.e., A + D, B + D, and C + D tests). The experiment was carried out in order to validate the effect of degradation kinetic for the single entailed in single PAH which varies from aromatic rings ranging from 2 to 5 as well as establish the influence of adding PAHs together with a 5-aromatic ring contaminant (i.e., benzo[a]pyrene). The assay was performed in a bioaugmented condition using two microbial inoculant derived from anaerobic digestion tests on lignocellulosic substrate. The result obtained showed that the two inoculants varied by enriched through the assay featured by experiments characterized by chronological re-inoculation on new substrate, for its successive treatment, every 24 and 96 h, respectively. This present study centralized on the effectiveness of PAHs degradation, characterization of the microbiological abundance, and pathway which provide a holistic approach on the bioremediation of soil contaminated with PAHs.

Simarroa et al. (2013) evaluated the effect of various in situ bioremediation treatments which entail natural attenuation, bioaugmentation, biostimulation, and bioaugmentation on creosote-contaminated soil. Some of the parameters assessed were evolution of bacterial communities, toxicity, creosote degradation, and microbial respiration. The result obtained indicated that the creosote reduced significantly all the treatments, and no single variation was discovered among all the treatments. Moreover, it was discovered that some certain PAHs were broken down to a larger extent through biostimulation. The domination of low temperatures at an average of 8.9 °C lowers the microbial creosote and the polycyclic aromatic hydrocarbon uptake and polycyclic aromatic hydrocarbon degradation (>60%) at the completion of the experiment while the level of toxicity remains constant through the experiment. The result obtained from the biostimulation indicated maximum microbial biodiversity by the termination of the biodegradation process, while the composition of all the treatment varies from all the treatments in comparison with the control assay. It was later discovered that some of the uncultured bacteria belong to the genera *Sphingomonas, Balneimonas, Pseudomonas, Pantoea*, and *Flexibacter*. It was also established that *Pantoea* and *Balneimonas* possess the capability to degrade PAH while *Pseudomonas* genus was the most of the species identified during the process of creosote biodegradation. The result affirmed that some bacteria possess an intrinsic potential to degrade the creosote without previous exposure.

Bento et al. (2003) assessed the effect of bioaugmentation, natural attenuation, and biostimulation on the degradation of total petroleum hydrocarbons available in the polluted soils with diesel oil. It was observed that bioaugmentation exhibits the maximum degradation which includes heavy (C23–C40) fractions of TPH (75.2%) and light (C12–C23) fractions (72.7%) while natural attenuation shows more activity when compared to the biostimulation. The highest dehydrogenase activity of 3.3-fold was detected from bioaugmentation of the Long Beach soil followed by 4.0-fold by the natural attenuation of the Hong Kong soil. It was also observed that the population of heterotopic and microorganisms that possess that capability to degrade diesel oil was not influenced by bioremediation treatment. It was also established that the application of inoculum of microorganism pre-selected from their own environment gave the best approaches for the ecorestoration of soil polluted with diesel oil.

The pollution of the soil with aromatic compounds has been identified as a serious environmental concern which could lead to mutagenic and carcinogenic properties. In view of this, Koul and Gauba (2014) wrote a comprehensive review on the application of bioaugmentation for the bioremediation of heavily contaminated soil. The authors stated that bioaugmentation has been utilized as a biotechnological techniques for enhancement of the biodegradative potentials of polluted soil using some microorganisms. The amendment of pre-grown microbial cultures improves the breaking down of heavy metal and organic compounds. The most significant factor in the selection of potential microorganism that could break down most of these contaminants as well efficaciously compete with original micro flora. It was also stated that the application of genetic engineered microorganism could enhance the stability of indigenous microorganisms without affecting their biodegradation potential. Bioaugmentation is commonly applied in the bioremediation of municipal wastewater. Moreover, it was stated that remediation industry practices employed bioaugmentation as a sustainable approach for the bioremediation of various generated pollutant from their industry because it is cheaper and affordable, and it could facilitate the process of bioremediation on the site.

It has been stated that microorganisms possess the capability to enhance plant growth–promoting capability and bioremediation of heavily polluted sited with heavy metals. In view of the aforementioned, Arunakumara et al. (2015) isolated phosphate solubilizing bacterial strain and tested their effectiveness in the bioremediation of the following strains such as Co, Pb, and Zn and their potential to fast track their uptake by *Helianthus annuus*. The level of heavy metal was performed using the agar dilution techniques while the rate of metal uptake and the influence of phosphate solubilizing bacterium in the enhancement of the heavy metal uptake was established in a pot experiment while batch experiment was utilized for the establishment of bacterial inoculation on the movement of metals in soil. The characterization of the isolated that could solubilize phosphate using 16S rRNA sequence evaluation showed that *Klebsiella oxytoca* JCM1665 was the best strain among many others. It possesses the capability to solubilize phosphorus in the absence and presence of metals. It was also established that the inoculation of strain JCM1665 of *Klebsiella oxytoca* led to the improvement of *H. annuus* (49%, 22%, and 39%, respectively in Co, Pb, and Zn contaminated soils) when compared to the control plants while there was improvement in the level of translocation and accumulation of Co, Pb, and Zn from roots to shoots Also, the water-soluble fraction of Co, Pb, and Zn in soil was improved by 51%, 24%, and 76%, respectively in inoculated soils when compared to the control without any inoculants. Their study showed that *Klebsiella oxytoca* JCM1665 possess metal mobilizing capability and could enhance plant growth promotion with improved phytoextraction activity most especially for the soil polluted with Co, Pb, and Zn.

It has been observed that the process of bioaugmentation could enhance the process of microbial diversity and the level of soil fertility apart from playing a crucial role in the bioremediation of heavily polluted soil. Festa et al. (2016) evaluated the influence of bioaugmentation with *Sphingobium* sp. AM strain on numerous soil microbiomes, contaminated soil (Phe), chronically contaminated soil (IPK), and pristine soil (PS). The study was carried out to establish the role of these microorganisms in the bioremediation of these polluted soil and their role in the improvement of the ecology that drives bacterial communities after each inoculation of these isolates. It was discovered that AM strain draft genome classifies genes for the metabolism of aliphatic and aromatic hydrocarbons. Moreover, it was detected that inoculation enhances the removal of phenanthrene during the whole treatment of Phe no observable degradation of any PAH was detected. Also, pyrosequencing evaluation enhances the diversity and richness of contaminated microbiomes, hence autonomously of PAH degradation enhancement, we detected traces of inoculant formation, signifying it could utilize other resources to persist. The rate of inoculation does not have any effect on the bacterial community of PS. It was also observed that the incubation conditions enhanced the level of orders from *Sphingomonadales* and *Actinomycetales* while inoculation resulted in the reduction of level of *Actinomycetales* while the addition of most diverse microbiomes with inoculants most especially to PS and Phe led to enhancement in the level of orders from *Rhizobiales, Sphingomonadales*, and *Burkholderiales*. It could be concluded that there is a synergetic effect between all the genera which showed that there may not be any relation with PAH degradation.

It has been observed that the extemporaneous, natural self-attenuation of the groundwater polluted with oil-derived pollutants most especially in the groundwater environment has been observed to be slow which might warrant numerous amplification activities that are obligatory to speed up the process. It has been observed that ex situ bioremediation is one of the best treatments for the bioremediation of polluted soil from which the ground is evacuated from its natural site and developed into piles suited in different clean-up sites. This permits easy amendment and regulator of the development parameters and consents for delightful other optimization activities such as bioaugmentation with specially prepared microorganism cultures. In view of the aforementioned, Kaszycki et al. (2011) evaluated the influence of soil-derived bacterial community utilized as inoculum in the bioaugmentation of organic compounds. After inoculation, it was discovered that the level of the soil bacteria population was enhanced by 16–42 times and extended the value of 3.6×10^6 cells g^{-1}. The designated optimization activities, pragmatic for the first stage of the longstanding bioremediation scheme, permitted to accomplish substantial pollution removal rates: over 3.5-fold at the site P1 and over five-fold at P2.

da Silva and Alvarez (2010) wrote a comprehensive review of bioaugmentation. The authors recounted that bioaugmentation has been a major priority in the bioremediation process, employed to enhance the aforementioned process in degrading recalcitrant pollutants in the ecosystem. That proper inoculate aid in improving the efficiency and activity of the bioremediation process. Nevertheless, the entire process also depends on external factors that might militate against its set objectives for environmental restoration. The authors opined that there is a need to improve on the qualities of the strains used in the bioaugmentation process in order to boost the normal genetic constituents of the microbes and enhance the catabolic enzyme specificity and gene adaptability against critical environmental conditions such as redox condition and pH that may affect in situ condition of bioaugmentation. A better understanding of the biology and the chemo-taxis response away and toward sourced contaminants of the microbes is very important, in order to predict and monitor the process of regulation and to improve the distribution and perfusion of the micro-biota. In conclusion, the authors are of the opinion that a part of re-engineering the microbes adapt to abiotic stress, the issues of biological stress, such as struggle for food might also hinder the biological process. They recommend the selection of inhibited species that specifically hinder the biological process and add specific strains of bacteriophages to buffer the stress faced by the microorganisms during the pigmentation process. More so, there is a need to improve on a biological model that will be employed for predictive analysis of catabolic enzyme genes and other markers of biological stress, to ensure a perfect clean-up process. This will inform certain ecological decisions and forestall future strategy for a better ecorestoration.

Mrozika and Piotrowska-Seget (2010) in a review looked at the clean-up of soils polluted with aromatic compounds using bioaugmentation approach. The authors stated that most mutagenic and carcinogenic health risks are associated with the impacts from aromatic compounds, especially the poly/long chain forms. That bioaugmentation has been proven to be more efficient in the decontamination pollutants through the introduction of specific fit consortia of microbes that will enhance the degradation capacity of already existed inoculum. The authors also stressed the need to avoid external and inter ecological and biological stressors that will militate against the biological degrading process. They suggested that the improvement of bioaugmentation could be attained by distributing suitable microbes that are powerless on several transporters of triggered soil and the re-engineering of microbial gene.

The process of the decontamination of oil, diesel, and fuel hydrocarbons in a cold or snowy situation has become one of the greatest challenges faced in biotechnology of pollutants.

Kauppi et al. (2011) tested and evaluated the relationship of bioaugmentation and biostimulation in the improvement of bioremediation of oil, diesel, and fuel hydrocarbons in polluted soil during a cold or snowy condition. The authors used a different assortment of microorganism inocula, aeration, bulking negotiator, and nutrient alga under field and laboratory settings. The rRNA genes of the consortia microbes were explored. The results of their study indicated that proteo-bacteria were the most well highly distributed microbes in the consortia. The biodegrading process was more efficient when aeration and nutrients were slowly released concurrently. The microbial inocula was unable to improve the remediation of the soil nor was a long-lasting consortia density noticed in the laboratory setting. However, in the field setting, the result showed that there was enough aeration and excess decrease of moisture when the bulking negotiator was employed. The findings from their study showed that bioaugmentation was not effective under cold condition. The authors concluded that the rate of biostimulation through enhancement of oxygen and nitrogen source increased the remediation potentials of the consortia microbes in the cold soil unlike bioaugmentation.

Taccari et al. (2011) tested and evaluated the bioaugmentation and biostimulation impacts of microbial consortium on the decontamination of petroleum diesel. The biological control test was investigated for 120 days. Different substrates (-β-cyclodextrin; biosurfactant, compost, guano, and microbial consortium) were combined or individually used by the microbial population. The results of the biological study indicated that the adding of the compost guano with the microbial consortium elevated the activities of the heterotrophic aerobic microorganism which was suspected to be strain of *Pseudomonas*. Bioaugmentation and biostimulation were noticed to be on the increase in direct variance with the increase of the microbial diversity, as well as the dry and wet mass (biomass contents). The diversity and biomass of the microbial community were later reinstated at the expiration of the bioremediation after a sudden drop, instigated through the xenobiotic stressor. There were similarity between the microcosms and the microflora density population with or without the addition of biosurfactant. However, a decrease of the petroleum hydrocarbon was noticed below the situation tested. The findings from their study showed that a combined substrate (compost guano and the bacterial consortium) was significant in the decontamination of the petroleum hydrocarbon about 96%, after 120 days of investigation.

Sludge from petroleum hydrocarbon, specifically oil mixture, has been known to contain recalcitrant pollutants. Ragheb et al. (2011) tested and evaluated the bioaugmentation potential of oil sludge using an enhanced strategy. The investigation lasted for 198 days. Two microbial consortia were used alongside with microcosms consisted with PAHs and alkanes isolated from an oil sludge and soil. The results from their study showed that about 30% degradation of the TPH (total petroleum hydrocarbons) from the oily sludge. Although, the degradation of the alkane content was slightly removed. While, the asphaltic and aromatic parts were significantly improved via the adding of the other consortium. The findings of their study showed that resin a polar compound was significantly enriched with asphaltene and aromatic application. However, their volume in terms of concentration was reduced to the normal concentration at the culmination of the incubation timing.

The decontamination of polluted soils containing PAHs has become an evolving biotechnology approach. Typical biotechnological techniques used currently are bioattenuation, biostimulation, and bioaugmentation. María et al. (2016) in a book chapter reviewed different bioremediation techniques (bioattenuation, biostimulation, and bioaugmentation) used in the degradation of PAHs in polluted soil. The authors stated that these current biotechnologies are considered favorable, because of the advantages (ecological friendly, cost-effective, and do not produce any noxious substance), which the conventional techniques do not have both in the field and laboratory settings. The authors in conclusion recommend agricultural management as a panacea to the end-point of major pollutant in conjunction with the aforementioned bioremediation techniques.

Cosgrove et al. (2010) tested and evaluated the potential effects of bioaugmentation and biostimulation on the bioremediation of polyurethane suppressed in a soil. The authors used microcosms obtained from soil alongside with Impranil for the biostimulation process, yeast extract, and polyurethane degrading fungi for the bioaugmentation process. The results showed that the extract from the yeast for biostimulation in combination with Impranil, improved about 62% of the decontamination of polyurethane suppressed in the soil as compared to the control, and also linked with 45% alleged improvement of the degradation of polyurethane by the consortium organisms. The results of the bioaugmentation of polyurethane with the fungi showed about 28% degradation potential when wheat was added to mycelium-rich inoculum. This indicated that the wheat acted as a biostimulation impact on the degradation of polyurethane. A further addition of several strains of *Mucor mycotina* sp., *Penicillium ochrochloron*, *Penicillium viridicatum*, and *Nectria haematococca* enhanced about 30–70% of degradation of polyurethane. This informs that both bioremediation techniques (bioaugmentation and biostimulation) are working in synergy to recital of the degradation of the pollutant. The findings from their study revealed that bioaugmentation however spurred the numbers of the native consortium microbial and fungi population for effective bioremediation process. They recommend both the techniques as feasible instruments for the degradation of environmental pollutants with polyurethane.

Population increase and technological developments have been linked to the major generation of environmental concerned pollutants. These imbalances as a result of these impacts have caused impending stress in the biotic community. However, several methods have been employed in remediating the ecological concerned pollutant. Goswami et al. (2018) in a review looked at the different potential strategies of remediation, environmental pollutants using bioaugmentation and biostimulation techniques. The authors stated that bioaugmentation has been proven efficient in the remediation of recalcitrant pollutants using strains of microbes as well as the biostimulation of the process using regulating nutrients that will enhance the efficacy of the microbial strains in the remediation of the rate of degradation of some environmental concerned pollutants. The authors, however, pinpointed that the co-eco-friendly nature of the two bioremediation techniques has yet to be ascertained and recommend the evaluation of the ecological and health impacts of these techniques.

The uncontrolled use of fungicides in agricultural activities has yielded to rebound of recalcitrant chemicals like Azoxystrobin in the agro-ecosystem. However, the ecorestoration of soil contaminated by this chemical can forestall a healthy environment for soil micro and macro biota. Baćmaga et al. (2017) tested and evaluated the bioaugmentation potential of soil fungicide pollutant—Azoxystrobin. The authors investigated this with the use of catabolic enzymes (alkaline phosphatase, acidic phosphatase, catalase, urease, and dehydrogenases) secreted by the four microbial consortium strains [KJ843149.1 (*Bacillus megaterium*) KF831381.1 (*Bacillus weihenstephanensis*), KC848897.1 (*Bacillus cereus*), and LM655314.1 (*Bacillus* sp.)] and two fungi strains [JN943451.1 (*Aphanoascus fulvescens*) and AB861677.1 (*Aphanoascus terreus*)]. The results indicated that the microbial consortium was able to increase the breakdown of azoxystrobin in the contaminated soils within 90 days of investigation by the four microbial (24%) and two fungi (78%) strains correspondingly. Azoxystrobin was degraded by *Aphanoascus fulvescens* and *Aphanoascus terreus* by 9% in the sandy-loamy soils. The findings of the study showed that the activity of the soil catabolic enzymes was altered/ increased, due to inoculation of the topsoil by the microbes and fungi strains, which is also an indication of that the augmentation process has attained its objective compared to the control. The entire process created a suitable environment and effective removal of azoxystrobin as well as improved the adverse impact on the soil micro and macro biota. More so, it created an avenue to utilize microbial organisms in the contaminated soils in the bioremediation process of azoxystrobin.

In conclusion, the authors recommend the strains of microorganisms and fungi as the potential candidates for the decontamination of soil fungicides—azoxystrobin.

Ghaly et al. (2013) tested and evaluated the biodegrading potentials of pyrene a congener of PAHs. The efficacy of the degradation relies on the bioaugmentation and biostimulation of the soil environment with mycobacterium and toting of food or nutrients to the degrading media. Results showed that there was an increase in the number of microbial cells (40, 58, 70, and 132) in the bioaugmentation, biostimulation, and control group correspondingly. However, a pause time (0.5 days) and growth rate (0.896 day^{-1}) were noticed when bioaugmentation and biostimulation were combined as a treatment at mean temperature of 41 °C and minimum–maximum temperature of 28–32 °C. This was consequent as a result of the non-compensation of the gas lost during the organic matter breakdown in the remediation of pyrene in the bioreactor. The amount of pyrene breakdown was shown by the reduction of the oxygen level/concentration and the rise of the carbon (IV) contents in the bioreactor exhaust as compared to the control. More so, the level of O_2 to CO_2 in the treatment groups, bioaugmentation, and biostimulation were the same. However, at day 7 trial period, the concentration of O_2 to CO_2 declined. The greatest reduction (84.29%) of pyrene was noticed in the biostimulation-bioaugmentation process, followed by 87.56% of the bioaugmentation process, 50% of the biostimulation process, and 37% of the control group. The findings of this study showed that there were various degradation rates in the microbial phases (stationary, exponential, and lag) when both the bioremediation processes were combined.

Garbisu et al. (2017) did a review of the biodegradation of soil pollutants using bioaugmented facilitated plasmid method. Unfortunately, microbial degradation of soil contaminants sometimes is ineffective due to the rapid reduction of microbial sustainability and richness. This is consequent on the genes that encrypt in the biodegradation of organic compound found in the plasmids of the microbial cell. A facilitated plasmid technique in bioaugmentation targets to excite the binge of pollutant degradation of DNA segment among native strains of soil microorganisms via the preface of plasmids found in the contributor gene pool. This will enhance the host's ability for an effective degradation process. In conclusion, the authors suggested that for the entire bioaugmentation facilitated plasmid process to be more effective, an in-depth knowledge of the soil native consortium and the environmental factors that may militate against the plasmid expression and acquisition should be of paramount interest in bioremediation prospective research.

Baneshi et al. (2014) tested and evaluated the impact of bioaugmentation in improving the flora decontaminating of pyrene and phenanthrene-selected PAH congeners. The authors stated that PAHs removal from the soil by phytoremediation is an effective technique suggested for a future utilization. *Onobrychis sativa* and Sorghum were combined with the specific microbial consortium to phytoremediation pyrene and phenanthrene. Polluted soil (1.5 kg) of proportion 100:300 mg was used and investigated for 120 days. The results showed that the flora were able to remediate the polluted soil and significantly decontaminate totally the pyrene (63%) and phenanthrene (74.5%) contents of the soil correspondingly.

When both plants were combined, the bioaugmentation efficiency improved for pyrene (74.1%) and phenanthrene (85%) as well as for sorghum (85.2%) and *Onobrychis sativa* (73.84%) correspondingly.

In the combined mode, the removal efficiency dramatically increased, leading to pyrene and phenanthrene removal efficiencies of 74.1% and 85.02% for *Onobrychis sativa* and 73.84% and 85.2% of sorghum, respectively. In conclusion, the authors recommend sorghum and *Onobrychis sativa* as typical bioaugmentation tools for the degradation of phenanthrene and pyrene from adulterated soil. In summary, they also suggested the utilization of indigenous plants for the biodegradation of soil recalcitrant pollutants such as PAHs congeners.

The introduction of disproportionate dependency on chemicals increase the level of industrialization with enhanced undiscriminating, discarding of specifically chlorinated solvents, triggering a variation of environmental problems. It has been observed that chlorinated solvents such as perchloroethylene and trichloroethylene possess the capability to pollute the groundwater that could led to several health and environmental hazards. Numerous approaches have been applied in resolving these challenges but only very few success have been recorded. Time edgings for remediation have a tendency to be time-consuming, generally measured in decades. In view of the aforementioned, Anjali (2018) wrote a comprehensive review on the application of genus Dehalococcoides as a bioaugmentation tool for the bioremediation of heavily polluted chlorinated solvents. The techniques that have been utilized in the present, past, and future recommendations in the application of bioaugmentation are highlighted.

Baek et al. (2007) evaluated the utilization of numerous bioremediation processes and microbial diversity for the ecorestoration of polluted soil with crude oil. Several treatments such as bioaugmentation (BA), natural attenuation (NA), biosurfactant addition (BE), and biostimulation (BS) while their combined treatment containing bioaugmentation, biostimulation, and biosurfactant addition, which were referred to as (CT), were applied in the biodegradation of process and the determination of the microbial level present in this communities. It was observed that CT treatment showed the highest CT treatment while there was no observable changes in the level of the available hydrocarbons after 120 days. It was observed that the total level of bacterial count improved during the first 2 weeks in all the treatments and later become unstable. The alkane monooxygenase gene fragment, alkB, and the bacterial communities were related by denaturing gradient gel electrophoresis (DGGE). The result obtained indicated that the DGGE evaluation of the CT and the BA treatments which entails *Nocardia* sp. H17-1 showed modest dominant population structure in comparison with the other treatments. Moreover, the Simpson dominance index (D) and the Shannon–Weaver diversity index (H') evaluated from 16S rDNA established a quantitative variation in the community structure after and before the application of bioremediation treatment as well as among treatment situations.

Olu-Arotiowa et al. (2019) assessed the ecorestoration of atrazine herbicide–polluted agricultural soil under numerous bioremediation approaches utilizing indigenous *Aspergillus niger, Pseudomonas aeruginosa*, *Bacillus subtilis* as a bioaugmentation agents while poultry droppings were applied as biostimulation agents. The result obtained due to the process of bioaugmentation with all the tested strains and the application of biostimulation enhances in maximum atrazine biodegradation which varies from 97 to 100%.The biodegradation half-life and modeling using first-order kinetic model were applied in establishing the kinetics of atrazine biodegradation in the soil. It was observed that the rate of the constants (k1) of atrazine biodegradation in the soil where bioaugmentation with *Aspergillus niger*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*, while the fungal and the bacterial consortium vary from 0.059 and 0.191 day^{-1}. Also, it was detected that the soil exposed to natural bioattenuation, biostimulation, and joint bioaugmentation and biostimulation are 0.026, 0.164, and 0.279 day^{-1}, respectively. The half-life ($t1/2$) of atrazine ecoretordation in soil when exposed to natural bioattenuation was affirmed to be 26.7 days. The best ecorestoration effectiveness showed the following strategies with the following treatments in the following trends like combined bioaugmentation and biostimulation > Bioaugmentation with bacterial–fungal consortium > Biostimulation with poultry droppings > Bioaugmentation with *Pseudomonas aeruginosa* > Bioaugmentation with *Bacillus subtilis* > Bioaugmentation with *Aspergillus niger* > Natural bioattenuation.

Soil co-contaminated with organics and metals has been identified to entail some significant challenges for remediation. The availability of metal contamination can prevent or destroy the activity of microbial degradation of organic pollutants such as operative in situ biodegradation most especially utilizing bioaugmentation. Pepper et al. (2002) evaluated the bioremediation process of 3-chlorobenzoate (3-CB) and 2,4-dichlorophenoxyacetic acid (2,4-D) available in two various soil entailing cadmium (Cd) contamination and without the presence of cadmium (Cd) contamination. The potential of bioaugmentation in facilitating the process of organic degradation in these processes was also evaluated. The authors also assessed the level of degradation could be linked to the plasmid transference to native microbial populations (gene bioaugmentation) or survival of the introduced organism used for the process of bioaugmentation. It was observed that 2,4-D-degrading bacterium, *Ralstonia eutropha* JMP134 improved the rate of 2,4-D degradation when tested in Brazito soil that was inoculated with a Cd-resistant bacterium. Moreover, it was also established that the application of *Escherichia coli* Dll, which does not possessed chromosomal genes which could be utilized for widespread 2,4-D mineralization, was utilized for the process of gene bioaugmentation in Madera soil. Furthermore, it was observed that an enhanced gene transfer of the plasmid to the native populations was recorded and the rate of 2,4-D degradation was improved in comparison to that of the control. Also, it was established that *Comamonas testosterone* was applied in the process of cell bioaugmentation which was shown to validate that it plays a crucial role in the rate of bioremediation of 3-CB in Madera soil while non-bioaugmented samples evaluated with Madera soil exhibited a total 2,4-D degradation but non-bioaugmented Brazito soils demonstrates partial 2,4-D degradation. Their study established that the application of gene bioaugmentation and cell bioaugmentation could be utilized for biodegradation of organic degradation in co-contaminated soils. Eventually, the bioaugmentation approach may be contingent on the amount of contamination and the period frame obtainable for remediation.

Burghal et al. (2015) evaluated the effect of autochthonous microflora for the bioaugmentation of hydrocarbon polluted soil after it has been biostimulated by the mixture of sheep and cow dung in the presence of sawdust. The experiment was carried out in a test biopile containing contaminated soil entailing petroleum waste 100 kg together with 1.5% sawdust as well as necessary minerals and water that improve the growth of necessary microorganisms. Aeration was supplied to the pile by drainage-pipe network to enhance the process of bioaugmentation for a period of 90 days. It was established that there was alteration in the bacterial communities and the total petroleum hydrocarbons. There was also a drastic decrease in the total level of total petroleum hydrocarbons from 52 to 10.6 g kg^{-1}. It was also revealed that the dominant microorganism available in the soil entails autochthonous microorganisms and Gram-positive bacteria mainly from actinomycete group that possess the capability to biodegrade to the maximum level of 1.6×10^7 cfu g^{-1} at 45 days. Their study revealed that ex situ (biopile) experiment was the best approaches because it is cost-effective, eco-friendly, sustainable for effective bioremediation of polluted soil. The list of numerous microorganism utilized for bioaugmentation purposes are listed in Table 15.1.

15.2.1 Specific Gene Involved in Bioaugmentation

It has been recognized that most of the microorganisms utilized for the process of bioaugmentation do not survive. They have introduced the application of natural gene transfer so as to establish the transfers of remediation genes into polluted environment. The introduction of recent advances like genome sequencing has helped in rapid advancement in the establishment of the role of horizontal gene transfer played in the bioremediation of heavily polluted environment (Ochman et al. 2000). The movement of horizontal gene transfer may take place through the process of transformation or conjugation which involves the conjugative transposons between microorganisms or exchange of genetic material or physical contact such as plasmids, transduction which involves mediation by bacteriophage, and the application of gene bioaugmentation which involves several remediation genes that are available in mobile form that could be liked to self-transmissible plasmid when compared to the out-of-date cell bioaugmentation techniques involved. (1) There is no necessity for long-term persistence of the introduced host strain. (2) Release of remediation genes into local microorganisms could endure and increase in the environment. It has been observed that the movement of plasmids through conjugation is the technology mostly premeditated with reverence to bioaugmentation (Christensen et al. 1998; Dejonghe et al. 2000; DiGiovanni et al. 1996; Herrick et al. 1997; Newby et al. 2000a, b; Top et al. 1999, 2002, 1998).

Newby et al. (2000a, b) evaluated the level of bioaugmentation within two various bacterial donors that possess the capability of conveying the

Table 15.1 List of microorganisms used for bioaugmentation purpose

SN	Lists of microorganisms	Functions	Substance/substrates degraded	References
1	*Verticillium* sp., *Aspergillus* sp., *Acremonium* sp., strain BIA (*Enterobacter agglomerans*), strain 4015 (*Chromobacterium* sp.), strains B1f, B5A and B3g (*Bacilllus* sp.) and *Aspergillus sydowii*	Bioaugmentation	Benzo(a)pyrene, dibenzo(a)anthracene, pyrene, anthracene, phenanthrene, and naphthalene	Silva et al. (2009)
2	*Penicillium funiculosum* and *Rhizopus* sp.	Bioaugmentation	Petroleum hydrocarbons	Mancera-López et al. (2008)
3	*Fusarium oxysporum*, *Microbacteriaceae bacterium*, *Gordonia polyisoprenivorans*, *Microbacterium* sp., *Bacillus cereus*, and *Mycobacterium fortuitum*	Bioaugmentation	PAH congeners (pyrene, phenanthrene, and fluorine)	Jacques et al. (2008)
4	Strains NM and M (*Pseudomonas aeruginosa*) and strain DM-04 (*Bacillus subtilis*)	Bioaugmentation	Crude petroleum oil hydrocarbon	Das and Mukherjee (2007)
5	*Pseudomonas* sp., *Acinetobacter* sp., and *Rhodococcus* sp.	Bioaugmentation	PAH congeners (pyrene, phenanthrene, and fluorine)	Yu et al. (2005)
6	*Acremonium* sp., *Verticillium* sp., *Aspergillus* sp., *Trichocladium canadense*, and *Fusarium oxysporum*	Bioaugmentation	HMW-PAHs (4–7 rings)	Silva et al. (2009)
7	*Aspergillus* sp., and strain ZWL73 (*Pseudomonas putida*)	Bioaugmentation	LMW-PAHs (2–3 rings) and 4-chloronitrobenzene	Silva et al. (2009)
8	Strain BS29 (*Gordonia* sp.), strains LEBM1 and LEBM3 (*Aspergillus* sp.), strain LEBM2 (*Aspergillus* sp.) and strain FDS-1 (*Burkholderia* spp.)	Bioaugmentation	Aromatic and aliphatic hydrocarbons, chlorobenzene, phenol, and fenitrothion	Hong et al. (2007), dos Santos et al. (2008)
9	Strain ATCC 39723 (*Sphingobium chlorophenoticum*)	Bioaugmentation	Pentachlorophenol	Dams et al. (2007)

(continued)

Table 15.1 (continued)

SN	Lists of microorganisms	Functions	Substance/substrates degraded	References
10	Strain WatG (*Pseudomonas aeruginosa*)	Bioaugmentation	Diesel oil	Ueno et al. (2006)
12	Strain ST41 (*Pseudomonas* sp.)	Bioaugmentation	Marine gas oil	Stallwood et al. (2005)
13	*Absidia cylindrosora*	Bioaugmentation	Fluorene	Garon et al. (2004)
14	Strain A6L (*Arthrobacter chlorophenolicus*)	Bioaugmentation	4-Chlorophenol	Jernberg and Jansson (2002)
15	Strain BR60 (*Comamonas testosterone*)	Bioaugmentation	PAHs and crude oils	Gentry et al. (2001)
16	Strain pDH5/Paw 340 (*Pseudomonas putida*)	Bioaugmentation	4-Chlorobenzoic	Massa et al. (2009)
17	Strain RW112 (*Cupriavidus necator*)	Bioaugmentation	Chlorobenzoates Aroclor 1221 and 1232	Wittich and Wolff (2007)
18	Strain LB400/ohb (*Burkholderia xenovorans*)	Bioaugmentation	Aroclor 1242	Rodrigues et al. (2006)
19	Strain RE (*Pseudomonas fluorescens*)	Bioaugmentation	2,4-Dinitrotoluene	Monti et al. (2005)
20	Strain MP (*Pseudomonas fluorescens*)	Bioaugmentation	2,4-Dinitrotoluene	Monti et al. (2005)
21	Strain KT2442 (*Pseudomonas fluorescens*)	Bioaugmentation	Naphthalene	Filonov et al. (2005)
22	Strain F113rifpcbrrnBP1::gfpmut3 (*Pseudomonas fluorescens*)	Bioaugmentation	Naphthalene and PCBs	Boldt et al. (2004)
23	Strain RHAI (*Rhodococcus* sp.)	Bioaugmentation	4-Chlorobenzoate	Rodrigues et al. (2001a, b)
24	Strain AtzA (*Escherichia coli*)	Bioaugmentation	Atrazine	Strong et al. (2000)
25	Strain B13STI/pPOB (*Pseudomonas* sp.)	Bioaugmentation	3-Phenoxybenzoic acid	Halden et al. (1999)
26	Rhodococcus sp., *Pseudomonas* sp., *Burkholderia* sp., and *Arthrobacter* sp.	Bioaugmentation	Petroleum hydrocarbons	Adebusoye et al. (2007)
27	Strain F92 (*Rhodococcus* sp.)	Bioaugmentation	Various petroleum products	Quek et al. (2006)
28	Strain BCRc14349 (*Pseudomonas putida*)	Bioaugmentation	Trichloroethane and phenol	Chen et al. (2007)
29	Strain CS2 (*Pseudomonas fluorescens*)	Bioaugmentation	Ethylbenzene and biphenyl	Parameswarappa et al. (2008)

(continued)

Table 15.1 (continued)

SN	Lists of microorganisms	Functions	Substance/substrates degraded	References
30	Strain F113rifPCB (*Pseudomonas fluorescens*)	Bioaugmentation	Polychlorinated and biphenyl	Brazil et al. (1995)
31	Strain B13STI/pPOB (*Pseudomonas putida*) and *Pseudomonas* sp.	Bioaugmentation	3-Phenoxybenzioc acid	Halden et al. (1999)
32	Strain AtzA (*Escherichia coli*)	Bioaugmentation	Atrazine	Strong et al. (2000)
33	Strain RHA1 (*Rhodococcus* sp.)	Bioaugmentation	4-Chlorobenzoate	Rodrigues et al. (2001a, b)
34	Strain F112rifpcbrmBP1::gfpmut3 (*Pseudomonas fluorescens*)	Bioaugmentation	PCBs	Boldt et al. (2004)
35	Strain KT2442 (*Pseudomonas putida*)	Bioaugmentation	Naphthalene	Nesbo et al. (2001)
36	Strain MP (*Pseudomonas fluorescens*)	Bioaugmentation	2,4-Dinitrotoluene	Monti et al. (2005)
37	Strain RE (*Pseudomonas fluorescens*)	Bioaugmentation	2,4-Dinitrotoluene	Monti et al. (2005)
38	Strain LB400/ohb (*Burkholderia xenovorans*)	Bioaugmentation	Aroclor 1242	Rodrigues et al. (2006)
39	Strain RW122 (*Cupriavidus nectar*)	Bioaugmentation	Aroclor 1221 and 1232 and chlorobenzoates	Wittich and Wolff (2007)
40	Strain PaW 340/pDH5	Bioaugmentation	4-Chlorobenzoic acid	Massa et al. (2009)

self-transmissible plasmid pJP4, possessing 2,4-D degradative genes to local soil bacteria. It was established that pJP4 plasmid was transferred to the soil through *E. coli* D11.156 or its inventive host, *R. eutropha* JMP134. It was established that *R. eutropha* JMP134 possess the capability of mineralizing 2,4-D, but *E. coli* D11 could not due to the absence of the chromosomal genes together with plasmid genes that enable total mineralization of 2,4-D. It was further established that it took 28 days for complete biodegradation in the presence of soil receiving *R. eutropha* JMP134 while it took 49 days for the complete biodegradation of non-bioaugmented soil as well as soil receiving *E. coli* D11 inoculant. It was also established that many transconjugants isolated from *E. coli* D11 amended soil were recognized as the inoculant organisms most especially those that possess the capability to degrade 2,4-D obtained from the soil receiving *R. eutropha* JMP134. Subsequent deprivation of the preliminary 2,4-D adjustment, the authors added supplementary 2,4-D to the soil. Afterwards, the amendment 2,4-D was degraded further swiftly in the microcosms that was treated with the *E. coli* D11 inoculant when compared to the soil that was treated with the non-bioaugmented soil and *R. eutropha* JMP134 inoculant.

Their study showed the significance of local microorganisms in the biodegradation of specific pollutant in addition of necessary genetic material through gene augmentation. Their study also affirmed the capability of bioaugmentation to change the local soil microbial gene pool.

Dejonghe et al. (2000) evaluated the effect of propagation of two numerous 2,4-D degradation plasmids available in the B (lower) and A (upper) horizon of a soil. The application of an auxotrophic *Pseudomonas putida* strain that poses either of the two plasmids lead to enhance population of transconjugants ($>10^5\ g^{-1}$) in B and A horizons. It was further revealed that the donor population reduces following the bioaugmentation to the soil while the growth of transconjugant populations could be linked to the degradative potential of 2,4-D. It was later observed that the process of bioaugmentation led to improved 2,4-D degradation in the B horizon which does not possess any local degrader population when compared to the A horizon which had a larger number of indigenous degrader population. Their study also established that gene bioaugmentation could be applied for bioremediation of heavily polluted soil. The application of mobile genes in bioaugmentation was also established in a review documented by Top et al. (1999).

15.3 Microbial Derived Materials that Could Enhance the Process of Bioaugmentation

The process of bioaugmentation could be enhanced through the addition of enzyme or biosurfactant when combined or added singly in addition to microbial inoculant. The application of biosurfactant has been established for the bioremediation of organic polluted material or heavy metal contaminated environment (Garcia-Junco et al. 2003; Hong et al. 2002, Maier et al. 2001, Mata-Sandoval et al. 2002, Sandrin et al. 2000, Sekelsky and Shreve 1999). They possess the capability to prevent the adverse effect of metal toxicity on microbial inoculants and enhance the level of organic substrates available for degradation (Sandrin et al. 2000; Rahman et al. 2003). Sandrin et al. (2000) established that the application of metal-complexing with the biosurfactant mainly from rhamnolipid for reducing metal toxicity in a model polluted system. The experiment was performed in the presence of naphthalene-degrading *Burkholderia* sp. together with naphthalene and Cd. It was revealed that the addition of rhamnolipid prevented the eliminated Cd toxicity after the addition of ten-fold concentration of the Cd. It was discovered that at a lower concentration the rhamnolipid decreases and exhibited no impact on Cd toxicity. The authors affirmed that the presence of rhamnolipid reduces Cd toxicity by enhanced naphthalene bioavailability, LPS release, and metal complexation. Some other scientists have validated the application of enzyme that was encapsulated in dead microbial cells or in their purified form for the reduction of contamination (Zhao et al. 2003, Zhou 2003, Zhou and Thompson 2002, Zhou and Tiedje 1995, Zouboulis et al. 2001, Wackett et al. 2002, Bhandari and Xu 2001).

Strong et al. (2000) applied bioaugmented atrazine-polluted soil with genetically engineered *E. coli* strain that possess the capability to over produce the enzyme referred to as atrazine chlorohydrolase which could dechlorinate atrazine. The authors applied chemical in the inhibition of the genetically modified microorganisms before introducing them to the field site for the purpose of reducing their regulatory concern (Wackett et al. 2002). It was discovered that the level of atrazine concentrations in the enzyme-treated plots was reduced by 52% when compared to the insignificant biodegradation in the control plots. The application of 52% will help in the mitigation of all the associated challenges with bioaugmentation which are needed for the sustainability of the microbial inoculants in hearse environment most especially in the field.

15.4 Conclusion and Future Recommendation

This chapter has provided a detailed information on the application of bioaugmentation in the ecorestoration of heavily polluted environment. The role of cell bioaugmentation, activated soil, and immobilized microorganism was also highlighted. The application of some specific enzymes and biosurfactant when combined with bioaugmentation was also highlighted. Moreover, the movement of horizontal gene transfer during the process of bioaugmentation such as transformation, conjugation, and transduction was also highlighted. Information on the gene bioaugmentation, rhizosphere bioaugmentation, and their utilization in the bioremediation of polluted soil was discussed in detail. The application of some beneficial microorganism with high bioaugmentation capability when applied at the rhizosphere of some plants has been discovered to hasten the process involved in the absorption of heavily metals and various contaminants available in a particular environment. Moreover, there is a need to carry out more field trial so as to validate all the result observed on the laboratory scale. This will be a strong basis for their eventual commercialization.

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