Chapter 24
Prominences on Xenobiotic Degradation Underneath of Ecological Sanitary

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24.1 Introduction

A xenobiotic is defined as foreign synthetic substrate or compound that occurs within an organism through unnatural resources, also synthesized by or arrived within the same organism. It may include ingredients which exist in abundant concentrations that are expected in nature either in organism or ecological level.

Toxic Concern on Atmosphere In recent past, human population had an unrestricted wealth in terms of land and forest resources. Presently, due to our sloppiness, greed, and compromising approach in occupying them, the ecological assets in the world show, in lesser degree (Vidali 2001). The quick growth of various industries in the past century has extremely increased the release of toxic waste effluents into water bodies along with groundwater (Sethy et al. 2011). Environmental pollution caused by the release of these wide range of compounds [i.e., persistent organic pollutants (POPs)] from industries is creating disturbance to the ecosystem (Gursahani and Gupta 2011), causing climatic changes, reduction of water levels in the ground as well as oceans, melting of ice caps, global warming, ozone layer depletion due to photochemical oxidation, etc. (Sharma et al. 2011; le Mellec et al. 2010), and this made ecologists to focus more on impacts of pollution and its reduction.

It has already been noticed that industrial effluent is not properly regulated; rather, they become accidental (e.g., chemical or oil spills) which can form toxic and persistent material in native environments. Materials in the ecosystem are

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derivative of either biogenic or anthropogenic sources. Anthropogenic compounds are synthetic in nature and play a key role in contaminating the ecosystem (Indu 2006). Xenobiotics are in fact anthropogenic compounds that existed in biological chains or in the defined ecological pyramid which are unnatural and are present in decimal concentration. The latent health risk of a xenobiotic compound is a purpose of its tenacity in the environment as well as the lethality of the synthetic type (Wilson et al. 1985). The matter of xenobiotics in the ecological system has been an advancement for investigation in ecological chemistry for years.

24.2 Genesis of Xenobiotic

The major straight source of xenobiotics is wastewater and solid residual releases from the industries like chemical and pharma, plastics, paper and pulp mills, textile mills, and agriculture (enhancement products like pesticides, herbicides, etc.) (Fig. 24.1a–e). Some of the common residual factors in the wastewater and other effluents are phenol, hydrocarbons, different dyes, paint effluents, pesticides and insecticides, etc. (Gayathri and Vasudevan 2010; Jame et al. 2010; Nagamani et al. 2011; Sridevi et al. 2011).

24.3 Removal of Xenobiotic

Numerous approaches like physicochemical and biological ways have been implemented in the degradation of xenobiotics. There is a proven fact that physicochemical approaches are costly and usually give undesirable yields which are latent and toxic in nature; eventually they require further treatment stages (Sridevi et al. 2011). Such methodologies habitually add fragmented compounds which are

Paper and pulp residues Chemical and Pharma effluents Plastic Waste

Paint and Dye effluents Solid waste

Fig. 24.1 (**a**–**e**) Different sources of industrial effluents. (**a**) Paper and pulp residues. (**b**) Chemical and pharma effluents. (**c**) Plastic waste. (**d**) Paint and dye effluents. (**e**) Solid waste

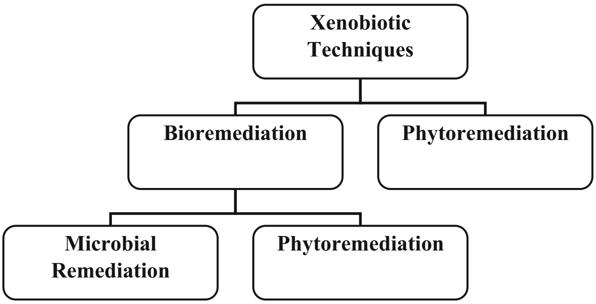

Fig. 24.2 Classification showing different xenobiotic techniques

difficult for further degradation and henceforth create the long-term damage in ecology. In order to get rid of these problems, several other sustainable and eco-friendly techniques have already been evolved, i.e., bioremediation, phytoremediation, etc. (Fig. 24.2).

24.4 What Is Bioremediation?

Microbial degradation of xenobiotics is one of the important ways to remove the environmentally harmful compounds. The potential of microorganisms to metabolize xenobiotic compounds has been recognized as an effective means of toxic and hazardous waste removal (Sridevi et al. 2011; Oaks et al. 2004).

Researchers have used biological system in bioremediation approach and defined as a process that includes microorganisms or their enzymes to retrieve the environment altered by pollutants to its original condition (Oaks et al. 2004). In another diaphragm, bioremediation is being termed as “a treatability technology that exploits biological activity to decrease the toxicity level” (King et al. 1997). Detoxification and mineralization are the vital happenings which occur simultaneously under the effect of biological waylays, where the excess waste material is supposed to be transformed into inorganic substrates, i.e., carbon dioxide, water, and methane (Reshma et al. 2011). Such substrates get accumulated stubbornly in the environment, though nature allows the biodegradation through multiple steps utilizing different biocatalytic systems or in benign presence of microbiota. The best examples are contaminated wastewater, ground- or surface waters, soils, sediments, and air where there has been either accidental or intentional release of pollutants or chemicals which are the sites where bioremediation occurs (Ali Elredaisy 2010; Aghamiri et al. 2011).

24.5 Significant Microbiota for Remediation

Microbiome symbolizes as a predominant biomass of our globe; on the contrary, the human population disturbs the ecology and triggers the xenobiotic influx on the planet. The significant microbes exhibit potential to degrade such lethal chemical, those that are listed as xenobiotic compounds. Microbial system uses their endo- and exo-secreted enzymes under the regime of preset metabolic pathways, exploiting them as novel carbon sources and henceforth cleaning the toxic substances (Singh et al. 2014). It is a proven fact that microbial populations show their eco-friendly behavior to overcome environmental pollution and to help in biodegradation of xenobiotic compounds. To meet such sustainable approach, microorganisms use dual modes of action for dilapidation of xenobiotics compound: (1) aerobic biodegradation and (2) anaerobic biodegradation. Aerobic biodegradation includes rich oxygen delivery systems; it is necessary to stock continuous oxygen due to biofouling at the level of remediation (Sharma and Fulekar 2009); moreover, bioreactor-based application is cost ineffective at the same time produces sludge which is highly expensive for further removal (Kumar et al. 1994a, b). Anaerobic habitats, including sludge digesters, groundwater, sediments, water-laden soils, gastrointestinal contents, feedlot wastes and landfill sites, and some xenobiotic compounds [e.g., tetrachloroethylene, polychlorinated biphenyls (PCBs), and nitro-substituted aromatics], can be effectively transformed or mineralized by anaerobic bacteria (Zhang and Bennett 2005).

In situ bioremediation procedure consists of basically three vital steps:

1. Bioattenuation: It is related to monitoring of natural progress of biodegradation to guarantee that contaminant declines with sampling time.
2. Biostimulation: The intentional stimulation of natural xenobiotic remediating microbes by electron acceptors, water molecule, nutrient addition, and/or electron donors.
3. Bioaugmentation: It is the addition of laboratory-grown potential bacteria that have suitable and biodegradative abilities.

Normally, the microbes use two pathways for biodegradation of xenobiotics, aerobic and anaerobic conditions.

In aerobic bioremediation, the basic equation will be:

$$\text{Xenobiotics} + \text{oxygen} \quad \overset{\text{Microbe}}{\rightarrow} CO_2 + H_2O + \text{left over residue}$$

In case of anaerobic bioremediation:

$$\text{Xenobiotics} \overset{\text{Microbe}}{\rightarrow} CO_2 + CH_4 + H_2O + \text{left over residue}$$

In aerobic biodegradation, CO_2 is produced along with some amount of water. In the absence of oxygen, anaerobic biodegradation process starts and methane gas is generated instead of CO_2. The conversion of biodegradable materials to gases like carbon dioxide, methane, and nitrogen compounds is called mineralization. Mineralization process is completed, when all the biodegradable biomass is consumed and all the carbon is converted into carbon dioxide (Kyrikou and Briassoulis 2007). Alkanes consisting long carbon chains and straight structures considered to be more prone to aerobic biodegradation. Aerobic degradation pathway of alkane degradation is the oxidation of the terminal methyl group into a carboxylic acid through an alcohol intermediate and after all completes mineralization through β-oxidation (Le and Coleman 2011). Aerobic biodegradation process of aromatic compound comprises of their oxidation by molecular oxygen; after oxidation steps, intermediates are the outcome, and then it enters into central metabolic pathways, including the Krebs cycle and β-oxidation.

Some xenobiotic pollutants are not mineralized by an aerobic degradation system because they are greatly recalcitrant owing to increase in halogenations in their structures. Replacement of halogen and nitro and sulfo groups on the aromatic ring increases the electrophilicity of the target molecule. These xenobiotic compounds resist the electrophilic attack by enzyme oxygenases in aerobic degradation process. Some of the recalcitrant that persists under aerobic condition are the polychlorinated biphenyls (PCBs), chlorinated dioxins, and some complex and banned pesticides like DDT (Alcock and Jones 1996). It is essential to overawe the high stubbornness of halogenated xenobiotic compounds from biosphere; in achieving these, the reductive attacks by anaerobic microorganisms are of boundless worth. On the other hand, anaerobic bacteria carried out reductive dehalogenation either by the complimentary reaction or by using a new type of anaerobic respiration. This procedure decreases the degree of chlorination and makes the product more available and manageable for mineralization process by aerobic bacteria (Ferguson and Pietari 2000). During anaerobic degradation process, the reductive dehalogenation is the first step of biodegradation of polychlorinated biphenyls (PCBs); dehalogenation process is carried out under anaerobic conditions where organic substrates act as electron donors.

There are a vast number of potential microbes, especially the bacteria, which carry out the bioremediation of xenobiotics. The common major groups of anaerobic bacteria that have the capability of biodegrading xenobiotic compounds are *Acidovorax* spp., *Bordetella* spp., *Pseudomonas* spp., *Sphingomonas* spp., *Variovorax* spp., *Veillonella alcalescens*, *Desulfovibrio* spp., *Desulfuromonas michiganensis*, and *Desulfitobacterium dehalogenans*, *D. oleovorans*, *G. metallireducens*, and *D. acetonicum*. Anaerobic sulfate-reducing bacteria (SRB) and methanogenic bacterial conditions can be useful to isolate pure culture of anaerobic bacteria to carry out xenobiotic degradation research work (Jiang et al. 2009; Zhang and Bennet 2005). Anaerobic microbes can also use and exploit substituted and intricate aromatic compounds in a way that do not disturb the benzene nucleus in the ring. On the other hand, sulfate-reducing bacteria (SRB) represent a huge group of anaerobic microorganisms that play a crucial role in numerous biogeochemical cyclic processes and also are able to biodegrade the crude oil (Ferradji et al. 2014). The sulfate-reducing bacteria are an obligated anaerobic bacteria, which utilize sulfate as final electron acceptor during the process of anaerobic respiration and, therefore, generate hydrogen sulfide (H_2S gas) by sulfate reduction. Anaerobic degradation process is also a renewable energy source; here the biogas is generated from the anaerobic digestion. It mainly consists of methane (CH_4) that can be collected easily and applied for eco-friendly power generation or as a fuel, which has been proved on a greater scale (Boetius et al. 2000).

24.6 Role of Microbial Enzymes in Bioremediation

Bioremediation is a microbial secreted enzymatic process which transforms a xenobiotic pollutant to innocuous products, which blends naturally with the environment; therefore, the toxicity is removed or reduced to a greater extent.

24.6.1 Enzymatic Activity

Potential enzymes like oxidoreductases, dehalogenases, monooxygenases, phosphotriesterases (PTEs), dioxygenases, oxygenases, etc. are reported from commercial fungal traits and exploited for bioremediation purpose.

These enzymes separate the chemical bonds and repost the electrons from reduced organic compound (called as donor) to another chemical substrate (known as acceptor). In due course of oxidation–reduction reactions, the chemical impurities or pollutants are supposed to be oxidized toward innocuous and harmless compounds (Karigar and Rao 2011). The oxidoreductases rinse the toxic xenobiotics products, i.e., phenolic or aniline compounds, either by the process of polymerization, or copolymerization with other substrates, or binding with the humic substances. The microbial enzymes have already been reported for bioremediation of azo dyes (Kumar et al. 2016; Husain 2006; Rani et al. 2014).

Enzyme-mediated transfer is a well-known phenomenon where one atom of molecular oxygen to the organic compound (Karigar and Rao 2011) in reversible manner has metabolic persuasion at cellular level in prokaryotes. Monooxygenases can be categorized into two subclasses based on the presence cofactor, flavin-dependent monooxygenases and P450 monooxygenases. Flavin-dependent monooxygenases contain flavin as prosthetic group and NADP or NADPH as coenzyme. P450 monooxygenases are heme-containing oxygenases that persist in both eukaryotes and prokaryotes. Monooxygenases act as biocatalysts in the bioremediation process and synthetic chemistry because they are highly regioselectivity and stereoselectivity on a wide range of substrates (Karigar and Rao 2011). Monooxygenases catalyzes enormous reactions such as desulfurization, dehalogenation, denitrification, ammonification, hydroxylation, biotransformation, and biodegradation of various aromatic and aliphatic compounds.

Dehalogenase plays an important role in the degradation of chlorinated pollutant. Some anaerobic microorganisms exploit dehalorespiration and use halogenated compounds as terminal electron acceptors (Le and Coleman 2011). An example of this process is the conversion of perchloroethylene (PCE), either dichloroethylene (DCE) (Schumacher and Holliger 1996), ethylene, or ethane which depends on the conditions. Researchers have reported the partial purification of two reductive dehalogenases from *Dehalococcoides ethenogenes* strain 195; both enzymes are membrane proteins. The first enzyme PCE-reductive dehalogenase reduces PCE to TCE and the second enzyme TCE-reductive dehalogenase reduces TCE, *trans*-DCE, *cis*-DCE, 1,1-dichloroethene, and vinyl chloride (Patil and Bagde 2012).

Phosphotriesterases are microbial isolated enzyme which hydrolyze and detoxify organophosphate pesticides (OPs). This reduces OP toxicity and decreases the ability of OPs to inactivate AchE (Shen et al. 2010; Theriot and Grunden 2010). These enzymes mainly hydrolyze phosphoester bonds like P–O, P–F, P–NC, and P–S, and these hydrolysis mechanisms include water molecule in the phosphorus center (Ortiz-Hernandez et al. 2003).

These are multicomponent enzyme systems that incorporate molecular oxygen to the substrate. On the basis of the complexity of the degradation pathways, the biodegradation phenomenon can be categorized into two types: (1) convergent mode and (2) divergent modes of degradation (Eltis and Bolin 1996). In the convergent mode, structurally varied aromatic compounds are converted to aromatic ring cleavage substrates catechol, gent sate, protocatechuate, and their derivatives. In divergent mode, a metal-dependent dioxygenase channels operate, and dihydroxylated intermediates are formed by one of the two possible pathways: the meta-cleavage pathway or the ortho-cleavage pathway (Takami et al. 1997).

These are classified under the oxidoreductase group of enzymes (EC Class 1) (Karigar and Rao 2011). Oxidation reaction is the major enzymatic reaction of aerobic biodegradation and is catalyzed by oxygenases. Oxygenases oxidize the substrates by transferring oxygen from molecular oxygen (O_2) and utilize FAD/NADH/NADPH as the co-substrate. Oxygenases metabolize organic compounds; they increase their reactivity and water solubility and cleave the aromatic ring (Arora et al. 2010). On the basis of the number of oxygen atoms used for oxidation, oxygenases can be further categorized into two groups: (1) monooxygenases and (2) dioxygenases, which have been discussed earlier.

24.7 Impending Aspects

Since last few decades, there has been a huge development in the field of the bioremediation of xenobiotic compounds. Several novel microbe-mediated bioremediation approaches have been reported targeting rare ecological niches and given interesting remediation pathways. Moreover, existing information on microbial exploitation is still under the scan. Biotransformation of xenobiotic compounds is yet to be reconnoitered owing to its multifarious nature. Efficacy of such xenobiotic compound for its biodegradation can be enhanced by addressing some relevant issues: (1) adapting tolerance mechanism to various xenobiotic materials, (2) the constitutive expression of catabolic candidate genes against the specific raw substrates, and (3) the kinetics and stability of the candidate enzymes which have recently been encoded. Even though usefulness and efficacy of the constructed organisms in terms of environmental pollution problem in ecosystem are yet to be uncovered and tested.

Numerous microbes biodegrade xenobiotic compounds through plasmids which encode for the catabolic genes and further transmit under specific conditions. To elucidate and develop the construct for such candidate genes and its further mobilization through the recombinant process, an improved construct of potential strains is required, and a proper, well-designed management is also a prerequisite. Due to the existing facts, the microbial degradation machinery is a bridging scope from the environmental monitoring point of view and ultimately leads to biodegradation of lethal compounds. In bioremediation process, presently, molecular techniques and approaches are being applied to characterize the genetic material of numerous bacteria from the several ecological samples. Existing data helps the researchers to compare with the standard and prevalent microbiological technique approaches; the molecular procedures facilitate us with more compatible and comprehensive explanation of in situ microorganism population and its response to concocted bioremediation and normal lessening processes. In addition to that, a dominant molecular procedure is required as metagenomic library which has been thrived for identification of the specific catabolic gene pool. In principle, the metagenomics approach is a culture-independent microbial genomic analysis; this approach is in function mode due to the existence of another powerful sequence-driven method. For entire microbial communities, modern tool and techniques which include the direct genetic investigation approach provide the access to recover unknown sequences from rare microbiota. The consistent and persistent contact with the pollutants and long exposure to their occurrence are the fundamentals of fight in contradiction of xenobiotic compounds; meanwhile such developments allow the advancement and evolution of new, more or less safe processes of xenobiotic remediation by microbiomes.

24.8 Conclusions

Microbial assortment, the abundance of species in ecological sites, delivers an enormous pool of resources which we can exploit for our benefit. Though, little is known about the true diversity of bacterial life. Despite the acknowledged value of microorganisms, our understanding of their diversity and many of their key roles in sustaining global life-support systems is still very scarce. This is because the vast majority of bacteria are non-culturable by standard methods, and we have only recently acquired the skills to explore this aspect of microbial biodiversity. Exploring the range of microbial biodiversity is the key to developing effective and environment-friendly “green” technologies. Bioremediation is one such process that exploits the catabolic abilities of microorganisms to degrade harmful and toxic xenobiotics. We have been able to restore what once were irreversibly polluted sites in some cases, attesting to the usefulness of this cleanup process. However, to maximize the potential benefits of microbial community in combating pollution problems, it is vital that we have fundamental understanding of a microbe’s degradative potential under various conditions, its biochemical systems, and its molecular biology.

Environmental problems caused by the industrial effluents are mainly due to accumulation of pollutants and other fragmented compounds, which in turn form into other substitutes (natural or manmade), finally forming a xenobiont. There is a quick need to degrade these xenobiotic compounds in an eco-friendly way. Various techniques like microbial remediation, phytoremediation, and photoremediation and their subtypes have been discussed. Each having their own ways of degrading these xenobionts also has a negative impact on the environment (side effects due to fragmentations and bioaccumulations). Photoremediation, a novel equipment-based technique, is rapid but also has a negative impact on the environment. Being a solar-driven technique, phytoremediation is restricted to particular sites containing contaminants. Although slow, on the whole, microbial bioremediation was found to cover a wide range of recalcitrant degradation and is known to be a better choice because of its nature of degradation.

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