

Chapter 8

Microbial Detoxification of Contaminated Land



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8.1 Introduction

Land pollution is a widely considered subject of global concern. Since the onset of industrialization, the problem of land pollution has been a matter of great concern and the issue has only been increasing every passing year. Land pollutants are the potent source of toxic and persistent organic pollutants (POPs) (Paul et al. 2019). Persistent organic pollutants are toxic chemical organic compounds that are highly resistant to environmental degradation. It adversely affects human health and the surrounding environment (Kevin 2021). These land pollutants are a storehouse of numerous contaminants which are taken up by the plants and animals. These organisms are later consumed by human beings which thereby affects and disturbs the equilibrium of the natural ecosystem. The toxic components of polluted land broadly involve heavy metals, petroleum hydrocarbons, polyaromatic hydrocarbons (PAH), pesticides, insecticides, halogenated and non-halogenated compounds (Saravanan et al. 2021; Gaine et al. 2021; Meagher 2000; Allen 2002). With rapid development of industry and agriculture, production and extensive use of artificial products have led to massive buildup of xenobiotics. Anthropogenic activities such as fossil fuel combustion, mining, corrosion, smelting, and waste disposal, greatly contribute to land pollution (Gaine et al. 2021; Thassitou and Arvanitoyannis 2001). According to the reports, approximately 2.5 million tons of pesticides are consumed throughout the world (Danila et al. 2020; FAO 2002). Based on the statistical data on total consumption of pesticides, Europe occupies the first position utilizing 45% of the

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pesticides, tailed by USA (24%) and others. The use of pesticides in Asia, particularly in India, has been increasing at an alarming rate. Currently, India as a pesticide producer holds twelfth position in the world and is the largest in Asia. Recent surveys have also confirmed that the presence of pesticides and groundwater aquifers are potent pollutants in water bodies and river beds (Tang et al. 2021; Shafi et al. 2020; Leong et al. 2007; Zakaria et al. 2003). These pollutants reach the water bodies in the form of toxic effluents and landfill leachate composed of persistent chemical components. Reports available in World Bank studies discussed the occurrence of dieldrin, polychlorinated biphenyls (PCBs) in rivers. The accumulation of these harmful contaminants by the aquatic and marine bodies has posed serious threats to their lives. The primary source of pesticides arises from the activities performed in the agricultural land (Zakaria et al. 2003). The presence of POPs in aquatic and terrestrial species has also been confirmed by various studies. According to Zakaira et al. (2003), dolphins are highly exposed to large amounts of DDT, chlordane, aldrin, and dieldrin in India. Based on reports, pesticide poisoning is one of the primary causes of increased death rates in developing countries (Zhong and Zhang 2020; WHO 1990). The linear rise in cancer cases and increasing death rate on exposure to high percentage of pesticides has been a matter of great concern (Hites 2021; Gong et al. 2021; WHO 1990; UNEP 1993). Major sources of xenobiotics are found in the production and manufacturing units of pesticide industries. Production farm workers, sprayers, and loaders are at high risk to pesticide consumption. This is because during formulation and application of the product the risk associated with the process is much more hazardous. Additionally, scientific reviews have also reported the contamination of heavy metals in agricultural land. These heavy metalloids found in the soil surface when taken up by the crops impose serious risks to crop growth and global food security (Rodríguez Eugenio et al. 2018). The heavy metalloids that are most widely available in the soil ecosystem are Pb, Cd, Cr, As, Cu, and Hg. Among these heavy metals, Cd is extensively encountered in agricultural lands where rice crops are grown. India, being an agrarian country and rice being the staple food; the concentration of cadmium continues to rise exponentially despite the implementation of various Environmental Regulatory Acts. Soil is the largest reservoir of carbon pool which holds almost five times more than the total mass of atmospheric carbon. The aggravating degradation of agricultural land because of the overload of contaminants might deteriorate the role and the mechanism of natural carbon cycle (Kopittke et al. 2019). Marrugo-Negrete et al. (2017), have also reported that the occurrence of heavy metals could also be derived from geogenic sources. The presence of geogenic heavy metals largely regulates the soil property and thus influences the soil quality (Clemens 2006). Thus, explosive increases in the level of contaminants over a short period of time have deteriorated the in-built self-remediating capacity of the environment, and hence the accumulation of pollutants. The use of living organisms as a model to undergo the process of biotransformation of toxic contaminants is a very effective bioremediation technique. Among all the bioremediation strategies, bioremediation assisted via microbes is a popular technique to transform highly toxic components into less toxic components (Garbisu and Alkorta 2003; Adhikari et al. 2004). These diverse ranges of microbial species could largely facilitate the

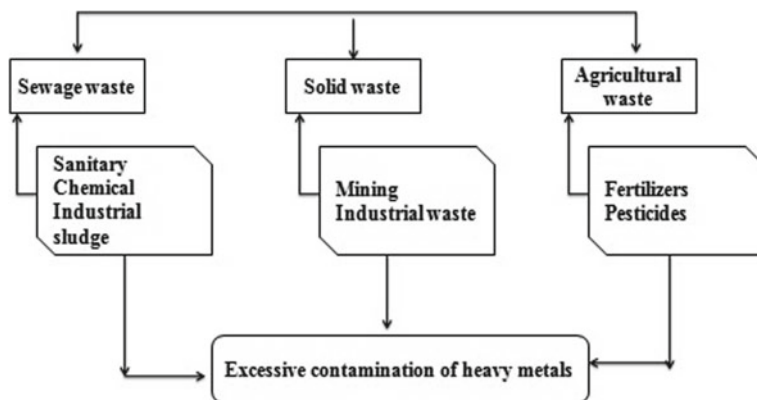


Fig. 8.1 An overview of all kinds of land waste

remediation of toxic contaminants both via active and passive processes (Bhatt et al. 2021; Scott and Karanjkar 1992). The microbial remediation process is conducted by various methods. Monitored natural attenuation and engineered microbial attenuation are the two popular techniques of microbial remediation. Usage of genetically modified microorganisms is another advanced biotechnological approach to degrade hazardous wastes (Dilek et al. 1998). Availability of carbon sources is the primary requirement of all the approaches to initiate the degradation process (Vidali 2001). Both indigenous and extraneous microorganisms are used for the remediation technique (Prescott et al. 2002). The mechanism of microbial remediation either participates in the oxidation–reduction reaction or is involved in the catalytic reaction that includes synthesis/degradation of organic compounds (Gadd 2000; Rajendran et al. 2003). The overview of all kinds of land wastes that undergoes microbial remediation is described in Fig. 8.1. *Pseudomonas putida* is the first patented microorganism registered in the year 1974 known for the degradation of petroleum (Glazer and Nikaido 2007). Presently, various reports are available on the usage of both aerobic and anaerobic microorganisms for the degradation of wide range of toxic components. Few potent examples of microorganisms that belong to the broadly classified groups mentioned above are: *Mycobacterium*, *Alcaligenes*, *Methylotrophs*, *Phanerochaete chrysosporium*, *Rhodococcus* (EPA 2003; Zeyauallah et al. 2009). Thus, this study is an attempt to highlight the effectiveness of microbial bioremediation and their associated mechanism of degradation.

8.2 Environmental Impact of Pesticides

Pesticides are globally used chemicals that are extensively used in agricultural land to safeguard the harvested crops from pests. Pesticides are classified into different types

Table 8.1 List of pesticides and their examples

Pesticides	Examples
Organophosphorus	Diazinon, dichlorvos, dimethoate, malathion, parathion
Carbamate	Carbaryl, propoxur
Organochlorine	DDT, methoxychlor, toxaphene, mirex, kepone
Cyclodienes	Aldrin, chlordane, dieldrin, endrin, endosulfan, heptachlor
Herbicides	Chlorophenoxy acids, hexachlorobenzene (HCB)
Nitrogen-based	Picloram, Atrazine, diquat, paraquat
Organophosphates	Glyphosate (Roundup)
Nitrogen-containing	Triazines, dicarboximides, phthalimide
Wood preservatives	Creosote, hexachlorobenzene
Botanicals	Permethrin, permethrin
Antimicrobial	Chlorine, quaternary alcohols

of chemicals based on their structural composition. It includes herbicides, insecticides, fungicides, and rodenticides (Table 8.1). The structural classification varies based on the presence of organochlorine, organophosphorus, pyrethroids, carbamates, and nitrogen-based compounds (Gilden et al. 2010). During 2001 amendment, Stockholm Convention has listed 12 persistent organic pollutants (POP) to cause adverse effects on the human health and ecosystem (Table 8.2). These POPs are sectioned under three different categories which include: pesticides, industrial chemicals, and by-products. The application, storage, and disposal approaches of POPs have posed a serious toxicity risk to living organisms and the environmental land (Fantke et al. 2012; Pieterse et al. 2015; Torres et al. 2013). The consumption of these pesticides has become such an inevitable practice that with each passing year the usage of xenobiotics has been increasing at an alarming rate (Abhilash and Singh 2009). As per reports, pesticide consumption was found to be highest in China followed by Korea, Japan, and India. Interestingly, among all the types of pesticides used in India, insecticides are mostly used which account to 76% as against 44% of global usage (Mathur 1999). These toxic recalcitrant compounds have become an issue of serious global concern because of their persistency, long-range transportability via air and water, and lipophilic; thus accumulate in the fatty tissues of living organisms (Torres et al. 2013; Pieterse et al. 2015). The impact of pesticides in these three components of ecosystems is described in detail. Even after banning few pesticides, significant amount of these toxic chemicals is observed in the major components of soil interaction system which includes: atmosphere, pedosphere, and hydrosphere.

Table 8.2 Persistent organic pollutants listed in Stockholm Convention amendment

Sl. No.	Item	Type of chemicals
	<i>2001 amendment</i>	
1	Aldrin	Pesticide
2	Dieldrin	Pesticide
3	Endrin	Pesticide
4	Chlordane	Pesticide
5	Heptachlor	Pesticide
6	HCB	Pesticide
7	Mirex	Pesticide
8	Toxaphene	Pesticide
9	DDT	Pesticide
10	PCBs	Industrial and by-product
11	Polychlorinated dibenzo-p-dioxins (PCDD)	By-product
12	Polychlorinated dibenzofurans (PCDF)	By-product
	<i>2009 amendment</i>	
1	Chlordecone (Kepone)	Pesticide
2	Lindane	Pesticide
3	α - HCH	Pesticide and by-products
4	B- HCH	Pesticide and by-products
5	Hexabromobiphenyl	Pesticide, industrial, and by-product
6	Tetra-BDE and penta-BDE	Pesticide, industrial, and by-product
7	Hexa-BDE and hepta-BDE	Pesticide, industrial, and by-product
8	PFOs and its salts	Pesticide, industrial, and by-product
9	PFOSF	Industrial
10	Pentachlorobenzene	Pesticide, industrial and by-product
	<i>2011 amendment</i>	
1	Endosulfan	Pesticide

8.2.1 Atmospheric Contamination

Pesticides once applied to the soil, it volatilizes from the soil and contaminates the atmospheric environment adversely (USGS 1995). The emission and evapo-transmission of pesticide particulates largely affect the air quality and human health (Gavrilescu 2005; Usman and Usman 2013). Usman and Usman (2013) monitored the percentage of DDT and HCH in air and it was observed to be $\sim 5.930 \text{ ng m}^{-3}$ and ~ 11.45 , respectively. Similarly, researchers recorded the concentration of DDT, chlordane, HCH, and endosulfan ranging from 250 pg m^{-3} to 6110 pg m^{-3} , 240 to 4650 pg m^{-3} , 890 to $17,000 \text{ pg m}^{-3}$, and 40 to 4650 pg m^{-3} respectively, in India (Gavrilescu 2005).

8.2.2 *Surface and Ground Water Contamination*

The primary cause of water body contamination is mostly due to agricultural and industrial runoff, soil erosion from contaminated sites, percolation through rainwater, and leaching (Barbash and Resek 1996). Widely reported insecticide and herbicide in the water bodies across the US are 2,4-D, diuron, chlorpyrifos, and diazinon followed by Trifluralin and 2,4-D (U.S. Geological Survey 1998; Bevans et al. 1998; Fenelon et al. 1998). Extensive use of these toxic chemical pollutants was found to be detrimental to the aquatic environment. According to USGS reports, 143 pesticides and 21 transformed products were recorded in the ground water of 43 states across the nation. In Bhopal, Madhyapradesh, India, 58% of groundwater was contaminated with organochlorine pesticide that exceeded the EPA standards (Kole and Bagchi 1995). Similarly, another study monitored and reported the concentration of endosulfan and DDT metabolites to be excessively high across all the states in India (Bakore et al. 2004). The main problem of ground water contamination with toxic chemicals is because it takes years to dissipate the contaminants (O'Neil et al. 1998; USEPA 2001).

8.2.3 *Land Surface Contamination*

Soil contamination is documented by many workers because of the occurrence of pesticides and transformed products derived from pesticides (Roberts 1998; Roberts and Hutson 1999). These hydrophobic compounds are highly persistent and tightly bound to the soil. The binding efficiency of the pesticides with soil largely depends on the soil-pesticide interaction. Generally, the higher the organic matter content in soil, the higher will be the adsorption properties of the pesticides and transformed products. The adsorption property of the ionizable pesticides is again inversely proportional to the soil pH (Andreu and Pico' 2004). The changes in the conformation of the pesticides include various metabolic pathways like hydrolysis, methylation, etc. These pathways generate toxic phenolic compounds as end products (Barcelo and Hennion 1997). The concentration of pesticides and their transformed products are characterized by determining water solubility, soil sorption constant, and partition coefficient of octanol/water. Indiscriminate use of pesticides in the soil has detrimental effects on the soil biota. The inherent population of bacterial and fungal communities is largely affected due to high dosage of pesticide application (Pell et al. 1988). These contaminants present in the soil inhibit the natural carbon and nitrogen cycle and reduce the growth and activity of the microorganisms involved in the process (Santos and Flores 1995). The organic compounds present in the pesticides target the tissues of the living organisms and disrupt the endocrine receptors and cause severe hormonal imbalance (Hurley et al. 1998). The other side effects associated with the accumulation of these harmful chemicals are immune-suppressive disorders, reproductive abnormalities, decreased memory, and cancer (Crisp et al. 1998; Hurley et al. 1998).

8.3 Sources of POP and Their Current Status

The complex status of persistent organic pollutants in the world needs scientific planning to harmonize the soil and revive its health. The chief components of POPs are polychlorinated dibenzodioxins (PCDD), polychlorinated biphenyls (PCBs), and polychlorinated dibenzofurans (PCDF). The Stockholm Convention identified 22 POPs as listed in Table 8.2. Among the toxic POPs, twelve POPs namely aldrin, dieldrin, endrin, chlordane, heptachlor, HCB, mirex, toxaphene, DDT, PCBs, PCDDs, and PCDFs are considered as “dirty dozens”. Additionally, twelve more compounds were added to the existing list, which included chlordecone, lindane, α -HCH, β -HCH, hexabromobiphenyl, tetra-BDE, penta-BDE, hexa-BDE, hepta-BDE, PFOs, and its salts, PFOSE, and pentachlorobenzene. The overdose of these compounds to combat disease and augment plant growth has caused serious threat to the soil ecosystem.

8.3.1 Pesticide Toxicity and Its Degradation

The persistence nature of pesticides is because of their compact physicochemical properties. The chlorinated pesticide is highly lethal. It imparts its toxicity at a significant level to the soil ecosystem disturbing the equilibrium of every living organism. The major routes of exposure to these chemicals are through food chain series, dermal contact, respiratory tract, etc. Microorganisms ubiquitously play an important role in the degradation wide range of POPs. The bioremediation ability of microorganisms depends on the structure and presence of functional group of the toxic group. The recalcitrant nature of the pesticides is also because of the presence of anionic species in the compounds. Microorganisms, with the help of their electron-donating and electron-accepting capability, can remediate the toxic contaminant. The degradation of these recalcitrant compounds by the microorganisms leads to the breakdown of parent compounds by producing carbon dioxide and water (Ref). The efficiency of this oxidation reaction depends on various environmental factors. This technique of decaying harmful pollutants using microbes can happen by two different processes. One being, the natural microorganisms stimulate the degradation of pollutants by utilizing the available nutrients in the contaminated land and the other being, isolating useful degrading microorganisms from one site and transporting into the polluted land. The latter process is termed as “bio-augmentation”. Apart from bioremediation, there are different terminologies used based on the target organism involved in the remediation strategy. Few microbes involved in the process of bioremediation is mentioned under Table 8.3. Among all the bacterial classes; *Sphingomonas* of alphaproteobacteria; *Burkholderia* of betaproteobacteria; *Pseudomonas*, *Acinetobacter* of gamma proteobacteria and *Flavobacterium* are considered to be efficient microdegraders (Mamta and Khursheed 2015; Kafilzadeh et al. 2015).

Table 8.3 List of microorganisms involved in the degradation of hazardous chemicals

Microorganisms	Toxic compounds
<i>Flavobacterium</i> spp.	Organophosphate
<i>Cunniughamela elegans</i> and <i>Candida tropicalis</i>	PCBs (Polychlorinated Biphenyls) and PAHs (Polycyclic Aromatic Hydrocarbons)
<i>Pseudomonas</i> , <i>Arthrobacter</i> , <i>Citrobacter</i> , <i>Vibrio</i>	Phenylmercuric acetate
<i>Alcaligenes</i> spp. and <i>Pseudomonas</i> spp.	PCBs, halogenated hydrocarbons and alkylbenzene, sulphonates, PCBs, organophosphates, benzene, anthracene, phenolic compounds, 2,4 D, DDT, and 2,4,5-trichlorophenoxyacetic acid, etc.
<i>Actinomycetes</i>	Raw rubber
<i>Nocardia</i> , <i>Pseudomonas</i>	Detergents
<i>Trichoderma</i> , <i>Pseudomonas</i>	Malathion
<i>Arthrobacter</i> , <i>Bacillus</i>	Endrin
<i>Closteridium</i>	Lindane
<i>Escherichia</i> , <i>Hydrogenomonas</i> , <i>Saccharomyces</i>	DDT
<i>Mucor</i>	Dieldrin
<i>Phanerochaete chrysosporium</i>	Halocarbons such as lindane, pentachlorophenol,
<i>P. sordida</i> and <i>Trametes hirsute</i>	DDT, DDE, PCBs, 4,5,6-trichlorophenol, 2,4,6-trichlorophenol, dichlorphenol, and chlordane
<i>Closteridium</i>	Lindane
<i>Arthrobacter</i> and <i>Bacillus</i>	Endrin
<i>Trichoderma</i> and <i>Pseudomonas</i>	Malathion
<i>Zylerion xylestrix</i>	Pesticides/Herbicides (Aldrin, dieldrin, parathion, and malathion)
<i>Mucor</i>	Dieldrin
Yeast (<i>Saccharomyces</i>)	DDT
<i>Phanerochaete chrysosporium</i>	Halocarbons such as lindane, pentachlorophenol
<i>P. sordida</i> and <i>Trametes hirsute</i>	DDT, DDE, PCBs, 4,5,6-trichlorophenol, 2,4,6-trichlorophenol, dichlorphenol, and chlordane
<i>Pseudomonas</i> spp.	Benzene, anthracene, hydrocarbons, PCBs
<i>Alcaligenes</i> spp.	Halogenated hydrocarbons, linear alkylbenzene sulfonates, polycyclic aromatics, PCBs
<i>Arthrobacter</i> spp.	Benzene, hydrocarbons, pentachlorophenol, phenoxyacetate, polycyclic aromatic
<i>Bacillus</i> spp.	Aromatics, long-chain alkanes, phenol, cresol
<i>Corynebacterium</i> spp.	Halogenated hydrocarbons, phenoxyacetates

(continued)

Table 8.3 (continued)

Microorganisms	Toxic compounds
<i>Flavobacterium</i> spp., <i>Methosinus</i> sp., <i>Methanogens</i> , <i>Azotobacter</i> spp.	Aromatics
<i>Rhodococcus</i> spp.	Naphthalene, biphenyl
<i>Mycobacterium</i> spp.	Aromatics, branched hydrocarbons benzene, cycloparaffins
<i>Nocardia</i> spp.	Hydrocarbons
<i>Xanthomonas</i> spp.	Hydrocarbons, polycyclic hydrocarbons
<i>Streptomyces</i> spp.	Phenoxyacetate, halogenated hydrocarbon, diazinon
<i>Candida tropicalis</i>	PCBs, formaldehyde
<i>Cunniinghamella elegans</i>	PCBs, polycyclic aromatics, biphenyls
<i>Pseudomonas</i> spp.	Benzene, anthracene, hydrocarbons, PCBs
<i>Alcaligenes</i> spp.	Halogenated hydrocarbons, linear alkylbenzene sulfonates, polycyclic aromatics, PCBs
<i>Arthrobacter</i> spp.	Benzene, hydrocarbons, pentachlorop henol, phenoxyacetate, polycyclic aromatic Aromatics, long-chain alkanes, phenol, cresol
<i>Bacillus</i> spp.	Halogenated hydrocarbons, phenoxyacetates
<i>Corynebacterium</i> spp.	Aromatics
<i>Flavobacterium</i> spp.	Aromatics, Naphthalene, biphenyl
<i>Azotobacter</i> spp.	Aromatics, branched hydrocarbons benzene, cycloparaffins
<i>Rhodococcus</i> spp.	Hydrocarbons
<i>Mycobacterium</i> spp.	Hydrocarbons, polycyclic hydrocarbons
<i>Nocardia</i> spp.	Phenoxyacetate, halogenated hydrocarbon diazinon
<i>Methosinus</i> sp.	PCBs, formaldehyde, biphenyl, polycyclic aromatics

8.4 Factors Responsible for Microbial Degradation

All micro-organisms have different metabolic rates and hence different growth patterns. The presence of different microorganisms contributes to adequate biodegradation since the process involves various microdegraders belonging to different bacterial classes. These variant microdegraders involve reactions like cleavage, oxidation, reduction, biotransformation, volatilization, biosorption, and bioleaching. Physicochemical parameters of the environmental matrix-like pH, oxygen, temperature, substrate availability, moisture content, types of carbon sources, largely regulate the metabolic features of the microbes which later influence the rate of degradation. Sites contaminated with pesticides often undergo leaching of particles. The leachate

has sufficiently high pH, resulting in the lowering of the degradation rate. Thus, the choice of pH depends on the selection of microorganisms chosen for the degradation process. Soil texture, permeability, and its bulk density are major soil properties that affect the rate of degradation. These soil properties are again determined by the moisture content, oxygen concentration, and nutrient availability in soil. Scientists have reported various mathematical models that describe the how the role of moisture content determines the degradation rate in polluted land (Raymond et al. 2001). It is reported that soil with low permeability hinders the bioremediation process. Anoxic sites with low oxygen concentration play a significant role in the process of bioremediation. The bioremediation process that takes place in sites contaminated with polyaromatic hydrocarbon is generally hindered because of low oxygen concentration since aerobic microorganisms are chosen for PAH degradation (Liu and Cui 2001). Limited oxygen concentration and low moisture content agglomerates the soil, thereby decreasing the bioremediation process (Cho et al. 2000). Availability of substrates/nutrients and their concentration largely affect the rate of degradation. For example: if the sites contaminated with PAH is supplemented with inorganic nutrients like nitrogen could enhance the degradation efficiency of most of the microorganisms (Zhou and Hua 2004).

8.5 Microbial Enzymes Used in Pesticide Bioremediation

Microbial degradation largely depends on enzymatic degradation. Microbial enzymes act as the biological catalysts that facilitate the conversion of persistent environmental pollutants to innocuous products (Table 8.4). These catalysts can be applied to a wide range of substances because of its chemical, regional, and stereo selectivity. It has great potential to effectively transform the pollutants in the biota by extensive transformations of the toxicological-based structural properties of the lethal compounds. According to Langerhoff et al. (2001), both aerobic and anaerobic degradations are essential to attain proper mineralization. Based on reports, aerobic degradation targets the cleavage of aliphatic and aromatic metabolites attached with

Table 8.4 Microbial enzymes involved in the degradation of various land contaminants

Enzyme	Used in industry
Dioxygenases	Synthetic industry, pharmaceutical industry
Laccases	Food industry, paper and pulp industry, textile industry, nanotechnology, synthetic industry, bioremediation, cosmetics
Peroxidases	Food industry, paper and pulp industry, textile industry, pharmaceutical industry
Oxidoreductases	Synthetic industry
Oxygenases	Synthetic industry
Monoxygenases	Synthetic industry

the compounds while anaerobic degradation results in dechlorination (Singh et al. 1999; Baczynski et al. 2010).

8.5.1 *Oxidoreductase*

Oxidases are the class of enzymes that utilizes molecular oxygen (O_2) as an electron acceptor to catalyze the oxidation–reduction reaction. The oxygen utilized in the process is either reduced to water (H_2O) or hydrogen peroxide (H_2O_2). Microorganisms such as bacteria and fungi undergo the detoxification process of toxic components via oxidative coupling (Bollag and Dec 1998). The biochemical reactions catalyzed by the enzymes help the microorganisms to derive energy and cleave the bonds to assist the transfer of electrons from one substrate to another. Thus, via oxidation–reduction mechanism, microorganisms contribute in converting the contaminants to harmless oxidized compounds (Gianfreda et al. 1999). Furthermore, oxidoreductases also participate in the detoxification of pesticides wherein phenolic/anilinic groups are attached to the moieties.

8.5.2 *Oxygenase*

Oxygenases belong to the group of oxidoreductase enzymes that involves oxidation of reduced substrates using FAD/NADH/NADPH as co-substrates. These enzymes actively participate in the cleavage of aromatic rings by introduction of molecular oxygen and regulating the metabolism of organic compounds. These classes of enzymes are also active against chlorinated aliphatics and halogenated methanes, ethanes, and ethyles by undergoing dehalogenation reactions (Fetzner and Lingens 1994). These enzymes are active against wide range of pollutants such as; herbicides, fungicide, insecticide (Fetzner and Lingens 1994; Fetzner 2003; Arora et al. 2009). Oxygenases are divided into two types based on the number of oxygen atoms involved in the process of oxygenation; namely monooxygenases and the other dioxygenases.

Monooxygenases include wide range of superfamily that catalyze substrates containing alkanes, fatty acids, and also steroids utilizing molecular oxygen. Due to wide range of stereo and region selectivity monooxygenases are often classified as biocatalysts (Cirino and Arnold 2002; Arora et al. 2009). Moreover, monooxygenases effectively degrade the aliphatic groups attached to the target compounds via hydroxylation, desulphurization, and denitrification (Arora et al. 2009). It comprises of two groups depending on the presence of cofactor; namely flavin-dependent monooxygenases and P450 monooxygenases. As the name suggests, flavin-dependent monooxygenases involve flavin as the prosthetic group whereas for P450 monooxygenases heme acts as the coenzyme in all living organisms. The reaction for flavin-dependent monooxygenases is catalyzed by NADP/NADPH. An

enzyme called methane monooxygenase enzyme is a promising degrader of hydrocarbons which includes methanes, alkanes, and alkenes (Fox et al. 1990; Grosse et al. 1999). Under aerobic conditions, monooxygenase undergoes dehalogenation; whereas for anaerobic conditions, it catalyzes reductive dechlorination (Jones et al. 2001).

Dioxygenases are multicomponent enzyme that participates enantiospecifically on wide range of substrates via oxygenation. Dioxygenases that act on aromatic hydrocarbon belong to the family of Rieske non-heme iron oxygenases. The mononuclear iron attached to the Rieske cluster is present in the alpha subunit. These microbial enzymes are present mostly in the soil that converts aromatic substrates to aliphatic products (Que and Ho 1996).

8.5.3 Cytochrome P450

The cytochrome P450 belongs to the family of hememonooxygenase which utilizes molecular oxygen to oxidize substrates and participate in the degradation process (Morant et al. 2003; Urlacher et al. 2004). It has a broad substrate range that includes 200 families for both prokaryotes and eukaryotes. Cytochrome P450 requires NAD(P)H, as a cofactor, which remains non-covalently bound to catalyze the reaction. One potential cytochrome P450 extracted from *P. putida* is known to have significant contribution in detoxifying chlorinated pollutants, namely pentachlorobenzene and hexachlorobenzene (Chen et al. 2002). Another variant of cytochrome P450 extracted from *Sphingobium chlorophenolicum* efficiently degrades hexachlorobenzene (Yan et al. 2006).

8.5.4 Peroxidase

Peroxidases are ubiquitously distributed in nature catalyzed by oxidation of organic and inorganic substrates and reduction of peroxides. The peroxidase enzyme can either be heme or non-heme proteins. These enzymes are produced from varied number of microorganisms such as *Bacillus subtilis*, *Citobacter* sp., *Bacillus sphaericus*, *Cyanobacteria*, *Streptomyces* sp., *Candida krusei*, *Anabaena* sp., and also yeasts (Hiner et al. 2002; Koua et al. 2009). Peroxidases extracted from fungal species work best on organophosphorus pesticides (Piontek et al. 2001). According to reports, *Caldariomyces fumago* is an efficient bio-degrader of organophosphorus-based substrates. These enzymes also help in transforming PAHs to less toxic products via oxidation (Hinter et al. 2002). The heme-based peroxidases have been classified into three groups on the basis of sequence comparison. Class I group of heme peroxidases is an intracellular enzyme that includes ascorbate peroxidase, cytochrome c, and also catalase-peroxidase. Class II group includes secretory fungal enzymes that is primarily involved in lignin degradation and Class III is a group of

peroxidases extracted from plants such as horse radish peroxidase, soyabean (Hiner et al. 2002; Koua et al. 2009). Non-hemeperoxidases comprise five different families namely thiol peroxidase, NADH peroxidase, alkylhydroperoxidase, and non-haem halo peroxidase. Among all the five different types of non-based peroxidase, thiol peroxidase is the largest to have two subfamilies in the group.

Microbial extracted peroxidase enzymes are further classified into lignin peroxidase enzyme, manganese peroxidase enzyme, and versatile peroxidase enzyme. Lignin peroxidase is extracted efficiently by white-rot fungus via secondary metabolism (Yoshida 1998). It is majorly involved in degradation of plant cell walls. The reaction converts hydrogen peroxide to water by accepting electrons from the oxidized form of LiP. This facilitates LiP to oxidize phenolic compounds into less toxic forms (Piontek et al. 2001). Manganese-based peroxidase is an extracellular protein secreted by basidiomycetes. This group of heme enzymes oxidizes Mn^{2+} which promotes the production of MnP and thus acts as substrates whereas MnP formed from Mn^{3+} helps in the chelation of oxalate compounds and helps in the biodegradation of xenobiotic compounds (Tsukihara et al. 2006). Microbial versatile peroxidases oxidizes Mn^{2+} , methoxybenzenes, and phenolic compounds similar to that of MnP, LiP. Unlike, lignin and manganese-based peroxidase this class of versatile peroxidase also participates in degrading non-phenolic dimers (Ruiz-Duenas et al. 2007).

8.5.5 *Laccases*

Laccases belong to the group of ubiquitous enzymes catalyzed by oxidation of reduced phenolic and aromatic substrates. It comprises multicopper oxidases extracted from wide range of prokaryotes as well as eukaryotes (Mai et al. 2000). The enzymes extracted from microorganisms is capable of producing both extracellular and intracellular laccases and thus proficient in oxidizing wide range of compounds such as aminophenols, polyphenols, methoxyphenols, diamines, polyamines, (Ullah et al. 2000; Rodriguez Couto and Toca Herrera 2006). The performance of the enzymes widely depends in pH and presence of chemicals such as halides, hydroxide, and cyanide (Xu 1996; Kim et al. 2002) in the environment.

8.5.6 *Hydrolases*

Hydrolases are broad group of enzymes used in pesticide bioremediation majorly involved in the catalysis of the hydroxylation of thioesters, C–H bonds, peptide bonds, esters, carbon–halide bonds, etc. Interestingly, this reaction is undergone in the absence of any redox cofactors. The hydrolytic enzyme is quite popular in the bioremediation process because of its availability, resistance against water-miscible solvents, economic viability, and eco-friendly properties.

8.5.7 Haloalkane Dehalogenases

Lindane, a highly persistent insecticide (an isomer of hexachlorocyclohexane) is widely used against pests. The bacterial genes involved in the degradation of hexachlorocyclohexane (HCH) have been studied extensively. Lin A and Lin B are the two key enzymes responsible for the biodegradation process. Lin A helps in the detoxification of γ -HCH whereas Lin B also known as haloalkane dehalogenase (Dhl A) is isolated from *Xanthobacter autotrophicus* (Nagata et al. 1993a, b). The enzyme also participates in the degradation of β -HCH and δ -HCH. The mechanism of β -HCH degradation is catalyzed by *Sphingomonas paucimobilis* whereas for δ -HCH *Sphingobium indicum* B90A and *Sphingobium japonicum* UT26 (Nagata et al. 2005; Sharma et al. 2006). The enzyme mediates the conversion of 2,3,5,6-tetrachloro-1,4-cyclohexadiene to 3,6-dichloro-2,5-dihydroxy-1,4-cyclohexadiene (Negri et al. 2007) (fig). The remediation of β -HCH via the enzyme LinB is initially converted to pentachlorocyclohexanol, which is further transformed to tetrachlorocyclohexanol. LinA enzyme, encoded by the Lin operon, mediates the very first step of dehydrochlorination of γ -HCH (Nagata et al. 1993a, b). This enzyme belongs to the family of scytalone dehydratase and naphthalene dioxygenase (Nagata et al. 2001). However, complete detoxification of γ -HCH involves both LinA and LinB. Raina et al. (2007) has observed another potent bacteria named *Sphingobium indicum* involved in significant reduction of HCH from the soil surface. Lin operon-mediated genes activate the mechanism of the remediation process.

8.6 Impact of Genetic Engineering in Pesticide Degradation

Several reports have described the importance on the genetic basis and the role of catabolic genes in pesticide biodegradation (Table 8.5). The genetically engineered microbial genes responsible for bioremediation are either extrachromosomal or genomic that work via recombinant DNA technology. In in-situ bioremediation technique, the most vital point is to understand how the mechanism of how the microbial genes interact with the environment to biodegrade the toxic pollutants (Hussain et al. 2018). The microbial enzymes isolated from plasmids, encoding opd gene have

Table 8.5 List of genetically modified organisms used in bioremediation

Microorganisms	Compound degraded
<i>P. putida</i>	Camphor degradation
<i>P. oleovarans</i>	Alkane degradation
<i>P. cepacea</i>	2,4,5-Trichlorophenoxyacetic acid degradation
<i>P. mendocina</i>	Trichloroethylene degradation
<i>P. diminuta</i>	Parathion (pesticide) degradation

varied genetic diversity. The *opd* gene is present in a highly conserved region in a bacterial plasmid which is widely responsible for the degradation of organophosphorus hydrolase (OPH) (Yan et al. 2006). The gene that encodes organophosphorus hydrolase has a promoter sequence that includes 996 nucleotides. Another important enzyme named, methyl parathion hydrolase (MPH) encoded by *mpd* gene is isolated from *Acrobacter* and *Brucella* widely participates in the biodegradation process. These microorganisms have interesting properties to respond to various kinds of stresses and adapt itself to the polluted environment. The molecular adaptation of these genes to achieve fitness in toxic environment draws attention to the development of strategies for the optimization of metabolic pathways and minutely characterizes the genes involved in the bioremediation process (Cho et al. 2004). The modified organisms developed by the insertion of gene of interest can perform the best only if it is introduced within its regulatory network (Cho et al. 2000). The degradation of organophosphate-based pesticides can be enhanced by site-directed mutagenesis. The modified organisms developed on the basis of variants of organophosphate can hydrolyze methyl parathion, an insecticide to 25 fold higher than the wild type (Cho et al. 2004). Cho et al. (2004) has also described in his paper the improvement in the performance of chlorpyrifos hydrolysis by the variants of OPH compared to the wild type. *Variovorax* sp. is well known for the degradation of Linuron, a phenylurea-based herbicide. The *libA* gene of *Variovorax* sp. strain SRS16 participates in the bioremediation process by undergoing hydrolysis. Another gene named Hyl A that also encodes for hydrolase enzyme is found in *Variovorax* sp. Strain WLD1 (Van Der et al. 1992). A herbicide named aryloxyphenoxypropanoate (AOPP) is degraded by *Rhodococcus ruber*, strain JPL-2. The *feh* gene that encodes carboxyesterase initiates the bioremediation (Zhang et al. 2002). Another xenobiotic metabolite, petachlorophenol and chlorpyrifos can be efficiently degraded by *Streptomyces* sp. A5 and *Streptomyces* sp. M7, respectively (Zhang et al. 2002).

8.7 Impact of Heavy Metal on Land Quality

With the development of the global economy, heavy metal contamination has been exponentially increasing leading to the deterioration of the ecosystem (Sayyed and Sayadi 2011; Prajapati and Meravi 2014; Zojaji et al. 2014). Excessive anthropogenic activities, improper management of chemical and industrial waste, mining activities, atmospheric deposition are various sources of heavy metal contamination (Zhang et al. 2011). In addition, the fly ash deposits by coal thermal power stations are a major anthropogenic source of heavy metal pollution across the globe (Pandey and Singh 2010; Pandey 2020). These toxic chemicals once accumulated in the body of living organisms disturb the entire food chain reaction. It risks the human life by attacking the cellular components and inducing stress even if it is accumulated at a very trace amount in the body (Ayangbenro and Babalola 2017; Wang et al. 2017). These harmful chemicals largely affect the liver and bones, damage the nervous system, blocks the functional property of essential enzymes (Moore 1990; Ewan

and Pamphlett 1996). Few metals which are listed as carcinogens are also noted to cause reproductive disorders. Reports are available on various physical and chemical methods to remove heavy metal (Azimi et al. 2017; Zamri et al. 2017; Fang et al. 2017). However, these technologies have many limitations which as a result generate toxic sludge as the by-product. Thus, an eco-friendly approach to bioremediate heavy metals is a worldwide accepted technology. Microbial-mediated bioremediation of heavy metals is in high demand because of their high-efficiency and cost-effective properties (Leal-Gutierrez et al. 2021; Pandey and Singh 2019). The microorganisms' work on the remediation process by transforming the active form of metals to inactive form. These organisms manage to build up resistance against heavy metals by changing their metabolic activity when the active form of metal act on the microorganisms. Heavy metals come in contact with the soil ecosystem through different pathways described in Fig. 8.1. Industrial activities like mining builds up elevated level of metal concentration in land and also in the wetlands (Bourrin et al. 2021; Leal-Gutierrez et al. 2021; DeVolder et al. 2003). Lead and zinc are the two excessively found metals during ore mining. These metals are later assimilated by the plants and thereby pose serious risk to the secondary level of organisms which includes human being and animals (Basta and Gradwohl 1998). Toxic metals such as chromium, arsenic, nickel, and lead are massively found in tanning, textile, and petrochemical industries. Extensive availability of these hazardous metals can cause serious threat to ecological equilibrium. Solid waste or sewage sludge such as animal waste and municipal solid waste leads to the accumulation of large amounts of heavy metals such as copper, arsenic, cadmium, lead, nickel, mercury, chromium, selerium, and molybdenum in soil (Basta et al. 2005; Sumner 2000). Generally, animal waste are considered to be organic manures for crop growth; however, induction of growth promoters in the diet could participate in the contamination of heavy metals such as arsenic, zinc, and copper in soil (Sumner 2000; Chaney and Oliver 1996). Thus, practice on application of animal waste as organic manure has become a matter of great concern. Additionally, toxic metals available in the land surface could also contaminate groundwater by leaching (McLaren et al. 2004). Fertilizers and pesticides are two sources of agricultural waste that generate large amount of metal deposition in soil. Large quantities of fertilizers (N, P, and K) are added in the crop field to enhance crop growth. However, along with crop growth these fertilizers adversely affect the soil quality and texture. Phosphate-based fertilizers largely build up the concentration of toxic metals in soil such as cadmium, mercury, and lead (Raven et al. 1998). Moreover, insecticides and fungicides also have heavy metals like Cu, Hg, Mn, Pb, or Zn in it. Insecticides used in fruit orchards contain high concentration of lead arsenate. Reports suggest that the pesticides used in banana crops in New Zealand and Australia are mostly arsenic-based (McLaren et al. 2004).

8.7.1 Microorganisms Mediated Heavy Metal Bioremediation Approaches

Microorganisms as bioremediators have gained enough popularity because of their outstanding performance in heavy metal detoxifying strategies. However, choosing an efficient approach is a major challenge (Fig. 8.2). Each of these approaches is discussed underneath.

- (a) *Biosparging*–Biosparging is an in-situ-based bioremediation technology where indigenous microorganisms are used to decontaminate polluted land. It is considered to be an advanced form of air sparging since nutrients are also pumped along with air in a saturated zone (USEPA 1995; Muehlberger et al. 1997). It takes about six months to two years to complete the process. The technique works efficiently in permeable soil.
- (b) *Bioventing*–The process of bioventing includes injection of air in a contaminated land. This technique degrades volatile organic contaminants and the effectiveness of this technique is reported under in-situ conditions (USEPA 1997). Bioventing is widely used for degrading petroleum products like diesel. The treatment process varies from six months to two years. However, limitation of this technology that this technique cannot be applied to sites with low permeability and high clay content (USEPA 1995).
- (c) *Biostimulation*–Biostimulation induces the activity of the microorganisms by addition of rate-limiting nutrients. These artificially added nutrients enhance the performance of the microorganisms by modifying the in-situ environmental

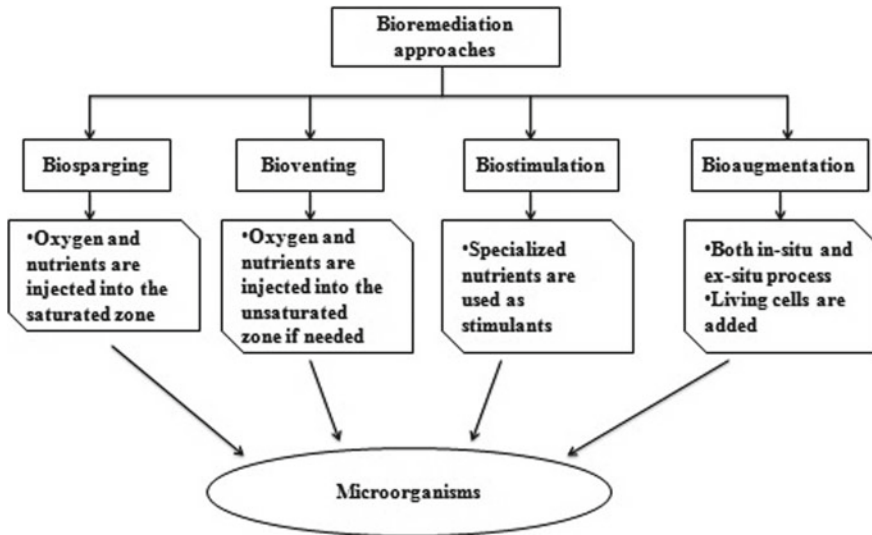


Fig. 8.2 An overview of bioremediation approaches catalyzed by microorganisms

condition which thereby augments the bioremediation potential of the inhabited microbial population (Nikolopoulou and Kalogerakis 2008; Prince 1997). Land contaminated with petroleum hydrocarbon has sufficient amount of C-source for the microorganisms, however, these lands are deficit of nitrogen and phosphorous. The richness of these environments could be stimulated by the addition of nutrients (Sarkar et al. 2005). Besides nutrients, temperature is another parameter that has a considerable effect on biodegradation. The temperature of an environment is correlated with other parameters such as viscosity and solubility properties in water (Atlas 1981). Additionally, biosurfactants are also used to increase the biodegradation rate (Bordoloi and Konwar 2009; Ron and Rosenberg 2002). Addition of nitrogen and phosphorous along with biosurfactants could stimulate the performance of the indigenous microorganisms (Nikolopoulou and Kalogerakis 2008). This could be considered one of the promising approaches to accelerate the process of microbial bioremediation (Baek et al. 2007).

- (d) *Bioaugmentation*—Bioaugmentation is one of the successful bioremediation strategies implemented to clean polluted land. The various approaches to perform bioremediation are: addition of pre-adapted a single bacterial strain or in consortium, addition of genetically engineered microorganisms (El Fantroussi and Agathos 2005). Thus, choosing an appropriate technique is quite a challenging task. For eg. if a land is contaminated with wide range of pollutants then a multi-component system could be a better representation of detoxifying land (Ledin 2000). According to study, a consortium-based approach is more advantageous than pure culture-based approaches since it has high metabolic activity (Nyer et al. 2002; Hussain et al. 2018). Alisi et al. (2009) has reported the reduction of isoprenoid and total hydrocarbon concentration to 60% and 75%, respectively in 42 days using microbial consortium. Additionally, Li et al. (2009) have documented that use of *Bacillus* sp., *Zoogloea* sp., and *Flavobacterium* as a microbial consortium in PAH degradation could enhance the degradation rate to 41.3%. Insufficient amount of substrates, competition among species could be few troubleshoot of the process. To overcome these drawbacks, application of carrier material could act as a physical support for the biomass as well as it could increase the survival rate of the organisms by providing better moisture and nutrients (Mishra et al. 2001).

Recent studies have shown that heavy metal removal by living/dead microorganisms are the most widely used technique in bioremediation (Table 8.6). The most primary mechanism of detoxifying heavy metals is by the adsorption of metal components through the slimy layers of bacteria with the help of functional groups like carboxyl, amino, phosphate, and sulfate. The microorganisms involved in the bioremediation process are known to utilize the metal adsorption techniques. For example, *Pseudomonas aeruginosa*, participates widely in the detoxification of mercury by adsorbing mercury ions. These ions once adsorbed by the microorganisms are accumulated by sulfhydryl groups present in cysteine-rich protein (Yin et al. 2016). *P. putida* is also one of the efficient mercury detoxifying organisms and with the help of

Table 8.6 List of microorganisms that utilizes heavy metals

Metals	Microorganism
Cu, Hg, Pb	<i>Ganodermaapplantus</i>
Cd, Pb	<i>Phormidiumvalderium</i>
Cd, Co, Cu, Ni	<i>Stereumhirsutum</i>
Ag, Hg, P, Cd, Pb, Ca	<i>Rhizopusarrhizus</i>
Cd, Cu, Zn	<i>Pleurotosostreatus</i>
Cd, Zn Zn, Ag, Th, U	<i>Aspergillusniger</i>
Cd, U, Pb, Co, Ni, Cd	<i>Citrobacter</i> spp.
U, Cu, Ni	<i>Zooglea</i> spp.
Cu, Zn	<i>Bacillus</i> spp.
U, Cu, Ni	<i>Pseudomonas aeruginosa</i>
Co, Ni, Cd	<i>Zooglea</i> spp.
Cd, U, Pb	<i>Citrobacter</i> spp.
Au, Cu, Ni, U, Pb, Hg,Zn	<i>Chlorella vulgaris</i>
Cd, Zn Zn, Ag, Th, U	<i>Aspergillus niger</i>
Cd, Cu, Zn	<i>Pleurotus ostreatus</i>
Ag, Hg, P	<i>Rhizopus arrhizus</i>
Cd, Pb, Ca	<i>Stereum hirsutum</i>
Cd, Co, Cu, Ni	<i>Phormidium valderium</i>
Cd, Pb	<i>Ganoderma applantus</i>
Cu, Hg, Pb	<i>Volvariella volvacea</i>
Zn, Pb, Cu	<i>Daedalea quercina</i>
Co, Ni	<i>Zooglea</i> spp.
Cd, U	<i>Citrobacter</i> spp.
Cu, Zn	<i>Bacillus</i> spp.
Au	<i>Chlorella vulgaris</i>
Ag, Hg	<i>Rhizopus arrhizus</i>
Th	<i>Aspergillus niger</i>
As	<i>E. coli</i> strain
Cd	<i>Alcaligenes eutrophus</i> ; <i>Stenotrophomonas</i> sp.
Cr	<i>Bacillus coagulans</i> , <i>B. Megaterium</i>
Cr, Cd, Ni	<i>Desulfovibrio desulfuricans</i>
Cu	<i>B. subtilis</i> , <i>Micrococcus luteus</i> , <i>Pseudomonas stutzeri</i> ; <i>Enterobacter</i> sp.
Hg	<i>Pseudomonas K-62</i>
Pb	<i>Bacillus</i> sp.; <i>P. Aeruginosa</i>
Zn	<i>Thiobacillus ferrooxidans</i> ; <i>Acinetobacter</i> sp.

(continued)

Table 8.6 (continued)

Metals	Microorganism
Cr, Cd	<i>Aspergillus</i> sp., <i>Rhizopus</i> sp.
Cr, Pb	<i>A. lentulus</i>
Cd, Cu, Pb	<i>Penicillium chrysogenum</i>
Cu(II), Pb(II)	<i>A. niger</i>
Cd, Hg	<i>Pleurotus sapidus</i>
Ni, Cd, Zn, Pb, Cu	<i>Rhizopus arrhizus</i>
Zn	<i>Trametes versicolor</i>

reductase, the microorganism participates in the reduction of Hg(II) to Hg⁰ (Wang et al. 2014). The use of mining sites is a global challenge due to the presence of heavy metals and needs urgent attention for their restoration (Ahirwal and Pandey 2021). Therefore, researchers revealed that the *Pseudomonas aeruginosa*-HMR1 is helpful to remove heavy metals and exhibits plant growth-promoting attributes, and can be used for mine land restoration for agro-forestry purposes (Bhojiya et al. 2021). Interestingly, bacteria named *Arthrobacter viscosus* can detoxify Cr(VI) by reducing it to Cr(III) from its surface in both living and dead forms. Quiton et al. (2018) have reported the use of *Staphylococcus epidermidis* biofilm for the elimination of Cr(VI) from solution. The removal of Zn(II) and Cd(II) is processed by *Rhodobacter capsulatus* and *Bacillus cereus*, respectively (Magnin et al. 2014). *Bacterial firmus* participates in the oxidation and reduction of As(III) and Cr(VI), respectively to decontaminate waste water from heavy metal pollutants (Bachate et al. 2012). The modification in the structural composition of heavy metals upon the action of reductase enzyme helps in the detoxification of heavy metals and thus enhances the bioremediation process via bacterial species. Additionally, extracellular polymeric substances (EPS) found on the microbial cell surface plays an important role in the adsorption of heavy metals. These EPS is made up of carbohydrates, lipids, proteins because of which ionic interaction is generated by the functional groups which thereby helps in the remediation process (Sheng et al. 2013; Wang et al. 2014). Heavy metal adsorption by exopolysaccharides catalyzed by the ionic interaction of the functional groups (carboxyl, phosphonate, amine, hydroxyl) is an energy-independent process that depends on adsorption isotherm models (Kim et al. 1996). Thus, the efficient soil microorganism is a new dimension and eco-friendly approach for sustainable agriculture and environmental development (Singh et al. 2011).

Fungi and algae are also widely known for their capability to accumulate heavy metals from the environment. The primary constituents of fungal cells are composed of chitin, glucuronic acid, phosphate, and polysaccharide. Fungi widely use the technique of metal speciation to implement bioremediation approaches (Lovley and Coates 1997; Eccles 1999). The mechanism involves the management of the transport of metal species via mobilization-immobilization method largely depending on the soluble and insoluble (White et al. 1998; Vachon et al. 1994). The carboxyl,

phosphoryl, amine, hydroxyl groups attached to the cellular components actively perform the ion exchange mechanism (Purchase et al. 2009). *Aspergillus niger* is a prolific Pb(II) removing fungus with the help of their biosorption capacities. Another fungal strain named, *Aspergillus fumigates* accumulates Cr(VI) following Freundlich isotherm (Ramrakhiani et al. 2011; Dhal and Pandey 2018). Trichoderma has been employed for bioremediating Cd(II) by Langmuir and Freundlich isotherm model (Amirnia et al. 2015; Bazrafshan et al. 2016). Similarly, *Saccharomyces cerevisiae* is reported to accumulate copper, zinc, and cadmium from a high alkaline environment. The process of accumulation by the microorganisms can be enhanced on addition of sodium chloride (Li et al. 2013). Algae are another group of microorganisms that has a very good property of adsorbing heavy metals. *Fucus vesiculosus* and *Cladophora fascicularis* help in the bioremediation of Pb(II) (Bilal et al. 2018; Poo et al. 2018). The peptide molecules present in the algal environments prevent the organism from heavy metal stress once accumulated (Bilal et al. 2018). *Saccharina japonica* and *Sargassum fusiforme* is well known for the removal of zinc, copper and cadmium (Poo et al. 2018; Rugnini et al. 2018).

8.7.2 *Bioremediation of Toxic Metals Through Microbial Biomass and Their Enzymes*

Bioremediation is an efficient technique for detoxifying harmful metals from soil system. This technique has offered various potential for its significant advancement. Further research on the mechanism of microbial remediation by various microbial communities could enhance the degradation of pollutants. The inoculation of microbial enzymes is another most favored approach used in the bioremediation processes. The defense pathway of microbial enzymes by changing the redox state of the ions present in the toxic components via oxidation–reduction reaction could efficiently decrease their toxicity (Prokop et al. 2003; Cho et al. 2004). The specialized genes present in the microorganisms regulate the activity of the microbial enzymes and prevent it from environmental stress. For example, *Bacillus* sp. and *Micrococcus* sp. have resistance against Hg and As ions, respectively (Mc Loughlin et al. 2005; Behrens et al. 2007).

Microorganisms have the capacity to produce high-yield biomass. This microbes-based biomass can be utilized as an effective adsorbent that could adsorb heavy metals from the pollutants (Yang et al. 2003). The process is initiated by the binding sites present in the cellular structures of the microorganisms. The two pathways associated with this process are: the active uptake mechanism and the passive uptake mechanism. When heavy metals get entrapped into the cellular membrane with the involvement of biological metabolic cycle it is referred as “active uptake” mechanism. Additionally, when the uptake of the heavy metal into the cellular structure is independent of the metabolic cycle it is termed as “passive uptake” mechanism. Scientific reports have suggested that microbial remediation via cells follows biphasic uptake of metals. This

means, the biosorption of heavy metals takes place rapidly at the initial phase and gradually the process slows down as the reaction proceeds. It is noteworthy to mention that the process of biosorption is quite sensitive to pH, ionic strength, and presence or absence of organic/inorganic ligands. These physiological parameters are practical limitations associated with this technique. However, these challenges can be met by proper strain selection with enhanced accumulation property. The efficiency of the process could be augmented by inoculation of consortium of strains with high metal biosorption properties (Pumpel et al. 2001). Generally, the naturally formed biomass does not have high adsorption capacity for heavy metal remediation. Thus, acid or alkali base treatments are used to enhance the adsorption capacity of the biomass (Cho et al. 2004). Under acid treatment, the biomass becomes positively charged which thereby generates a strong electrostatic attraction between biomass and heavy metals by opening up additional adsorption sites (Jackson et al. 2006, 2005). Similarly, under alkali treatment, the negative charge of the biomass is increased and thus can generate attraction of the positively charged heavy metals (Jackson et al. 2005; Afriat et al. 2006). Sodium hydroxide, sodium bicarbonate, disodium carbonate is widely used in alkali treatment (de Souza et al. 1996). Thus, optimization of the process in regard to biomass growth, cell development, and constant metal removal property would make the process technically advanced and economically viable.

8.8 Future Prospects

Bioremediation is no doubt a high-efficiency and low-cost technique. Compared to all other remediation technologies, microbe-associated bioremediation is the most feasible approach to pollutant degradation. The usage of appropriate consortium species based on the nature of contaminants has proved the technique to be far more profitable in degradation of complex substances. Considering the environmental factors such as pH, temperature, and ionic strength the rate of bioremediation process is altered and makes the technique quite a challenging one. Despite its wide application, there are still few bottlenecks that need further elucidation to enhance the bioremediation process. Treatment technologies that can deal with the toxicity of pollutants need a special attention in genetic engineering. The overexpression of genes involved in the remediation process can augment the rate of removal efficiency and microbial resistance against pollutants. Research on enzymatic function and their genomic advancement could be another prospect of potential for microbial remediation. This machinery of this technique follows either of the two paths among which one catalyzes the reaction in presence of catalyst and the other describes the quantitative efficiencies under controlled environment. One very important criterion of enzymatic degradation is the requirement of high catalytic efficiency under low substrate concentration. However, implementation of bioremediation technology independent of any cofactors makes the technique more viable and cost-effective. Cofactors free enzymatic method eliminates the regulatory issues involved in genetically modified technologies. This accelerates the solubility and hence the expression

of the enzymes. Moreover, use of consortium microorganisms could be another efficient mode of microbial remediation that needs further research. Thus, this process of remediation is a promising and ideal way to greener pastures. Regardless of any mode of microbial bioremediation that is used, the technology offers an efficient and cost-effective way to treat contaminated land.

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