Chapter 25
Microbe-Mediated Removal of Xenobiotics for Sustainable Environment

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Abstract Xenobiotics are man-made chemical compounds widely used in pesticides, dyes, drugs, explosives and other industrial chemicals. Poor waste disposal practices, intensive agricultural practices and fossil fuel combustion are some of the reasons that lead to the release of these compounds into natural ecosystem such as soil and water. These cause serious damage to aquatic and terrestrial ecosystems due to noxious nature of chemical compounds. Xenobiotics when build up in soil kill beneficial microbes of soil that are accountable for soil fertility. Degradation of xenobiotic compounds is being done using physical and chemical means; however, these methods consequently result in the formation of lethal intermediates and end products. Therefore, microbial remediation is adopted as a sustainable emerging technique to eliminate these pollutants from nature. The present study highlights on the involvement of several bacterial and fungal genera in catabolism of recalcitrant xenobiotics. Moreover, phytoremediation approach employing plants for treating chemically contaminated soil is also discussed.

Keywords Xenobiotics · Microbial remediation · Phytoremediation · Mycoremediation · Bioremediation

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25.1 Introduction

Xenobiotic chemicals are distant to the biosphere, are effectively lethal and are the cause of undesirable physiological and/or ecological effects, consequently leading to disease conditions in humans, other living creatures and pollution, respectively (Olicón-Hernández et al. 2017). Environmentally predominant synthetic xenobiotic compounds include hazardous pollutants such as pesticides, fuels, solvents, alkanes, hydrocarbon derivatives, synthetic polymers, dyes, plastics, etc. (Sharma et al. 2018). Antibiotics, steroids and biomedical waste mixtures also are an example of man-made xenobiotic compounds. Xenobiotic substances are becoming progressively a major problem as they are relatively new substances that resist degradation, mainly because of their recalcitrant nature. Being foreign to the organisms, xenobiotics are not easily recognized by the microbes in nature, and hence these compounds do not enter common metabolic pathways, thereby remaining persistent in nature. Such chemical contaminants are referred as persistent organic pollutants (Bhatt et al. 2019). The accumulation of persistent pollutants affects the environment and survival of higher as well as lower eukaryotes. Moreover, their recalcitrant nature is a major risk factor that poses threat to human health as it disrupts normal cellular pathways that play a significant role in their development and reproduction (Gangola et al. 2018; Baker et al. 2019). Nevertheless, prolong exposure with these xenobiotics may even cause neurological damage, immunosuppression and cancer (Kalyoncu et al. 2009).

Thousands of persistent organic pollutants exist in the atmosphere and hold an extended half-life of several days in air, or about a decade in soil and in living organisms. For instance, xenobiotic organic pesticides DDT, BHC, polychlorinated biphenyls, halogenated aromatic compounds and other such compounds that mainly partake agricultural and industrial routines are discarded in the environment in huge amounts globally. The chemical pesticides are being used to eliminate insect-borne diseases for healthier harvest, so as to meet the growing demand of food. Although these substances are advantageous to humans, their prolonged presence in the biosphere poses perilous effects, as such chemical compounds are not easily biodegraded and hence their concentration gradually upsurges with time (Varsha et al. 2011). These chemical compounds build up in natural reserves (soil, air and water) and also found to be accumulated in plants, animals and humans; thus, they remain present in the ecosystem for decades (Breivik et al. 2004).

25.2 Points of Xenobiotic Discharge

The prime or direct sources of xenobiotics entering into the environment are effluents from (1) chemical and pharmaceutical industries which include alkylphenols, octylphenol and biphenyls such as bisphenol A, stilbene, genistein, estrogens, etc. These pharmaceutically active complexes are controversial endocrine disruptors which disturb the physiological stability in animals and are bothersome to human health (Asgher et al. 2008). (2) Paper and pulp bleaching are major sources of release of harmful polychlorinated phenols (PCPs) and other organic compounds and include dyes like azo dye and crystal violet (D’Souza et al. 2006). Effluent discharge from textile industries and paper printing where synthetic dyes are majorly being used, adversely affects the aquatic life (Kumari et al. 2014). (3) Mining releases heavy metals into biogeochemical cycles. (4) Fossil fuel combustion and gas plants produce polycyclic aromatic hydrocarbons like naphthalene and benzo (a)pyrene, which are noxious and possibly carcinogenic xenobiotics (Gojgic-Cvijovic et al. 2012). (5) Intensive agriculture practices use enormous amount of chemical fertilizers, herbicides and pesticides such as chlorinated aromatic compounds and their derivatives: DDT, chlordane and lindane. Bioaccumulation and biomagnification of pesticides lead to toxic behavioural effects on animals and the human race.

25.3 Classes of Xenobiotic Compounds

On the basis of chemical composition, the recalcitrant xenobiotic compounds that are released from different industrial residual wastes, viz. paper and pulp remnants, dye effluents, chemicals, plastics and pharma waste, are categorized into the following types.

25.3.1 Halocarbons

These are volatile compounds comprised of different numbers of halogens (Cl, F, Br, I) in place of hydrogen atoms. These compounds are mainly used in the preparation of organochlorine pesticides, i.e. insecticides (DDTs and its metabolites [toxaphene, chlordane, etc.], BHC, lindane, etc.), herbicides (dalapon, 2,4-dichlorophenoxyacetic acid, *2*,*4*,5-trichlorophenoxyacetic acid, etc.) and fungicides (Qadir et al. 2017). Volatile compounds when escaped into the environment cause damage to the ozone layer, and when deposited in soil and leach into waterbodies, they lead to biomagnification (Bharadwaj 2018).

25.3.2 Polychlorinated Biphenyls (PCBs)

PCBs (2-chlorobiphenyl, 4,4′-dichlorobiphenyl, 2,2′,5,5′-tetrachlorobiphenyl, etc.) are prevalent toxic pollutants, inert in nature and highly stable mixtures resistant to extreme temperature and pressure (Tigini et al. 2009a). These compounds are covalently linked with two benzene rings having chlorine in place of hydrogen atoms. These are broadly used in plasticisers, in electrical equipment like capacitors and insulator coolants in transformers, etc.

25.3.3 Synthetic Polymer

Synthetic polymers are high molecular weight compounds also known as plastic polymers which include polystyrene, polypropylene, polyethylene, polyvinyl chloride, etc. Polyamide such as nylon, is expansively being used for wrapping materials, in garments, etc. (Shrivastava 2018).

25.3.4 Alkylbenzylsulfonates

These are anionic surfactants (branched alkylbenzene sulfonates and linear alkylbenzene sulfonates) broadly used in formulation of detergents. They have hydrophilic sulfonate group present at one end, as a result of which they are resistant to degradation by microorganisms, while at another end hydrophobic alkylbenzene tail is present, making it recalcitrant if it is branched (Bharadwaj 2018).

25.3.5 Oil Mixture

Oil is a natural product, is insoluble in water, possesses some toxic constituents and thus is a recalcitrant. Microbial degradation of oil has varying rates of degradation based on the complexes present in it. However, biological means of degradation of large oil spills over the water surface is ineffective, resulting in severe pollution problems (Qadir et al. 2017).

25.4 Hazardous Effect of Xenobiotic Compound

Xenobiotic compounds are potentially perilous to both lower and higher eukaryotes and even to humans. Exposure to xenobiotic pesticides increases the risk of diabetes, neurological disorder and several skin diseases and extended exposure may even cause cancer (Qadir et al. 2017). As described in the reports by Kelce et al. (1995), scientific inferences from environmental impact assessment studies depict that persistent organic pollutants are a major hazard that causes impairment of brain function, reproductive dysfunction and endocrine disruption. Besides, their recalcitrant nature leads to their gradual accumulation in the environment with time, thereby entering into the food chain and hence upsetting the ecosystem (Bharadwaj 2018).

25.5 Microbial Remediation of Xenobiotics

The use of chemicals that are noxious to human beings and that damage the wilderness of nature is prevalent. Industries manufacture different chemical compounds to satisfy the need of people for better living of life. Although the usage of pesticides, paints, plastics, pharmaceuticals and textiles that contribute to xenobiotics cannot be neglected from our daily needs, steps should be taken to eliminate these xenobiotic chemicals from the environment. Degradation of organic pollutants using physical and/or chemical route is economically not feasible; besides, chances of undesirable toxic intermediates and end products being formed are high. For this purpose, exploitation of microorganisms is the most competent, sustainable and feasible way to achieve efficient degradation of xenobiotic pollutants (Fig. 25.1). Microbes are likely used because of their rapid growth rate and that they possess complex enzymatic machinery that helps to degrade complex toxic compounds to innocuous or less harmful degraded products. Microorganisms also have evolved excellent biochemical control mechanisms so as to utilize pollutants as a source of carbon and energy and degrade them. However, microorganisms fail to degrade all chemical compounds for the reason that they are unable to break certain chemical bonds present in them (Gangola et al. 2019).

25.5.1 Bacterial Remediation

Successful bioremediation requires potent microbial strains which can withstand and degrade hazardous pollutants. Bioremediation mechanisms include both isolation of naturally occurring xenobiotic-degrading microbes from heterogenous microbial population and genetically engineered microorganisms. Biodegradation is affected by various factors like hydrophobic nature of hydrocarbons, bioavailability and predominant environmental conditions. Microorganisms can enhance the hydrophobicity of the cell surface by inherently changing their outer membrane so as to facilitate the uptake of hydrocarbons (Shukla and Singh 2020). Microbial communities get colonized at contaminated sites as they metabolize recalcitrant xenobiotic compounds (Galvão et al. 2005). Several aerobic (*Pseudomonas*, *Bacillus*, *Escherichia*, *Serratia*, *Gordonia*, *Moraxella*, *Micrococcus*, *Sphingobium*, *Pandoraea*, *Rhodococcus*), anaerobic (*Desulfovibrio*, *Desulfotomaculum*, *Methanospirillum*, *Methanosaeta*, *Pelatomaculum*, *Syntrophobacter*, *Syntrophus*) bacteria, methanotrophic and methanogenic bacteria, cyanobacteria and sphingomonads possess xenobiotic degradative potential (Varsha et al. 2011; Sinha et al. 2009).

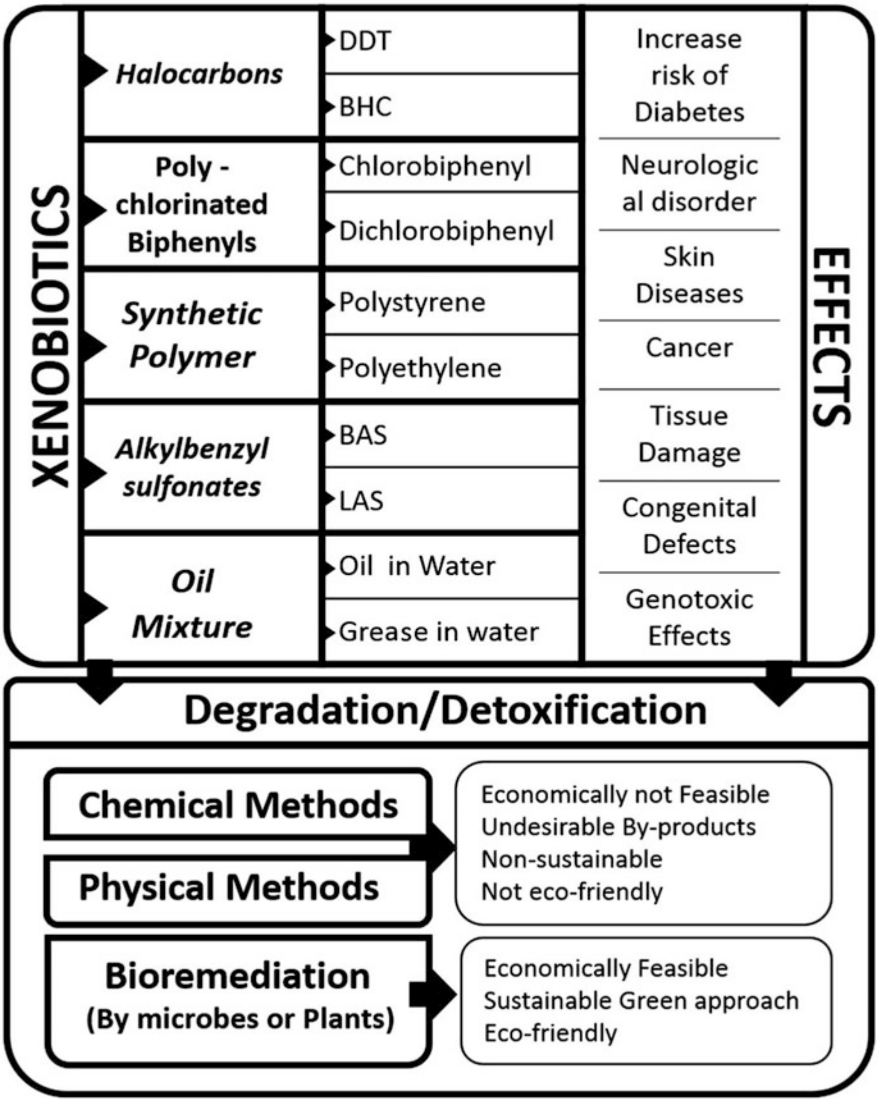

Fig. 25.1 Xenobiotic compounds, its types, effects and various strategies for degradation and detoxification (*BAS* branched alkylbenzene sulfonates, *LAS* linear alkylbenzene sulfonates)

A soil bacterium *Serratia* sp. strain DS001 utilized methyl parathion (an organophosphate insecticide), 4-nitrocatechol, *p*-nitrophenol and 1,2,4-benzenetriol as sole carbon and energy source and effectively metabolized methyl parathion, signifying the role of enzyme parathion hydrolase in degradation (Pakala et al. 2007). Yao et al. (2006) reported efficient degradation of phenolic compounds using H_2O_2 as oxidizer and an enzyme extracted from *Serratia* sp. AB 90027 as a catalyst. Using this enzyme/H_2O_2 treatment, high COD removal efficiency was also achieved. Kafilzadeh et al. (2015) in their studies collected samples from the sediments of Kor River, Iran, from the area with high agricultural activity and isolated five bacterial genera (*Klebsiella*, *Acinetobacter*, *Alcaligenes*, *Flavobacterium* and *Bacillus)* that were able to metabolize endosulfan, a lipophilic insecticide. Degraded metabolites of endosulfan produced depicted less toxicity when compared with endosulfan itself.

Extremophiles such as halotolerant and thermotolerant *Bacillus* sp. strain DHT also possess the capability of utilizing fuels, crude oil, several pure alkanes and polycyclic aromatic hydrocarbons as a sole carbon and energy source (Kumar et al. 2007). Thermophilic bacterium *Brevibacillus borstelensis* degraded polyethylene xenobiotic compounds for the sole source of carbon (Hadad et al. 2005). Efficient catabolism of phenanthrene and crude oil was attained by M19F and M16K strains of *Bacillus subtilis* on day 28 and day 18 post-inoculation, respectively (Oyetibo et al. 2017). Furthermore, *Bacillus drentensis* strain S1 isolated from sewage sample proved its potential in biodegradation of drug acetaminophen (paracetamol) (Chopra and Kumar 2020). A pure strain of *Pseudomonas* sp. YATO411 in an immobilized and a freely suspended system exhibited biodegradation of benzene and toluene, indicating its potential to catabolize high concentrations of these xenobiotics (Tsai et al. 2013). Additionally, bacteria of *Pseudomonas* species have been testified for partial and complete decomposition of fungicides, pesticides, aliphatic or polycyclic aromatic hydrocarbons, recalcitrant dyes, phenolic compounds and hexavalent toxic heavy metals (Poornima et al. 2010; Wasi et al. 2010; Joe et al. 2011). *Rhodococcus erythropolis* bacteria presented its potentiality in bio-desulfurization of crude oil (Amin 2011). An idea of “Super Bugs” also has been advanced to catabolize an extensive array of xenobiotic pollutants (Furukawa 2003). A metabolic by-product, known as “microbial concrete”, is produced by urease-producing bacterial strains, namely, *P. aeruginosa*, *P. mirabilis* and *Micrococcus*. Microbial concrete is employed for remediation and re-establishing the buildings (Reddy and Yang 2011). Cyanobacterial mats also were exploited for cleaning up contamination of oil spinoffs (Bordenave et al. 2009; Raeid 2011). Sarkar et al. (2017) reported 98% removal of total petroleum hydrocarbons with the aid of microcosms bioaugmented with *Enterobacter*, *Pandoraea* and *Burkholderia* strains.

Owing to the refractory properties of xenobiotics, their bioavailability is very low and thus their accessibility might be difficult under subsurface environment, and therefore they are persistent in the environment, escaping the metabolism by microorganisms. Consequently, organisms having degradative potential can be genetically manipulated to enhance mobility so as to access these pollutants (Díaz 2004). A major restraint of bioremediation process is optimal physico-chemical conditions that are obligatory for accurate metabolic working of microorganisms, as a prerequisite for degradation of xenobiotics, which may be tough to achieve in natural environment (Singh and Ward 2004). In several studies, syntrophic bacterial consortia are being used for degradation of xenobiotics, as single microorganism in some cases may not be able to perform all metabolic activities required (Díaz 2004). Therefore, when mixed bunch of bacteria works in combination, the dead-end products from one organism will be then broken down by another bacterium (Singh and Ward 2004). Biodegradation of chrysene, a persistent polycyclic aromatic hydrocarbon, was achieved by employing bacterial consortium consisting of *Bacillus* sp., *Rhodococcus* sp. and *Burkholderia* sp. Chrysene was utilized as a sole source of carbon and energy by the consortium (Vaidya et al. 2018). Researches also reported for possibility of application of a microaerophilic mixed bacterial consortium for complete mineralization of azo dyes methyl orange and Congo red (Dissanayake et al. 2021). Furthermore, anaerobic microbial consortia also are reported for anaerobic degradation of levofloxacin (Shu et al. 2021).

25.5.2 Myco-Remediation

Fungi are characterized as one of the most diverse collections of microorganisms that exhibit a significant part in nature as decomposers, mutualists or pathogens (Schmit and Mueller 2007). Mineralization exploiting fungus may be accomplished by direct metabolism, where a xenobiotic component may be totally degraded by fungi to non-toxic end products like inorganic chemical compounds and CO_2 (Mougin et al. 2009). Amongst diverse groups of fungi, white rot fungi can degrade several xenobiotics, including polycyclic aromatic hydrocarbons, persistent pesticides, dyes, chlorinated phenols and pharmaceuticals (Vanhulle et al. 2008). Besides, a wide variety of fungal species that assist in degradation of recalcitrant xenobiotic compounds are listed in Table 25.1.

25.5.3 Phytoremediation

Phytoremediation, termed as green remediation, agro- or botano-remediation, is a technique that employs plants for treating chemically contaminated soils (Wenzel et al. 1999), thereby reducing the concentration of hazardous compounds (Utmazian and Wenzel 2006). Plants are used because they can endure reasonably elevated amounts of xenobiotic chemicals deprived of noxious effects (Briggs et al. 1982). Moreover, plants have a potential to take up these chemicals and convert them into less toxic compounds (Bock et al. 2002). Therefore, it is one of best green routes to target chemical pollutants present in the environment for their removal. Enzyme secreted within plants aids in degradation of chlorinated compounds, herbicides and other organic pollutants; this method of removal of pollutants is termed as phytotransformation (Shukla et al. 2010). Likewise, rhizodegradation also leads to degradation and detoxification of recalcitrant pollutants present in soil with the aid of plant roots. The process is employed for catabolism of chlorinated solvents, surfactants, PCBs, petroleum hydrocarbons and various pesticides (Goyal and Basniwal 2017). For elimination of toxic metals from the soil, a method known as phytomining is employed, in which metal ions are absorbed by plant roots. If

Table 25.1 List of fungal species that possess xenobiotic degradation potential

Xenobiotic compounds	Fungi degrading xenobiotics	References
Pesticides		
Diazinon	*Aspergillus niger* MK640786	Hamad (2020)
Chlorpyrifos, profenofos and methyl parathion	*Aspergillus sydowii* CBMAI 935	Soares et al. (2021)
DDT	Consortium *of fungus Fomitopsis pinicola* + bacterium *Ralstonia pickettii*	Purnomo et al. (2020a)
	Xerocomus chrysenteron	Huang and Wang (2013)
	P. aeruginosa + *P. ostreatus*	Purnomo et al. (2017)
Allethrin	*Fusarium proliferatum* CF2	Bhatt et al. (2020a)
Chlorfenvinphos	*Penicillium citrinum*, *Aspergillus fumigatus*, *Aspergillus terreus* and *Trichoderma harzianum*	Oliveira et al. (2015)
Endosulfan and chlorpyrifos	*Cladosporium cladosporioides*, *Phanerochaete chrysosporium*, *Trichoderma harzianum*, *Trichoderma virens*, *Trametes hirsuta* and *Trametes versicolor*	Bisht et al. (2019)
PCBs		
Aroclor 1254	*Phanerochaete chrysosporium*	Eaton (1985)
3,3′,4,4′-Tetrachlorobiphenyl, 2,3,3′,4,4′-pentachlorobiphenyl, 2,3′,4,4′,5-pentachlorobiphenyl, 3,3′,4,4′,5-pentachlorobiphenyl and 2,3′,4,4′,5,5′-hexachlorobiphenyl	*Phlebia brevispora*	Kamei et al. (2006)
2-Chlorobiphenyl, 4,4′-dichlorobiphenyl and 2,2′,5,5′-tetrachlorobiphenyl	*Aspergillus fumigatus* MUT 4026, *Penicillium chrysogenum* MUT 4021, *Fusarium solani* MUT 4020, *Penicillium digitatum* MUT 4079, *Scedosporium apiospermum* MUT 641 and *Scedosporium apiospermum* MUT 631	Tigini et al. (2009b)
PCBs	*Pleurotus sajor-caju* LBM 105	Sadañoski et al. (2019)
Dye		
Methyl orange	*Gloeophyllum trabeum*	Purnomo et al. (2020b)
Triphenylmethane dyes	*Penicillium simplicissimum*	Chen et al. (2019)

(continued)

Table 25.1 (continued)

Xenobiotic compounds	Fungi degrading xenobiotics	References
Acid red 88	*Achaetomium strumarium*	Bankole et al. (2018a)
Reactive blue 4, Remazol brilliant blue and acid blue 129 (AB129)	*Trametes hirsuta* D7	Alam et al. (2021)
Scarlet RR dye	*Peyronellaea prosopidis*	Bankole et al. (2018b)
Polycyclic aromatic hydrocarbons		
Mixture of four polycyclic aromatic hydrocarbons	*Fusarium oxysporum*	Marchand et al. (2017)
Phenanthrene, anthracene and pyrene	*Trematophoma genus*	Moghimi et al. (2017)
Anthracene	*Trichoderma harzianum*, *Cladosporium* sp., *Aspergillus sydowii*, *Penicillium citrinum* and *Mucor racemosus*	Birolli et al. (2018)
Anthracene, anthrone, anthraquinone, acenaphthene, fluorene, phenanthrene, fluoranthene, pyrene and nitropyrene	*Cladosporium* sp. CBMAI 1237	
Phenanthrene, anthracene and pyrene	*Coriolopsis caperata*, *Fomes fomentarius*, *Pluteus chysophaeus*	Hadibarata and Yuniarto (2020)
Benzo[a]pyrene	*Penicillium canescens*, *Cladosporium cladosporioides*, *Fusarium solani* and *Talaromyces helicus*	Fayeulle et al. (2019)
Anthracene and dibenzothiophene	*Penicillium oxalicum*	Aranda et al. (2017)
Estrogenic xenobiotics		
Bisphenol A, estrone, 17β-estradiol, estriol, 17α-ethinylestradiol, triclosan and 4-*n*-nonylphenol	*Pleurotus ostreatus* HK 35	Křesinová et al. (2018)
4-*t*-Octylphenol	*Fusarium falciforme*	Rajendran et al. (2017)
Testosterone and 17α-ethynylestradiol	*Lentinula edodes*	Muszyńska et al. (2018)

required, metals can be extracted by incineration of plants to yield ash and assimilation of plant incinerations will help metal reuse (Shukla et al. 2010). *Thlaspi caerulescens*, *Alyssum murale*, *Alyssum markgrafii*, *Bornmuellera baldacii* subsp. *markgrafii* and *Leptoplax emarginata* are examples of some plants involved in phytomining (Aboudrar et al. 2007; Bani et al. 2007, 2009). Trends in phytoremediation also use transgenic or genetically engineered plants that help to elevate tolerance and metabolism of xenobiotic chemicals for remediation by plants (Sonoki et al. 2011). In contrast to conventional mechanical methods being used, phytoremediation is an eco-friendly and economically feasible approach to clean up polluted groundwater (Bhatt et al. 2020b). Nevertheless, it is a time-consuming process as it is reliant on the growth of the plants. Additionally, for successful remediation by means of this green technique, it is necessary to look for right plant for right pollutant (Goyal and Basniwal 2017; Bhandari and Bhatt 2021).

25.6 Conclusion and Future Prospects

Environmental pollution is escalating due to increased global industrialization, leading to generation of hefty portions of xenobiotic wastes that present potent health hazard to mankind. Degradation of recalcitrant chemicals is thus the need of the hour so as to lessen contamination of these environmental pollutants. In this context, remediation strategies employing microorganisms, plants and their enzymes have gained substantial attention. As cited in this chapter, microbial remediation using bacteria and fungi and phytoremediation are capable of mineralization of xenobiotics to innocuous or less toxic end products. Though microbial-based systems for removal of pollutants are slow, it offers several pros over physico-chemical methods of remediation, as it is an economically feasible and eco-friendly method. Furthermore, comprehensive studies on the utility of these microorganisms and their enzyme machineries and studies on cloning and expression of genes appear to be compelling tools to decrease the levels of toxic chemicals and to understand the mechanism of biodegradation, respectively. Thus, it can be concluded that elimination of toxic pollutants from the environment through green approach is only possible with the assistance of microorganisms and plants.

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