Microbial Degradation of Xenobiotic Compounds

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Abstract

Xenobiotic compounds are extraneous chemicals accumulated in the environment that are posing a threat to the biosphere. The highly recalcitrant nature of xenobiotic compounds makes them resistant to biological degradation. However, numerous microorganisms have been extensively explored for their competency in the degradation of such compounds. Polycyclic aromatic hydrocarbons, nitroaromatic compounds, aromatic hydrocarbons, as well as halogenated aliphatic, azo compounds, *s*-triazines, and organic sulfonic acids, are essential classes of pollutants with xenobiotic structural characteristics. These compounds must be assessed for their degradation extent employing efficient microbes. In natural environments, the surrounding 'environment's physicochemical attributes may influence and indeed constrain overall biodegradation performance. Moreover, during microbial degradation, several biotic and abiotic factors such as pH,

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temperature, etc., influence the process. The current chapter presents an overview of the microbial degradation of xenobiotics. A systematic approach for efficient degradation and factors affecting the overall process is comprehensively discussed. Further, the current challenges and opportunities concerning the degradation of distinct xenobiotics are also discussed.

Keywords

Biodegradation · Xenobiotic · Mineralization · Co-metabolism · Pesticides · Chlorinated hydrocarbons · Haloaromatics · Azo compounds · Nitroaromatic compounds

7.1 Introduction

In the last few decades, unprecedented population growth and precipitous industrial development have accelerated the use of new synthetic compounds. These anthropogenic compounds, which are unknown to the living species, are collectively called as xenobiotic. These human-made compounds (inorganic, organic, or metal-containing) with the artificial chemical structure are not a natural component of the biosphere and appended into the environment by artificial means. Hence, synthetic organic compounds (xenobiotics) have become a threat to the environment (Jeffries et al. 2018). In the Greek language, Xenos means "strange" and biotics means "life-related" (Ojo 2007). These strange, exogenous synthetic substances have "unnatural" structural features to which microorganisms have not been exposed during evolution (Greń 2012). Most of the xenobiotic compounds are toxic, and unprecedented release of them in the environment instigates a global concern. The xenobiotic compounds mainly include pesticides, herbicides, fertilizers, pulp/paper bleaching agents, drugs, fuels, solvents, carcinogens compounds, antibiotics, synthetic azo dyes, refrigerants, and other organic compounds (Jha et al. 2015). Xenobiotic compounds are also called recalcitrant, as they resist natural decomposition and remain in the environment for a longer time (Jha et al. 2015). The unusual chemical and physical properties of xenobiotics compounds make them recalcitrant to biodegradation (Godheja et al. 2016). The chemical structural factors, such as the type, number, the relative position of bonds, and the nature of substituents, are mainly accountable for the xenobiotic character that resists microbial enzyme's attack (Anawar et al. 2017). The bond energy of carbon and halogen bond (-C-X) is extremely high and needs a large amount of cleavage energy. Hence, the presence of halogens and other groups, such as nitro, sulfonate, methoxy, amino, etc., as a substitute makes synthetic organic compounds non-degradable (Knapp 2003). The aromatic nature, cyclic structure, and branched linear chains further enhance xenobiotic nature. Furthermore, the high stability, insolubility in water, and considerable molecular weight are the other prime factors for the nondegradability of these compounds (Phale et al. 2019). Based on structural moiety or characteristics, xenobiotic compounds can be classified into six different types. The halocarbons are the primary type of xenobiotic compounds having halogen atoms, namely, CI,

Br, F, or I. The *primary sources* of halocarbons are paints, condenser units of cooling systems (Freons, CCI_3F), insecticides (dichloro-diphenyl-trichloroethane (DDT), lindane, etc.), and herbicides (dalapon) and solvents (chloroform, $CHCl_3$). The paper mill effluent also contains different halocarbons, such as pentachlorophenol, tetra-chloro-guaiacols, tetra-chloro-catechol, etc. The *second type* of xenobiotic compounds comprises of two benzene rings covalently linked and substituted with halogens commonly recognized as polychlorinated biphenyls (PCBs). The primary sources of PCBs are mainly plasticizers employed in the synthesis of plastics, coolants used in transformers, and heat exchange fluids (Godheja et al. 2016). The synthetic polymers (polyethylene, polystyrene, polyvinyl chloride, nylons, etc.) used as garments, wrapping materials, etc., comprise the *third type* of xenobiotic compound. The sulfonate ($–SO_3$) group bearing detergents, commonly called alkyl benzyl sulfonates, are the *fourth type* of xenobiotic compound. The oil mixtures, due to their toxicity and insolubility in water, become recalcitrant and constitute the *fifth type* of xenobiotic compounds. Pesticides and herbicides, such as organophosphorous, benzimidazoles, methyl parathion, and morpholine, having an aliphatic or aromatic cyclic ring structure with different groups as a substitute forms the *sixth type of xenobiotic* compounds (Anawar et al. 2017).

The persisting nature of xenobiotics compounds in the environment for long time results in bioaccumulation or biomagnification. The accumulation of harmful synthetic compounds has more prolonged stability in soil that triggers impediments in soil ecosystems. Similarly, the long-lasting occurrence of extensively used aromatic herbicides such as triazines used for constraining the growth of broad-leaved weeds in agricultural fields and urban and recreational areas, alter the soil environmental conditions (Gouma et al. 2014; Cook 1987). Similarly, freshwater and marine ecosystems are also affected by xenobiotic compounds. These toxic compounds are entered into the food chains and accumulated in the high concentration level. The non-degradable pesticide and herbicides straightway influence organisms, soil quality, and also reaches nearby freshwater bodies (Abatenh et al. 2017a). Drugs and antibiotics are foreign to the human body and can be considered as xenobiotics. These compounds can trigger disorder multiple cellular communication pathways directly linked to the growth, development, and normal physiological function (Greń 2012). The crucial components of the cell which interact with xenobiotics are proteins, lipids, and DNA, the latter leading to a mutation that can lead to cancer. Many of the xenobiotic compounds, such as substituted phenolic compounds, phthalates, etc., behave as endocrine disruptors, causing preterm birth, early weaning, and altering the quality of semen, while adversely affecting menstruation cycle and duration of lactation in humans.

The traditional methods employed for the remediation of xenobiotic contaminated sites include chemical treatment, low temperature-induced thermal desorption, incineration, and photocatalytic treatments (Greń 2012). Apart from these, biological methods, such as bioremediation and phytoremediation, are found to be more efficient, safe, economical, and sustainable than thermal and chemical processes (Jha et al. 2015; Anawar et al. 2017). Bioremediation is most widely utilized to remove xenobiotic compounds through degradation, immobilization, or

detoxification of these hazardous materials while employing suitable microorganisms (Greń 2012; Abatenh et al. 2017a). The bioremediation technique encompasses numerous microbial species possessing different degradation mechanisms to eradicate the toxic contaminants from the environment. Microbial degradation of xenobiotics compounds is a natural tactic to confiscate environmental pollution (Poursat et al. 2019). The more comprehensive range of growing conditions and high tolerance to chemical contaminants make microbial degradation an efficient green method for mitigating nondegradable xenobiotics pollutants. The detoxification of xenobiotics compounds through microbial metabolism has been well studied. The comprehensive understanding of microbial transformations of xenobiotic compounds (mainly synthetic organic compounds) with a brief discussion of the removal of different toxic compounds and the factors influencing the processes have been discussed in this chapter.

7.2 Microbial Biodegradation

The nutritional requirements of microorganisms are usually organic carbon as a source of energy. This nutritional versatility of microorganisms can be exploited for the degradation of pollutants (Abatenh et al. 2017b). The well-organized detoxification process of contaminants by breaking down to less or non-toxic elements or completely mineralized or transformed into carbon dioxide in the environment employing microorganisms are termed as bioremediation. Usually, bacteria, fungi, and archaea are bioremediators that can be employed as biological agents to carry out bioremediation (Strong and Burgess 2008).

7.2.1 Different Microbes for Xenobiotics Degradation

Nearly half of the biomass of earth is microorganisms representing high diversity in the biosphere (Sinha et al. 2009). 'Microbes' vast adaptability propounds a simple, economic, and greener approach to mitigate environmental pollution while facilitating the biodegradation of xenobiotic compounds. Additionally, microorganisms are an integrated part of biogeochemical cycles. Due to high adaptability, microbes can grow at extreme heat, subzero temperatures, dry conditions, and in the absence of oxygen, hence playing an active role in the biosphere's sustainable development (Srivastava et al. 2014). Figure 7.1 depicts the possible ways of biological transformations of xenobiotics in the environment (Greń 2012).

The catabolic activities can be carried out by bacteria-mediated degradation or fungal-mediated degradation and are crucial in converting complex toxic organic compounds into less or completely nontoxic residues (Srivastava et al. 2014). Both aerobic and anaerobic bacterial genera are actively employed to bioremediate a wide range of xenobiotic compounds. Various aerobic bacteria strains like *Escherichia*, *Pandoraea*, *Bacillus*, *Moraxella*, *Pseudomonas*, *Rhodococcus*, *Sphingobium*,

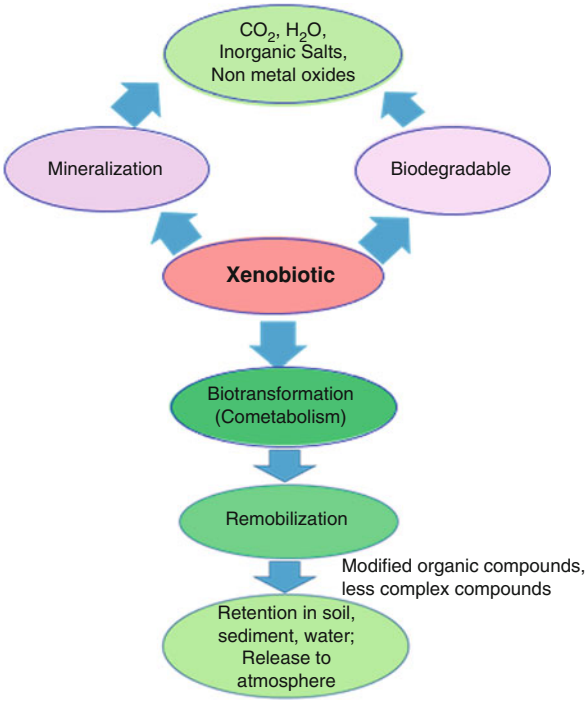

Fig. 7.1 Possible ways of biological transformations of xenobiotics in the environment (Greń 2012)

Gordonia, *Micrococcus*, etc., are employed in the degradation of a wide range of xenobiotic compounds (Van Ginkel 1996; Gangola et al. 2018; Bhatt et al. 2019). Anaerobic bacterial strains, such as *Methanosaeta*, *Pelatomaculum*, *Methanospirillum*, *Desulfotomaculum*, *Syntrophobacter*, *Syntrophus*, *Methanotrophic*, *Methanogenic*, *Cyanobacteria*, etc., are also vastly used in bioremediation (Novotný et al. 2018; Benn and Zitomer 2018). In microbial degradation of organo halogenated compounds such as dichlorodiphenyltrichloroethane, pentachlorophenol, 1,2,3,4,5,6-hexachlorocyclohexane, etc., highly toxic compounds are primarily reduced to less chlorinated intermediates. *Flavobacterium* and *Pseudomonas* strains have been investigated to degrade, specially organo-phosphorous pesticides, aromatic or aliphatic hydrocarbons, phenols, and dyes (Jeffries et al. 2018; Ortiz-Hernández et al. 2003). *Dehalococcoides* sp. has been reported for the decomposition of highly toxic chlorinated hydrocarbons pollutants (Chrast et al. 2019; Saibu et al. 2020). Similarly, polyaromatic hydrocarbons are highly toxic to the ecosystem and also to human health. Various bacterial strains *Pseudomonas*, *Arthrobacter*, *Mycobacterium*, *Sphingomonas*, *Alcaligenes*, etc., are reported as potential agents for the bioremediation of various aromatic hydrocarbons, such as naphthalene, substituted benzene, substituted aniline, xylene, anthracenes, phenolic compounds, etc. (Patil and Yadav 2018; Mpofu et al. 2020; Miyazawa et al. 2020; Tusher et al. 2020). Three enzymes, *esterases*, *permeases*, and *dioxygenases*,

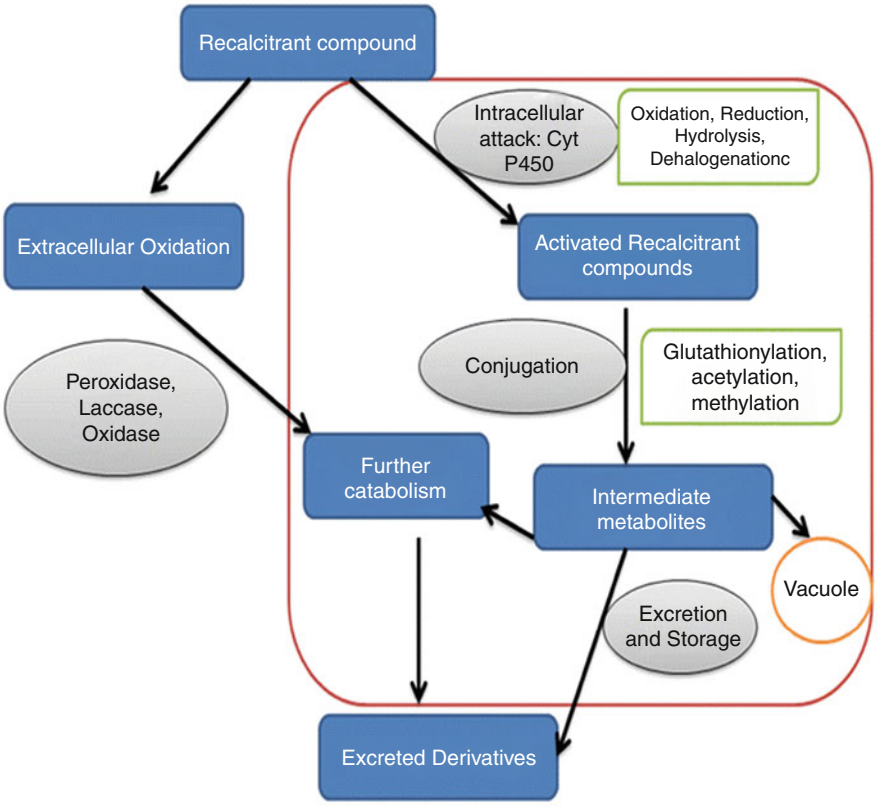

Fig. 7.2 Fungal degradation of xenobiotic compounds (Copyright© 2016, Springer Nature. All rights reserved, reprinted with permission) (Deshmukh et al. 2016)

consecutively degrade phthalate isomers (Vamsee-Krishna and Phale 2008). Bacillus bacterial species effectively mineralize benzimidazole compounds and oil spills (Xu et al. 2018). The dyes get bioaccumulated in natural environments due to high chemical and protolithic stability and pose a negative impact on the food chain. Synthetic and azo dyes used in textile industries can be degraded by bacteria strains, such as *Anoxybacillus*, *Bacillus*, *Exiguobacterium aurantiacums*, *Sphingomonas* sp. *Xanthomonas* sp., etc. (Takon 2019).

The fungi induced biodegradation (Fig. 7.2) of xenobiotic compounds is termed as mycoremediation (Ceci et al. 2019). The fungal-mediated biodegradation is more robust than the bacterial degrading processes, as the fungi can nurture in the presence of high concentrations of toxic organic pollutants (Akhtar and ul Mannan 2020). The rapid colonization of substrates due to 'fungi's mycelial nature facilitates deep infiltration into the pollutant molecule and completely mineralizes it (Bielčik et al. 2019). The degradation of organic pollutants by fungi from aqueous solution is facilitated through the adsorption process. The fungal species, such as

basidiomycetes and ascomycetes, degrade polycyclic aromatic hydrocarbons (PAHs) through laccases, a copper-containing enzyme (Viswanath et al. 2014; Arregui et al. 2019). The petroleum hydrocarbons are completely mineralized by *Pleurotus pulmonarius* an edible rot fungus, *Morchella conica*, and *Tylospcno fibrilnsa* of *Mycorrhizal* species (Liu et al. 2020). The fungal species, such as *Aspergillus niger*, white-rot fungus, *Phanerochaete chrysosporium*, etc. are widely employed in the bioremediation of fertilizers and pesticides (Deshmukh et al. 2016).

To enhance the efficiency of biodegradation of xenobiotic compounds, isolation of bacterial strains with unique catabolic capabilities and genetically modification of degradative pathways is essential (Tahri et al. 2013). The development of engineered strains with superior biodegradation capability becomes a challenge to the scientific community. Genetically engineered microorganisms (GEMs) can be developed by modifying enzyme specificity and affinity, construction pathway, bioprocess development, monitoring, and control (Tahri et al. 2013). From the past decades, genetically engineered microorganisms emerge as an efficient option for the complete degradation of xenobiotic compounds. Table 7.1 depicts a summary of bacterial and fungal genera efficiently utilized in the biotransformation of xenobiotic compounds.

7.2.2 Parameters Influencing the Rate of Biodegradation

The regulation and optimizing of microbial degradation of xenobiotic compounds is a complex process. Its efficiency depends on various factors such as the chemical nature and concentration of pollutants, their availability to microorganisms, 'microorganisms' nature, and environmental factors (Abatenh et al. 2017a). The slow rate of microbial degradation of contaminants is associated with the environmental factors, such as pH, temperature, low moisture, presence of oxygen/other electron acceptors, nutrient contents, and also the chemical nature of pollutants (Knapp 2003).

7.2.2.1 Chemical-Specific Factors

State/Solubility/Hydrophobicity

The microbial degradation is an enzyme-mediated reaction, generally taking place in an aqueous medium. The most important factors affecting the degradation are the substrate's (pollutant's) solubility, state, and hydrophobicity. The 'substrate's physical condition is crucial for biodegradation as many of the liquids and solids recalcitrant organic compounds have low solubility, which may resist degradation. Even the surface area of pollutants affects the rate of biodegradation. The finely divided small particles with greater surface area enhance the attachment of degrading microbes on pollutants and further enhance the degradation rate (Jain et al. n.d.). The hydrophobic property of some organic compound's steers bioaccumulation in the fatty tissues of higher organisms. A hydrophobic organic compound may cause slight damage at lower trophic levels; however, it gets drastically high toxicity for higher trophic levels in the food chain (Jha et al. 2015).

Table 7.1 Bacterial and fungal genera efficiently utilized in the biotransformation of xenobiotic compounds

Compounds	Bacteria	References	Fungus	References
Pesticide				
DDT	*Alcaligenes eutrophus*, *Dehalospirilum multivorans*	Sinha et al. (2009)	*Ph. chrysosporium*, White-rot fungi	Ojo (2007)
2,4-Dichlorophenoxyacetic acid (2,4-D)	*Flavobacterium Arthortbacter*, *Pseudomonas cepacia*, *Alcaligenes eutrophus*	Sinha et al. (2009), Ortiz-Hernández et al. (2003)	*Umbelopsis isabellina*	Nykiel-Szymańska et al. (2018)
Atrazine	*Nocardia*, *Pseudomonas*, *Rhodococcus*	Sinha et al. (2009), Ambrosoli et al. (2005)	*T. versicolor* , *Pl. ostreatus*, *Ph. Chrysosporium*, *Pleurotus pulmonaris*	Knapp (2003), Phale et al. (2019)
Parathion	*Flavobacterium*, *Pseudomonas dimuta*, *Acenetobactor* sp., *Pseudomonas* sp., *Enterobacter* sp. and *Photobacterium* sp.	Jha et al. (2015), Knapp (2003)	*Aspergilus niger* AN400	Abo-Amer (2011)
Diazinon	*Flavobacterium*	Abo-Amer (2011)	*Apergillus niger* MK640786	Abo-Amer (2011)
Fenthion	*Bacillus*	Gangola et al. (2018)	White-rot fungus *Phanerochaete chrysosporium*	Rani et al. (2011)
Carbofuran	*Achromobacter*, *Pseudomonas*, *Flavobacterium*	Jha et al. (2015), Knapp (2003)	Genus *Gliocladium*	Parte et al. (2017)
Lindane	*Bacillus* sp., *Chryseobacterium joostei*	Gangola et al. (2018)	*Ph. Chrysosporium*, *Pleurotus florida*, *Phanerochaete eryngi*	Ulčnik et al. (2013)
Halogenated organic compounds				
Vinylchloride	*Dehalococcoides* sp.	Yoshikawa et al. (2017), Saiyari et al. (2018)	*Aureobasidium pullulans*	Webb et al. (2000)
PCE (trichloroethylene)	*Dehalococcoides ethenogenes*195	Novotný et al. (2018)	*Graphium*, *Trametes versicolor*, *Ganoderma lucidum*, and *Irpex lacteus*,	Marco-Urrea et al. (2008)
Aromatic hydrocarbons compounds				
Napthalene	*Pseudomonas putida*	Nwinyi et al. (2016)	*P. chrysosporium* and *T. harzianum*	Ghosal et al. (2016)

PCP (pentachlorophenol)	*Psedomonas* sp.	Wald et al. (2015)	*T. versicolor*, *Absidia* and *Cunninghamella*	Patil and Yadav (2018), Bhatt et al. (2019)
3CBA (3-Chloeo benzoic acid)	*Arthrobacter* sp.	Jeffries et al. (2018), Mpofu et al. (2020)	White rot and *Ectomycorrhizal*	Ghosal et al. (2016)
1,4 DCB	*Alcaligenes* sp.	Benn and Zitomer (2018), Kurt and Spain (2013)	*Pl. ostreatus*	Marco-Urrea et al. (2009)
2,3,4-Chloroaniline	*Pseudomonas* sp.	Yoshikawa et al. (2017), Nwinyi et al. (2016)	*Basidiomycetes*	Bielčik et al. (2019)
2,4,5-T (2,4,5-trichlorophenoxy acetic acid)	*Pseudomonas* sp.	Liang et al. (2014)	*Eupenicillium* sp.	Itoh et al. (2013)
Fluoranthrene	*Pseudomonas cepacia* AC1100	Nwinyi et al. (2016), Wald et al. (2015)	*Penicillium janthinellum* VUO 10,201	Boonchan et al. (2000)
Pyrene	*Mycobacterium* PYR-1, *Sphingomonas paucimobilis*	Sinha et al. (2009), Yang et al. (2013)	*Trichoderma harzianum*, *Phanerochaete chrysosporium*, *Pleurotus ostreatus*, *Crinipellis stipitaria*	Ghosal et al. (2016)
Xylene	*Penicillium chrysogenum*, *Pseudomonas putida* *Phanerochaete chrysosporium*, *Dechloromonas* sp. (RCB)	Takon (2019), Wald et al. (2015)	*Cladophialophora* sp.	Tahri et al. (2013)
4-Chlorophenol	*Alcaligenes* sp.	Miyazawa et al. (2020)	*R. rhodochrous*	Ghosal et al. (2016)
Dioxins	*Dehalococcoides* sp.	Sinha et al. (2009), Tusher et al. (2020)	*Basidiomycetous*	Nakamiya et al. (2005)
RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine)	*Desulfovibrio* sp.	Sinha et al. (2009)	*Cladosporium resinae*, *Cunninghamella echinulata varelegans*, *Cyathus pallidus* and *Phanerochaete chrysosporium*	Ghosal et al. (2016)

(continued)

Table 7.1 (continued)

Compounds	Bacteria	References	Fungus	References
Benzene	*Dechloromonas* sp.	Jeffries et al. (2018), Strong and Burgess (2008)	*Cladophialophora* and *Cladosporium*	Tahri et al. (2013)
Phthalate compounds				
Phthalate	*Burkholderiacepacia*DBO1	Sinha et al. (2009), Vamsee-Krishna and Phale (2008)	*Aspergillus*, sp., *Fusarium* sp., and *Penicillium* sp.	Steliga (2012)
Azo dyes				
Reactive Dark Blue K-R	*Exiguobacterium* sp.	Sarkar et al. (2017)	*Penicillium* sp. QQ	Steliga (2012)
Reactive Green 19	*Proteus vulgaris*, *Micrococcus glutamicus*	Liang et al. (2014)	*Trametes versicolor* U97	Singh and Singh (2017)
Acid Orange 7	*Aeromonas caviae*, *Protues mirabilis* and *Rhodococcus globerulus*	Singh and Singh (2017)	*Enterococcus faecalis*	Singh and Singh (2017)
Sulfonated di-azo dye reactive red HE8B, RNB dye	*Bacillus* sp. ETL-2012, *Pseudomonas aeruginosa*, *Bacillus pumilus* HKG212	Gangola et al. (2018)	*Pl. ostreatus*, *Micrococcus glutamicus* NCIM-2168	Ferraz et al. (2011)
Acid Orange 7	*Aeromonas caviae*, *Protues mirabilis* and *Rhodococcus globerulus*	Singh and Singh (2017)	*Enterococcus faecalis*	Singh and Singh (2017)
Red HE3B, Remazol black 5B, red HE7B	*Providencia* sp., *Pseudomonas aeuroginosa Alcaligenes faecalis* and *Commamonas acidovorans*	Rieger et al. (2002)	*Aspergillus niger*, *Aspergillus fumigatus*, *Aspergilus terreus* and *Penicillium* sp.	Singh and Singh (2017), Wesenberg et al. (2003)
Organophosphorous compounds				
Chlorpyrifos	*Flavobacterium* sp. ATCC2755,1 *Pseudomonas diminuta*, *Micrococcus* sp, *Enterobacter* sp.	Ortiz-Hernández et al. (2003), Nabil et al. (2011)	*Phanerochaete chrysosporium*, *Pencillium brevicompactum*, *Trichoderma harzianum*, *Aspergillus sydowii*-CBMAI 934, *Penicillium raistrickii* CBMAI 931	Kumar et al. (2018)

Fenitrothion	*Flavobacterium* sp., *Arthrobacter aurescenes* TW17, *Burkholderia* sp. NF100	Ortiz-Hernández et al. (2003)	*Aspergillus parasiticus*, *Trichoderma viride*	Rani and Devi (2018)
Ethoprophos	*Flavobacterium* sp., *Pseudomonas putida*, *Enterobacter* sp	Ortiz-Hernández et al. (2003), Karpouzas et al. (2005), Mardani et al. (2017)	*Fusarium oxysporum*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Fusarium moniliforme*, *Trichothecium*	Kumar et al. (2018), Singh and Walker (2006)
Cadusafos	*Flavobacterium* sp.	Karpouzas et al. (2005)	*Gliocladium*	Javaid et al. (2016)

Size and Shape

In general, the degradation of pollutants is an enzyme-induced biochemical process. The degradative enzymes are located inside a microbial cell and within the cytoplasmic membrane, acting as a selective permeability barrier to the foreign molecule. Further, the formation of an enzyme–substrate complex is necessary to initiate any enzymatic degradation reaction (Jain et al. n.d.). The inability of most of the recalcitrant compounds in complexing with the enzyme's active site due to their size, and probably their shape becomes a crucial factor in affecting the rate of degradation, and this can be overcome by employing techniques using extracellular/isolated enzymes (Knapp 2003).

Toxicity

Various xenobiotic compounds are toxic to some or more extent to most of the microorganisms. The chloro or nitro derivatives of phenols and fungicides are highly toxic to many microorganisms, as they kill susceptible microbes or merely inhibit their growth (Knapp 2003). For example, toluene is highly toxic to most of the bacteria and damages their membrane structure. Many nontoxic chemicals kill microbes while inhibiting a specific key enzyme during the degradative pathway.

Concentration

When the xenobiotic compounds present in less amount in the ecosystem, the lack of biodegradation is observed, it may be due to low concentrations of compounds that do not provoke the formation of the enzymes required to degrade it. Even low organic compound concentrations may not provide a sufficient amount of carbon and energy to microorganisms. It was reported that 75% mineralization of herbicide 2,4-Dichlorophenoxyacetic acid (2,4-D) in stream water took place in just 8 days when the concentration of 2,4-D was between 0.00022 and 0.022 g/L. However, when the concentration was less than 0.0000022 g/L, only 10% mineralization was observed (Knapp 2003).

Molecular Structure

Although xenobiotic 'compounds' fate depends on the entire molecule properties, the presence of different subunits may affect the degradation rate process to some extent. Microbial enzymes quickly disintegrate the functional 'groups' presence, including carboxylic acids, esters, and amide bonds, as all these structural moieties are vulnerable to hydrolysis and do not require any special coenzymes. Whereas amine compounds are less susceptible to degradation than amides due to the requirement of oxygenase, oxidases, or dehydrogenases enzyme and specific co-factors (Abatenh et al. 2017a). The presence of some structural moiety hinders the degradation process. For instance, the quaternary carbon atom (carbon atom that has attached to four other carbon atoms), impervious to degradation as they resist the formation of a carbon–carbon double bond (Knapp 2003). Moreover, the degree of branching also affects biodegradability due to the formation of quaternary carbon (Jha et al. 2015). The presence of diazo linkage, aromatic sulfonic acids, and polychlorinated aromatic or aliphatic moiety is less vulnerable to biodegradation

due to their xenobiotic nature. Similarly, the presence of several substituents also affects the rate of biodegradation. It is clearly understood with the aerobic degradation of the chlorinated phenoxy-acetate herbicides (such as 2, 4-D and 2,4,5-trichlorophenoxy acetic acid (2,4,5-T)). Many microorganisms are capable of degrading 2,4-D. The presence of one extra chlorine atom in 2,4,5-T makes it more resistant to mineralization. Similarly, the position of substituents also affects the rate of microbial degradation. The ortho and para isomers of disubstituted aromatic compounds showed a similar type of the degradability due to similar electron pattern. In contrast, meta isomers of disubstituted aromatic compounds showed varied degradability.

Type of Microbe

In case of bacterial degradation, the Gram-positive and Gram-negative bacteria respond differently to organic, inorganic, and metal-bearing substrate. Hence, the type of bacterial strain also affects the rate of degradation. Due to diverse metabolism, Gram-positive bacteria can adhere to a wide range of aromatic hydrocarbons, metal-bearing substrate, and biopolymers (Narancic et al. 2012). Gram-positive bacteria produce spores as a response when it comes in contact with toxic chemicals and extreme physical conditions. The most utilized bacteria for bioremediation are *Bacillus anthracis* and *Streptomycetes* sp. followed by *Rhodococcus*, *Arthrobacter*, *Gordonia*, and *Nocardia genus* for the disintegration of aromatic organic compounds like benzene and its derivatives, naphthalene, and biphenyls, etc. On the contrary, the presence of lipopolysaccharide outer membrane in Gram-negative bacteria makes them more permissive to toxic compounds (e.g., PAHs).

7.2.2.2 Environment-Specific Factor

pH

The metabolic processes that occur during microbial degradation of xenobiotic compounds include bio-sorption, a pH-dependent phenomenon. The contaminated site's pH also affects the net negative charge on the microbial cell surface and alters the isoelectric point in the solution. Hence, even a slight change in pH affects the rate of metabolic processes. The acidic pH increases the solubility of metal ions, which further intensifies metal ions' adherence to get attached to the microbial cell surface (Strong and Burgess 2008). The overall efficiency of microbial degradation enhances at lower pH (Rehan 2016). Additionally, ligands' ionic state such as carboxyl residue, phosphoryl residues, S–H groups, and amino acid groups changes with change in pH value, thereby altering the degradation process(Srivastava et al. 2014).

Physical Factors

The critical physical factor affecting the rate of bioremediation is *temperature*. It either enhances or slows down the bioremediation process. The survival of microorganisms entirely depends on temperature. In low-temperature environments, such as polar regions, there will only be a constrained and specified microflora,

which is responsible for the decrease in the degradation process"s capability. The microbes metabolically get inactive at a sub-zero temperature in this region due to the ceasing of the transportation phenomenon within the microbial cells leads to even freezing of the entire cytoplasm. The other physical factor affecting the rate of biotransformation is pressure. The environmental conditions, such as high pressure at the abyssal depths of the oceans, constrain the microflora (Abatenh et al. 2017b).

Nutrient Availability

For growth and reproduction, microorganism needs various nutrients. The carbon–nitrogen–phosphorus (C:N:P) ratio is a crucial factor in the balancing nutrient and the biodegradation efficiency (Singh and Singh 2017). The addition of specific nutrients in appropriate quantity is the best possible way to increase the efficiency of bioremediation. In a few particular biodegradations, some particular nutrients, such as metals as a cofactor, are required to carry specific enzymatic conversions. The degradation process facilitated by the cyanocobalamin enzyme of microorganisms that contain vitamin B12 as a prosthetic group/cofactor requires cobalt (Liu et al. 2014). Similarly, white-rot fungi require manganese when manganese peroxidase is used in the biodegradation process (Viswanath et al. 2014). Few microorganisms need growth factors, such as specific amino acids, nucleotides, or vitamins/cofactors. Few nonessential cofactors could also enhance the rate of particular degradation. For example, flavin nucleotides (FAD) addition increase the rate of anaerobic reduction of azo dyes (Viswanath et al. 2014).

Presence of Oxygen

The microorganisms facilitate the degradation process through aerobic and anaerobic modes. The oxygen requirement diverges from one organism to another based on their degradation route. It is observed that hydrocarbon metabolism gets enhanced in the presence of oxygen. Generally, the aerobic mechanism is the most versatile metabolism. However, various investigations in anaerobic mechanisms have been carried out to mineralize a wide range of xenobiotic compounds, such as chlorinated phenols, PAHs, and PCBs. It is noted that the more highly chlorinated compounds are more vulnerable to anaerobic degradation through the reductive chlorination mechanism. Whereas the less chlorinated compounds are more susceptible to aerobic degradation (Singleton 1994). The microbial degradation of lignin and lingosulfonates in wood pulping is only facilitated in the presence of oxygen (Knapp 2003). In certain biodegradative reactions, degradation of polychlorinated organic compounds, such as dichloro-diphenyl-trichloroethane (DDT) or lindane, is effectively carried out anaerobically. In some cases, the degradation process gets hindered in the presence of O_2. For example, in the mineralization of azo dyes, electrons get expended in the reduction of oxygen rather than the cleavage of the target compound's diazo bond resulting in a decrease in the efficiency of the biodegradation process.

Inhibitory Materials

Some toxic compounds act as inhibitors, which may either inhibit the degradation process or kill microbes in the process. The xenobiotic compounds such as phenol, toluene, or cyanide hinder a wide range of microbes. Some natural compounds (e.g., phenolic components derived from decomposing plant tissues, or heavy metals from ores) are also encumbering the degradative processes. Phenol and 'toluene's high concentration is highly toxic to microorganisms, whereas, at low concentrations, they usually degrade. Even higher salinity inhibits microbial growth.

On a broader scope, to enhance the understanding of the essence of microbial degradation of toxic xenobiotic contaminants in the environment based on the type of xenobiotic compound, microbes used, aerobic and anaerobic degradation, and utilization of genetically engineered microbes are needed to be considered.

7.3 Microbial Degradation of Different Xenobiotics

7.3.1 Polycyclic Aromatic Hydrocarbons

The toxicity, low bioavailability, genotoxicity, poor biodegradability, and carcinogenic nature of polycyclic aromatic hydrocarbons (PAHs) exemplify them as organic pollutants. PAHs enter in the environment and accumulate in food chains while emerging as hazardous environmental pollutants posing a threat to public health. PAHs are aromatic compounds consisting two or more fused benzene rings in angular, linear, and cluster arrangements (Cerniglia 1992; Schützendübel et al. 1999). The US Environmental Protection Agency (US EPA) listed 16 PAHs as the most hazardous and human carcinogen pollutants. Chemically, PAHs are non-polar colorless to pale yellow or light greenish solids with a pleasant odor and comprise nitrotoluene and chlorinated organic compounds, such as pentachlorophenol, polychlorinated biphenyl, and chlorinated dioxin. PAHs are broadly spread as pollutants in the air, soil, freshwater reservoirs, sediments, surface water, and groundwater systems (Chang et al. 2014). Anthropogenically, PAHs are emitted into the environment during the incomplete combustion of coal, tar, petrochemical, gaseous fuel, automobile exhaust, etc. during industrial and other human activities or accidental discharge (Megharaj et al. 2011). Naturally, PAHs are formed during forest fires, volcanic eruptions, etc. (Abdel-Shafy and Mansour 2016). PAHs are formed as a waste product during the pyrolysis of organic substances at high temperatures. The type of product formed depends on the starting material's chemical nature and transformation temperature (Megharaj et al. 2011). Among PAHs, phenanthrene compound photosensitized human skin and caused mild allergies. Benzo (pyrene) was identified as highly carcinogenic for human beings. The high covalent binding of PAHs to DNA, RNA, and proteins can be correlated with carcinogenicity (Santarelli et al. 2008). Few transformation products of PAHs are more hazardous than parent PAHs. For example, at the cellular level, enzyme cytochrome P450 monooxygenase oxidizes PAHs into epoxides in the human body. These epoxide products are highly reactive and alter normal cells into a

cancerous one. PAHs also alter the functioning of hormone metabolizing enzymes of the thyroid glands and harm the reproductive and immune system (Rubin 2001). The bioremediation of PAHs using bacterial, fungal, and algal species depends on the surrounding conditions, such as nutrients, number and species of the microorganisms employed, chemical nature of the PAHs, etc. The different bacterial species were explored for the degradation of PAHs, including *Pseudomonas*, *Achromobacter*, *Flavobacterium*, *Alcaligenes*, *Nocardia*, *Bacillus*, *Arthrobacter*, and *Acinetobacter*, (Table 7.1). Similarly, various fungal strains employed for the degradation, including *Aureobasidium*, *Candida*, *Rhodotorula*, and *Sporobolomyces* (found in marine environments), *Trichoderma*, *Mortierella*, etc. (found in marine and soil environments).

There are two bacteria-mediated approaches (*aerobic and anaerobic*) depending on oxygen involvement in PAHs' complete mineralization using microorganisms. The aerobic catabolism of PAHs is carried out by hydroxylation and oxygenolytic mediated aromatic ring cleavage. In this type of degradation, the oxygen acts as a final electron acceptor and a co-substrate. The *aerobic bacterial degradation* of PAHs embracing either monooxygenase or dioxygenase enzymes mostly under aerobic conditions. The detailed mechanism of dioxygenase mediated biodegradation was elaborated by several studies (Müller et al. 1998). Dioxygenase enzyme, a multicomponent enzyme, generally comprises reductase, ferredoxin, and terminal oxygenase subunits (Mallick et al. 2007). The general mechanism of aerobic degradation of PAHs consists of two steps; first, *cis*-dihydrodiol is formed due to hydroxylation of an aromatic ring via dioxygenase. The product *cis*-dihydrodiol further aromatized by dehydrogenase enzyme and forms a diol intermediate. These so formed diols also undergo ortho-cleavage or meta-cleavage by dioxygenases, forming catechols that are further converted as TCA cycle intermediates (Rubin 2001). Mallick et al. proposed a biodegradation mechanism of phenanthrene carried by *Staphylococcus* sp. strain PN/Y isolate from petroleum-contaminated soil (Mallick et al. 2007). The metabolic pathway for phenanthrene initiates with deoxygenation of phenanthrene at 1,2-position followed by meta-cleavage of phenanthrene-1,2-diol break down at meta-position to 2-hydroxy-1-naphthoic acid. The formed acid product then undergoes a series of reactions to yield catechol, which is further metabolized by catechol-2,3-dioxygenase to 2-hydroxymuconaldehyde acid (TCA cycle intermediates). The bacterial degradation of PAHs also proceeds through the *cytochrome P450-mediated pathway*, which involves the production of *trans*-dihydrodiol (Rubin 2001; Harvey 1996). Moody et al. have proposed the mechanism of the degradation of more carcinogenic benzo-[*a*]pyrene by *Cytochrome* P450 using *Mycobacterium vanbaalenii* PYR-1 strain (Fig. 7.3) (Moody et al. 2004). In the degradation of benzo-[*a*]pyrene mediated by *Microsomal cytochrome* P450 enzymes; first 10-oxabenzo[def]chrysen-9-one get formed, which further gets converted into benzo[*a*]pyrene *cis*-9,10-dihydrodiol through deoxygenation at C-9 and C-10. The successive meta cleavage and aromatic-ring closure form the dihydroxy intermediate, i.e., 10-oxabenzo[def]chrysen-9-one. In another route, benzo[*a*]pyrene get oxidized to *cis*- and *trans*-11,12-dihydro-11,12-dihydroxybenzo [*a*]pyrene mediated by *M. vanbaalenii* PYR-1. The *Cytochrome* P450 and *Epoxide*

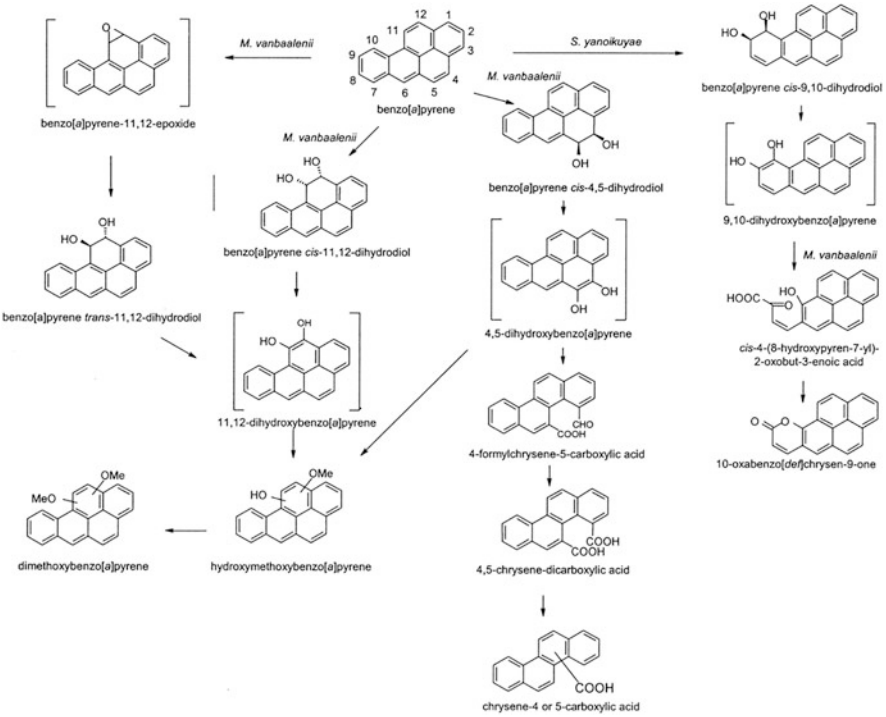

Fig. 7.3 Pathway for the degradation of benzo[*a*]pyrene by *M. vanbaalenii* PYR-1. Compounds in brackets are hypothetical intermediates (Copyright© 2004, American Society for Microbiology. All rights reserved, reprinted with permission) (Moody et al. 2004).

hydrolase act on benzo[*a*]pyrene *trans*-11,12-dihydrodiol and convert it into benzo [*a*]pyrene 11,12-epoxide. To explore the metabolic ability of 15 different bacterial isolates segregated from petroleum oil-contaminated sites, they were investigated at controlling factors concerning PAHs' degradation rates (Saeed et al. 2011). It was reported that the maximum of PAHs was optimally degraded at 15°C at an oxygen level of 4 ppm. Simarro et al. studied the impact of different parameters such as C/N/P ratio, sources of the nitrogen, carbon, and iron, the iron concentration, and pH on biodegradation on naphthalene, phenanthrene, and anthracene with a degrading bacterial consortium C2PL05 (Simarro et al. 2011). The high biodegradation efficiency was observed when the optimized factors were at the ratio (100:21:16) for C/N/P using 0.1 $mmol^{-1}$ concentration of $NaNO_3$ and $Fe_2(SO_4)_3$ at pH 7 on a mixture of glucose and PAHs (as carbon source).

The unavailability of oxygen in an environment poses a crucial challenge to microorganisms to exploit aromatic compounds as growth substrates (Fuchs et al. 2011). The lack of molecular oxygen in anaerobic conditions bestows redox potential as a critical factor for biodegradation of PAHs in soils, sediments, and aquifer systems (Karthikeyan and Bhandari 2001). Various studies highlighted the efficient

anaerobic degradation of fluorine, anthracene, phenanthrene, naphthalene, acenaphthene, etc. (Carmona et al. 2009; Cui et al. 2008). PAHs" anaerobic degradation usually involves the reductive destruction of the aromatic ring using nitrate, sulfate, or ferric ions as a final electron acceptor (Carmona et al. 2009). The bacterial degradation of PAHs *under anaerobic conditions* becomes possible when PAHs are bioavailable in a sufficient amount, and the presence of particular inorganic electron acceptors (nitrate, sulfur, etc.) in the native microflora encompasses degradative enzymes with genetic setup for encoding. The reduction of nitrate (NO_3^-) into different nitrogen oxides and molecular dinitrogen using bacteria is known as *microbial denitrification* in which nitrate assist as a terminal electron acceptor in the oxidation of PAHs (Karthikeyan and Bhandari 2001). Various studies highlighted the PAHs degradation under nitrate-reducing conditions in a different anoxic habitat, such as soils, lakes, rivers, oceans, petrochemicals contaminated site, oil spillage, sewage sludge, etc. (Ambrosoli et al. 2005; Liang et al. 2014; Chang et al. 2002; Dou et al. 2009; Wang et al. 2012). Both low- and high-molecular-weight PAHs are mineralized by nitrate-reducing facultative anaerobes. The microbial mineralization of naphthalene under denitrifying conditions in pristine and oil-contaminated soil slurry was successfully reported by Al-Bashir et al. (Al-Bashir et al. 1990). The complete mineralization of naphthalene and 2-MN in contaminated arctic soil at low temperatures under nitrate-reducing conditions using genera like *Acidovorax*, *Bordetella*, *Pseudomonas*, *Sphingomonas*, and *Variovorax* was investigated (Eriksson et al. 2003). Lu et al. highlighted that the 2- and 3-ring compounds among the 16 priority PAHs are mineralized by sediment enrichment culture under denitrifying conditions rather than sulfate-reducing prerequisites (Lu et al. 2012). The microbial degradation of highly toxic benzo(*a*)pyrene, a class 2A carcinogen identified by the International Agency for research in cancer (IARC), is well studied. The benzo(*a*)pyrene, fluoranthene, and phenanthrene were completely mineralized by using *Pseudomonas* sp. JP1 form river sediment (Yang et al. 2013). Qin et al. has studied *Microbacterium* sp. strain activity under the denitrifying products nitric oxide and nitrous oxide as electron acceptors and disintegrated around 84.2% of benzo(*a*)pyrene at the BaP/nitrate ratio of 1:33 in 10 days (Qin et al. 2017). The sulfate-reducing bacteria are ubiquitous in freshwater, groundwater and marine sediment, volcanic mud, and anaerobic sludge (Muyzer and Stams 2008). The common sulfate-reducing bacteria are *Deltaproteobacteria*, *Clostridia*, *Nitrospirae*, *Thermodesulfobacteria*, and *Thermodesulfobiceae*. During anaerobic degradation of PAHs, the reduction of sulfate occurs with the formation of hydrogen sulfide (H_2S) (Meckenstock et al. 2016). The naphthalene and 2-methylnaphthalene were successfully degraded by N47 (derived from the soil of a contaminated aquifer near Stuttgart, Germany) comprised of an unidentified member of *Deltaproteobacteria* in association with 7% of *Spirochaetes* members (Safinowski and Meckenstock 2004). Rothermich et al. successfully revealed the *in situ* mineralization of 14C-naphthalene and 14C-phenanthrene in petroleum-contaminated, anoxic, and sulfidogenic harbor sediments (Rothermich et al. 2002). The biotransformation of fluorene and phenanthrene could be carried through a series of hydration and hydrolysis reactions followed by decarboxylation with

subsequent p-cresol and phenol (Tsai et al. 2009). Himmelberg et al. have reported TRIP1, a new sulfate-reducing enrichment culture belong to the *Desulfobacteraceae* family for phenanthrene degradation (Himmelberg et al. 2018). The initial reaction involves the degradation *via* carboxylation to 2-philanthropic acid as the primary intermediate. The intermediate is then converted into corresponding CoA ester by the enzyme 2-phenanthroate-CoA ligase.

Apart from bacterial degradation, some fungi are also used to degrade PAHs by co-metabolizing PAHs into diverse oxidized products and CO_2. The bacterial degradation of PAHs mainly incorporates *Dioxygenase* enzymes and partially monooxygenase-mediated reactions, whereas fungal degradation only involves monooxygenase enzymes. The fungal mineralization of PAHs follows various enzymatic pathways, and the efficiency of this biotransformation mostly depends on fungal species and growth conditions (Cerniglia 1992). Both of the ligninolytic fungi/white-rot fungi and non-ligninolytic fungi are employed for the degradation of PAHs. The metabolic activity of ligninolytic fungi implicates lignin peroxidase (LiP), manganese peroxidase (MnP), and laccases (a phenoloxidase enzyme) are capable of mineralization of xenobiotic compounds. The aromatic ring present in PAHs structure is oxidized by hydroxyl free radical produced by ligninolytic enzymes, and PAH-quinones and acids are formed. The activity of non-ligninolytic fungi such as *Cunninghamella elegans* includes P450 monooxygenase like enzymes that facilitate initial oxidation of PAHs. The ring epoxidation reaction is catalyzed by P450 monooxygenase. This catalytic reaction yields arene oxide, which is unstable and immediately converted into *trans*-dihydrodiol through epoxide-hydrolase catalyzed reaction (Tortella et al. 2005). The arene oxide formed during the metabolic activity of cytochrome P450 can also be reorganized into phenolic derivatives via non-enzymatic reactions in conjugation with sulfate, xylose, glucuronic acid, or glucose (Pothuluri et al. 1996). The other ligninolytic fungi, (brown-rot fungi) such as *Flammulina velutipes* and *Laetiporus sulphureus*, metabolize PAHs like fluoranthene, phenanthrene, and fluorine and mostly produces hydrogen peroxide to degrade hemicelluloses and cellulose (Li et al. 2010). The high degradation of low-molecular-weight (LMW) PAHs (2–3 ring compounds) was perceived in *Aspergillus* sp., *Trichocladium canadense*, and *Fusarium oxysporum(*Silva et al. 2009*)*. The complete degradation of high-molecular-weight (HMW) PAHs (4–7 rings) was reported in, *Acremonium* sp, *Aspergillus* sp., *Verticillium* sp., *and T. canadense*.(Kumar et al. 2018) The total mineralization of PAHs by fungi insinuate that it can be utilized as a valuable candidate for the degradation of PAHs in contaminated sites.

The biodegrading potential of naturally occurring microorganisms can be boosted by enhancing the metabolic activity or broad substrate specificity of certain enzymes associated with PAH-degrading pathways. A modified microorganism (GMM) or *genetically engineered* microorganism (GEM) is produced by altering genetic material using genetic engineering techniques or recombinant DNA technology (gene conversion, gene duplication, plasmids mediated gene delivery, etc.) are employed to enhance mineralization of PAHs pollutants in the environment (Megharaj et al. 2011). The engineered *Pseudomonas putida* developed by molecular techniques

produced catechol 2, 3-dioxygenase (C23O; EC 1.3.11.2), which decomposed polycyclic aromatic hydrocarbons (PAHs) (Mardani et al. 2017; Xia et al. 2005). 2, 3-Dioxygenase is a key enzyme, mainly a nonheme iron dioxygenase for the disintegration of PAHs by altering catechol into 2-hydroxymuconic semialdehyde (Mesquita et al. 2013).

7.3.2 Azo Compounds

Around 60–70 % of dyestuffs utilized in textile and other industries, such as paper, food, cosmetics, etc., are azo dyes. Azo dyes have complex aromatic structural moiety comprising one or more (–N=N–) azo bonds that are not found naturally, making them xenobiotics (Lourenço et al. 2006). Almost 3000 different diverse azo dyes are synthesized and utilized for coloring purposes and accountable for the generation of substantial effluent waste (Singh and Singh 2017). The effluent majorly containing dye from various industrial practices directly expulsed into freshwater bodies has become a severe concern. The azo dye loaded effluent impart several ill effects on living systems, which include decreased aquatic photosynthesis, deplete dissolved oxygen, and pose toxic effect (carcinogenic and mutagenic) on flora, fauna, and humans. Azo dyes generally comprise azo linkages, linking phenyl, naphthyl rings substituted with functional groups like nitro, chloro, sulfonate, hydroxyl, triazine amine, methyl etc. (Asad et al. 2006). The azo dyes containing a single azo bond are termed as monoazo dyes (e.g., disperse blue 399, reactive yellow 201, acid orange 52). Diazo dyes comprise of two azo bonds (acid black 1, reactive brown 1, amido black, brown 2). Triazo dyes (direct blue 78, direct black 19) and polyazo dyes (direct red 80) are commonly used. The presence of a functional group, a number of azo bonds, type, and configuration, poses an impact on its degradative capacity (Rani et al. 2011). The degradation of xenobiotic and recalcitrant natured azo dyes is widely carried out to uncover diverse microorganisms" role in azo dyes" bioremediation. Various bacterial strains from various ecological niches such as soil, water, fecal matter, contaminated food materials, etc., are employed for complete mineralization of azo dye. The competence of microbial degradation of azo dyes relies on the adaptability and the activity of the selected microbes. A large number of bacterial and fungal species has been reported for the mineralization of various azo dyes in the last two decades and are listed in Table 7.1. Various studies reported the bioremediation of azo dyes using pure bacterial cultures, such as *Proteus mirabilis*, *Pseudomonas luteola*, *and Pseudomonas* sp., etc. (Pointing and Vrijmoed 2000; Wu and Der Jean 2012; Waghmode et al. 2011). Certain dyes are more hazardous than their degradation products. Recent studies highlighted that the complete mineralization of dye is not achieved as carcinogenic intermediates are produced during degradation (Rieger et al. 2002). The synergistic metabolic activities of consortia (mixed bacterial strains) culture enhanced the degree of mineralization. In a bacterial consortium, the different bacterial strains may react with the dye molecule at different positions or decompose intermediate metabolites (Tony et al. 2009). The consortia comprised of *P. rettgeri* strain HSL1 and *Pseudomonas* sp. SUK1 are

employed to degrade Reactive Black 5, Reactive orange 16, Direct Red 81, and Disperse Red 78 azo dye (Bumpus 1995). In the degradation of an azo dye, the primary step is reductive cleavage of azo bond that results in the formation of aromatic products under an anaerobic environment (Bumpus 1995). The nitro group of azo dyes are mutagenic and forms toxic products, such as 1, 4-phenylenediamine, *o*-toluidine, etc., during degradation, whereas sulfonated azo dyes have low or no mutagenic effect (Ferraz et al. 2011). Similarly, the extensively used Methyl Red is also mutagenic. It can be degraded to form *N*,*N*-dimethyl- phenylenediamine (DMPD), which is toxic and mutagenic in nature (Ayed et al. 2011). The genotoxic metabolites, 40-aminoacetanlide or 5-acetamido-2-amino-1-hydroxy-3,6-naphthalene disulfonic acid, get formed during the breakdown of acid violet 7 by *Pseudomonas putida* (Abdel-Shafy and Mansour 2016). Acid Violet 7 azo dye cause lipid peroxidation, chromosomal abnormalities, and inhibition of enzyme *Acetylcholinesterase*. The other azo dye, Disperse Blue 291, poses genotoxic, mutagenic, cytotoxic effect and is responsible for DNA fragmentation in human hepatoma cells (Tsuboy et al. 2007).

The microbial degradation of azo dyes is facilitated by enzymes such as azoreductase, laccases, lignin peroxidase, manganese peroxidase, and hydroxylases. Some aerobic bacterial strains (e.g., *E. coli* contain flavin reductase) can break the azo bond through the reduction process carried by *Azoreductases* in oxygen-rich environments (Fig. 7.4). Enzyme *Azoreductase* needs electron donors (redox mediator) such as FADH or NADH to carry out redox reaction to break azo bond resulting in toxic intermediate aromatic amine under an aerobic environment. In the next step, the membrane-bound azoreductase enzyme completely mineralizes the intermediate amino acids (Sarkar et al. 2017). The bacteria consume azo dye as a carbon or nitrogen source and also form a product from glucose (Lima et al. 2014). The laccases enzyme (phenol oxidase) comprises multicopper atoms with less substrate susceptibility and is an active enzyme capable of degrading numerous aromatic xenobiotic compounds (Kalyani et al. 2012). Various *bacterial strains*, such as *Pleurotus ostreatus*, *Schizophyllum commune*, *Sclerotium rolfsii*, *Neurospora crassa*, etc., can be employed for the degradation of azo dye. Phenol oxidase (laccase) uses copper ion as a mediator to oxidize the aromatic amine. Laccase follows a free radical-mediated mechanism for mineralization of azo dye and produces phenolic degradative products. *Laccase* oxidizes the phenolic rings using electron, and phenoxy radicals get formed, which immediately oxidized to produce carbonium ion. Simultaneously, the nucleophilic attack of water makes a 4-sulfophenyldiazene and benzoquinone in the process (Camarero et al. 2005). Further, phenyldiazene radical get produced from the oxidation of 4-sulfophenyldiazene with loss of molecular nitrogen producing sulfophenyl hydroperoxide (Singh et al. 2015a). A particular bacterial strain, *Extremophiles* (e.g., *Exiguobacterium*), is used to treat high saline dye effluent, as common bacteria species could not withstand the high temperature and high salinity (Ambrósio et al. 2012; Ng et al. 2017).

The studies revealed that bacteria degradation of azo dyes under aerobic conditions is restricted due to hindrance in the azo bond cleavage (Ola et al.

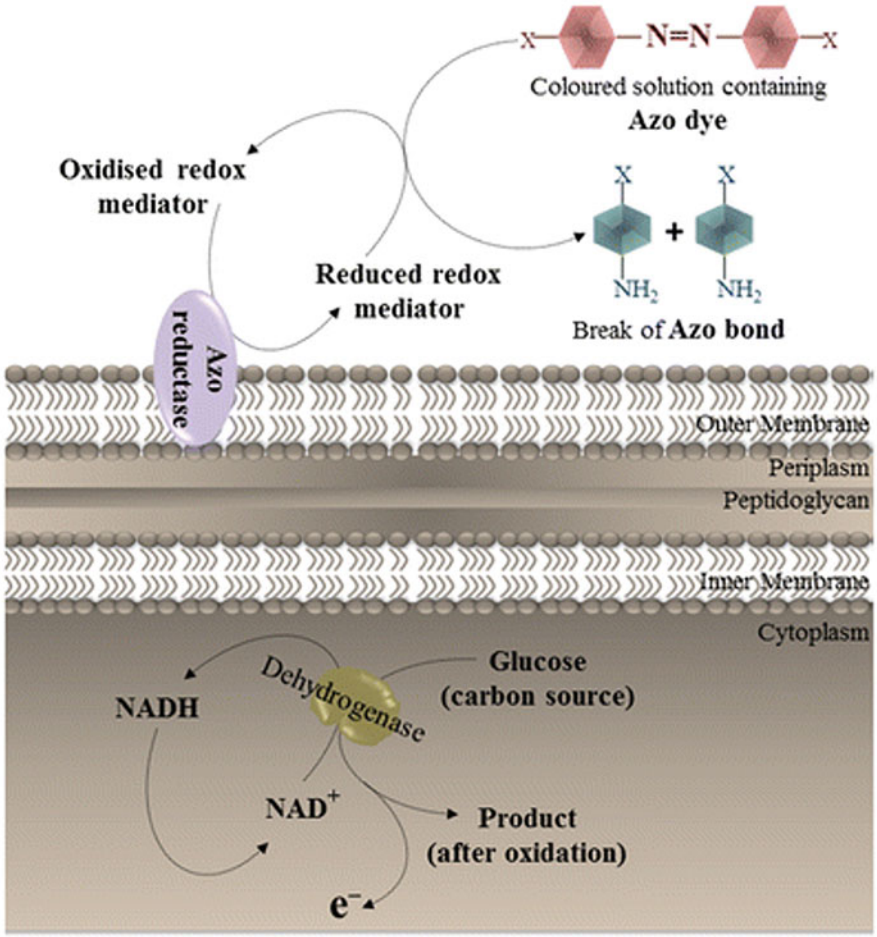

Fig. 7.4 Mechanism of *Azoreductase* action in the reduction of azo dyes (Copyright© 2017, Springer Nature. All rights reserved, reprinted with permission) (Sarkar et al. 2017).

2010). Besides, azo dyes are disintegrating into intermediate compounds but not completely mineralized in the presence of oxygen. By employing aerobic–anaerobic degradation, azo dyes can be completely mineralized (McMullan et al. 2001). In the coupled process, the first azo bond breaks up with the formation of aromatic amines under anaerobic conditions, and then ring cleavage in amino acids facilitated by nonspecific enzymes occurs under aerobic conditions (Lu and Liu 2010). In this way, coupled anaerobic treatment followed by aerobic treatment completely disintegrate azo dyes (Singh and Singh 2017; Bumpus 1995). It was observed that the high efficiency is obtained using mixed bacterial culture rather than the pure strain.

Fungal facilitated degradation is a potential alternative to bacterial degradation of azo dyes. The ligninolytic fungi of class basidiomycetes are extensively used in the

degradation of azo dyes due to their capability to alter metabolic reactions based on varying carbon and nitrogen sources. The white-rot fungi, *Phanerochaete chrysosporium*, is the utmost employed for bioremediation. Instead of it, other fungal species, such as *Trametes (Coriolus) Versicolor*, *Pycnoporus sanguineus*, *Aspergillus flavus*, *Pleurotus*, *Coriolus Versicolor*, *Aspergillus ochraceus*, *Bjerkandera adusta*, *Rhizopus oryzar*, etc. have been highlighted for degradation of azo dyes (Saratale et al. 2009; Fu and Viraraghavan 2001). Generally, white-rot fungi produce lignin peroxidase, manganese-peroxidase, and laccase enzymes competent in mineralizing complex toxic aromatic compounds, such as PAHs, dyes, and steroids compounds (Revankar and Lele 2007). The factors governing the rate of degradation of azo dyes mainly are nutrient availability, time, pH, stirring speed, temperature, oxygen supply, and the number of additives added (Singh and Singh 2017). Therefore, all these parameters should be optimized in order to maximize the rate and extent of degradation.

Thus, biological systems embracing microorganisms such as bacteria and fungi bestow an inexpensive and sustainable method for the degradation of various complex azo dyes. However, the effectiveness of biological treatment for removing azo dyes relies on the adaptability and metabolic activity of selected microorganisms.

7.3.3 Organophosphorus Compounds

Organophosphate pesticides (OPs) are the heterogeneous compounds that are extensively used commercial pesticides comprised of phosphoric acid derivatives. Around 140 OP compounds being utilized as growth regulators and pesticides. The commercially available pesticides paraoxon, diazinon, chlorpyrifos, parathion, coumaphos, isofenphos, parathion, dichlorvos, parathion-methyl, profenophos, etc. have been used broadly, and their various degradation tactics have been studied extensively (Ortiz-Hernández and Sánchez-Salinas 2010). Organophosphorus compounds (generally called organophosphate pesticides) are the degradable esters, amides, or thiols derivatives of phosphoric, phosphonic, phosphinic, or thiophosphoric acids. Presently, the widely used organophosphate pesticides are chlorpyrifos, parathion, fenitrothion, ethoprophos, profenofos, etc. (Table 7.1). Chemically, organophosphorus compounds contain two organic groups and cyanide, thiocyanate, or phenoxy groups as side chains (Balali-Mood and Abdollahi 2014). The rate of biodegradation of organophosphorus compounds is affected by various environmental parameters, such as pH, temperature, and sunlight availability. The high solubility makes these compounds more vulnerable to human and animals and instigates severe health hazards. Due to this fact, the removal of organophosphate compounds is extensively studied, and more efficient sustainable treatment technology is being investigated. Typically, the microbial biodegradation mechanism of organophosphates proceeds through the cleavage of P–O alkyl and aryl bonds by a hydrolysis reaction, which is the enzyme-mediated process. The enzymes involved in these hydrolysis reactions are *hydrolase*, *phosphotriesterase*, *phosphatase*, and

carboxylesterases (Singh and Walker 2006; Lu et al. 2013). Various bacterial and fungal strains have been isolated from contaminated sites and verified as an efficient biological tool for transforming organophosphates (Table 7.1).

Chlorpyrifos (*O*,*O*-diethyl *O*-3,5,6-trichloro-2-pyridyl phosphorothioate) pesticides are the most widely applied for the controlling breeding and populace mosquitoes, flies, and other household pests (Kim and Ahn 2009). The removal methods of chlorpyrifos have been largely studied (Wang et al. 2019). The half-life period (an essential property of xenobiotics) of chlorpyrifos in soil and water is 38 and 2118 days (Wang et al. 2019). It is degraded by isolated microbes derived from agricultural soil, industrial sludge, activated sludge, effluents, etc. Chlorpyrifos is degraded co-metabolically and catabolically by bacteria isolated from different kinds of matrices (Chishti et al. 2013). The bacterial strains, such as *P. putida*, *Pseudomonas stutzeri*, *Pseudomonas aeruginosa*, *Pseudomonas nitroreducens*, and *Pseudomonas fluorescence*, *Bacillus aryabhattai*, *Stenotrophomonas* sp, *Leuconostoc mesenteroides*, *Lactobacillus brevis*, *Lactobacillus plantarum*, and *Lactobacillus sakei*, etc., are used for mineralization of chlorpyrifos (Nabil et al. 2011; Verma et al. 2014). Pailan et al. reported the highest degradation rate of chlorpyrifos and parathion at an optimal concentration of 200 mg mL^{-1} by isolate obtained by West Bengal agricultural soil, India, and was found to be an efficient candidate (Pailan et al. 2015). *O*,*O*-diethyl *O*-[6-methyl-2-(1-methylethyl-4-pyrimidinyl)] ester insecticide commonly called Diazinon used in home gardens and on farms to control a wide variety of leaf-eating insects, cockroaches, ants, and fleas in residential localities (Seo et al. 2007). The high solubility in water and 40 days long half-life period illustrated it as toxic to the biosphere. Abo-Amer demonstrated high metabolic activity of the *Enterobacteriaceae S. marcescens* derived from the Saudi Arabian agricultural field against such compounds (Abo-Amer 2011). Similarly, Seo et al. successfully used *Arthrobacter*, and *Mycobacterium* strains that were isolated from the petroleum-contaminated soil sample of Hawaii (Seo et al. 2007). The organophosphate pesticides are used at tremendous scale as insecticides need more enhanced degradation strategies to reduces the chances of their accumulation and related health hazards. Engineered microorganisms should have been designed to increase the potential ability of microbial degradation processes for organophosphorous compounds.

7.3.4 Halogenated Hydrocarbons

Halogenated hydrocarbon or halocarbons are the hydrocarbon compounds where at least one hydrogen is substituted by the Group 17 (Group VIIa—the *halogen* elements are astatine (At), bromine (Br), chlorine (Cl), fluorine (F), iodine (I), and tennessine (Ts)). Some of the halocarbons produced naturally during the halogenation reaction, such as the combustion of biomass during the forest fire. However, most of the halocarbon present is synthetic and intentionally produced by humankind as principle products or by-products. Halocarbons in which hydrogen forms the carbon-hydrogen bond replaced with one of the halogen species shows more

structural and chemical stability. The halogenation process of aliphatic hydrocarbons and aromatic hydrocarbons is extensively used to produce different economically favorable chemicals, used in millions of tons globally per year. Due to structural and chemical stability, these halocarbons have found their extensive use in day-to-day products, mainly in lubricants, fire extinguishers, solvents, insulators, paints, varnishes, plasticizers, pesticides, etc. Halogenated hydrocarbons are part of the synthesis of polyurethanes and polycarbonates as an intermediate. The chemicals, such as herbicides and pharmaceuticals, contain a variety of halogenated hydrocarbons. Also, the chemical in metal-cleaning, fire-extinguishing compounds, rubber, plastic, paint/varnish, healthcare, textile, fungicides, and insecticides comprise of saturated halogenated hydrocarbon (Bhat and Vaidyanathan 1995). One of the major adverse impacts of these halogenated hydrocarbons is their unregulated release into the environment. Most of these compounds are considered carcinogenic and can harm humans and other animals.

7.3.4.1 Biodegradation of Halo-Aliphatic Compounds

Chlorinated aliphatic hydrocarbons are the large group of contaminants that found their way to the environment. It causes several adverse effects on the environment as well as on human health. Due to the toxicity and carcinogenicity of chlorinated hydrocarbons, there is an urgent need for these compounds" extensive remediation process. Dehalogenation is one of the notable routes for the degradation of these compounds. Dehalogenation by the use of microorganisms gives an excellent alternative. There have been extensive studies performed to establish the various methods to identify and isolate a variety of microorganisms capable of degrading chlorinated hydrocarbons (Leisinger 1996). Trichloroethylene (TCE) is mainly used as a solvent in the paper industry, textile industry, paint removers, typewriter correction fluids, and automobile industry. Many years of TCE usage has led to the accumulation of this hazardous chemical in the environment, especially to the groundwater bodies. TCE is a common groundwater contaminant and considered a potential carcinogen and mutagen (Leisinger 1996). Early research for biodegradation of TCE was based on the postulate that if mono-oxygenase enzymes of methanotrophs oxidize and dechlorinate halogenated methane, it might be possible that methanotrophs may also degrade TCE. Wilson et al. have shown a significant decrease in the TCE concentration 2 days after using exposed soil containing methanotrophs. In the study, the unsaturated soil sample was exposed to natural gas to favor methanotrophs' growth and then packed in the glass column, to which water containing TCE was passed through the column (Moriyama et al. 1988). Similarly, Fogel et al. reported TCE degradation into CO_2 and water-soluble products by mixed culture of methane utilizing bacteria. These bacteria were isolated from the sediment soil sample. These microbes were exposed to methane to enhance the growth of methane-degrading bacteria (Fogel et al. 1986). Pursuing on the same path, Little and coworkers isolated a pure strain of microorganism Strain 46-1 (Type-1 methanotrophic bacterium) was capable of degrading TCE in the presence of methane or methanol. The isolated strain was able to degrade TCE into CO_2 and water-soluble products under anaerobic conditions. Strain 46-1 was found to convert

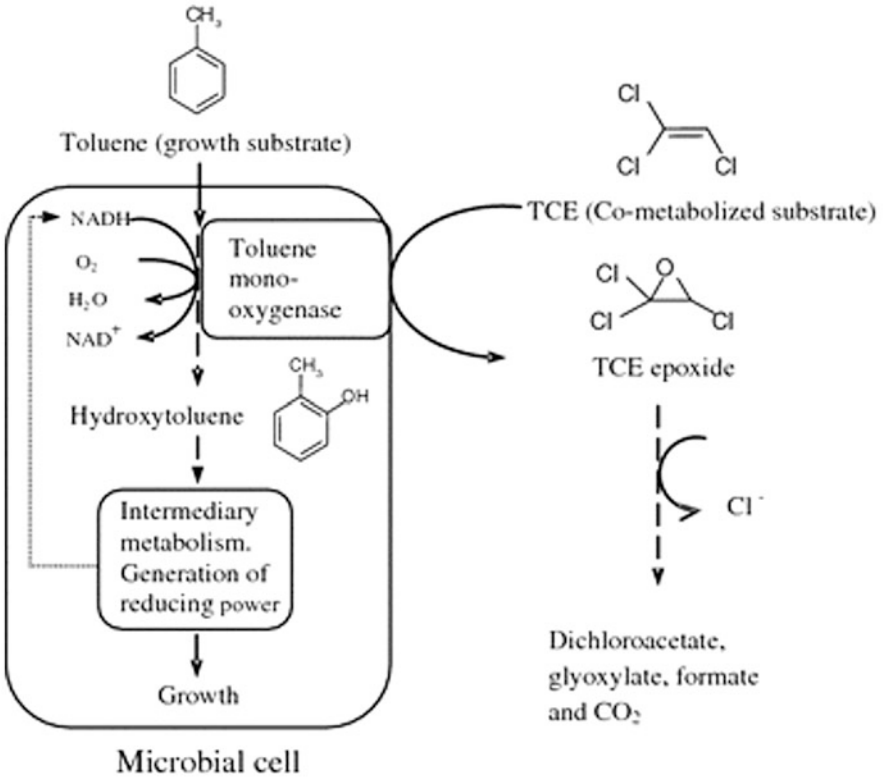

Fig. 7.5 Proposed mechanism for degradation of TCE by toluene-oxidizing bacteria (Copyright© 2012, Springer Nature. All rights reserved, reprinted with permission) (Suttinun et al. 2013)

about 40% of supplied TCE when incubated for 20 days. Among the converted 40%, 15.1% were the water-soluble products, and 11.4% were CO_2 (Little et al. 1988). Apart from methanotrophs, several other studies reported the bacterial degrading TCE using toluene and phenol as a growth substrate (Suttinun et al. 2013; Li et al. 2014). The bacteria produce toluene monooxygenase, which helps in oxidizing TCE in the co-metabolism system (Fig. 7.5). Some of the commonly used TCE-degrading bacteria are *Pseudomonas*, *Burkholderia*, *Methylosinus*, *Nitrosomonas*, *Alcaligenes*, *Acinetobacter*, *Mycobacterium vaccae*, *Nocardioides* sp. CF8 (Arp et al. 2001; Amin et al. 2014; Alpaslan Kocamemi and Çeçen 2006; Halsey et al. 2005). Many monooxygenases and dioxygenases producing microorganisms have been reported, which can facilitate TCE degradation. Some of the examples are *Nitrosomonas europaea* (ammonia monooxygenase), *Pseudomonas* sp. JS150, *Burkholdria cepacia* G4, *Pseudomonas putida* F1,*Pseudomonas fluorescens* CFS215, *Pseudomonas* sp. JS150, *Pseudomonas* sp. W31 (toluene 2-monooxygenase) *Pseudomonas fluorescens* CFS215, *Rhodococcus* sp. L4 (isopropyl benzene/toluene dioxygenase),

Pseudomonas butanovora Rhodococcus erythropolis BD2, *Pseudomonas* sp.W31 (toluene dioxygenase), *Mycobacterium vaccae*, *Nocardioides* sp. CF8 (butane monooxygenase), etc. (Alpaslan Kocamemi and Çeçen 2006; Halsey et al. 2005; Suttinun et al. 2009; Leahy et al. 1996; Yang et al. 1999; Lange and Wackett 1997; Morono et al. 2004). Sullivan et al. established *Methylosinus trichosporium* OB3b for the TCE degradation (Sullivan et al. 1998).

7.3.4.2 Biodegradation of Haloaromatic Compounds

Structurally, halogenated aromatic compounds contain one or more atoms of halogens (chloride, fluoride, bromide, and iodide) and a benzene ring. In industry, many organic compounds are used with the modified chlorinated aromatic moiety. Such compounds may be modified with one or more functional groups. Such compounds are vastly used as herbicides, pesticides, pharmaceuticals, and disinfectants (Marier 1982). Chlorinated aromatic hydrocarbons are the PCBs and used as hydraulic fluids, insulating fluids for electricity transformers and capacitors, coolants, cutting oils, adhesives, stabilizing additives in plastics, etc. Most PCBs are toxic compounds and are classified as persistent organic pollutants (Tilson and Kodavanti 1997). Some of the derivatives of chlorobenzene (especially monochlorobenzene and dichlorobenzenes) have been used as chemical intermediates and solvents in the manufacturing industry. Hexachlorobenzene is the raw material for a plasticizer for polyvinyl chloride, synthetic rubber, porosity controlling agent, and military's pyrotechnic compositions in electrodes' manufacture. Benzyl chloride is used to manufacturing quaternary ammonium chlorides, dyes, tanning materials, pharmaceutical, and perfume. Chloronaphthalenes have been used as lubricant additives, heat transfer media, dielectric fluids, solvents, and electric insulating material (Kurt et al. 2012). There are severe health hazards as these halogenated aromatic compounds are accumulated in the environment and come in contact with the human population. Toxicity of the halogenated aromatic hydrocarbons has been related to gastrointestinal and neurological symptoms (nausea, headaches, and central nervous system depression) as well as acute irritation of the eyes, mucous membrane, and lungs, reproductive disorders, liver dysfunction (hepatitis, jaundice, and porphyria), and acne (chloracne) (Moldoveanu 2019). Given the hazardous nature and severe health effect, there is a need to find and assess the methods to remove or degrade these halogenated aromatic hydrocarbons.

Chlorophenol contains at least one chlorine atom and at least one hydroxyl group at the benzene ring and is a highly toxic and has cytotoxicity, carcinogenicity, and mutagenic properties (Arora and Bae 2014a). Depending on the structural varieties, it can be generally grouped in five major classes as mono-chlorophenols (MCPs), poly-chlorophenols (poly-CPs), chloro-nitrophenols (CNPs), chloro-aminophenols (CAPs), and chloro-methyl phenol (CMPs). Chlorophenols are the vital constituent of pesticides, herbicides, solvents, pulp-paper industry, and dyes. Extensive use of these compounds and inadequate waste distribution leads to the accumulation these compounds on a hazardous scale. To address the issue, many researchers have been working on microbial degradation, as microorganisms can completely degrade it. Chlorophenols follows the general degradation mechanism of halogenated

aromatic hydrocarbon. First, monooxygenase catalyzes hydroxylation on either ortho-position or para-position of the benzene ring structure. If hydroxylation occurs at para position, then chlorocatechols is formed and if hydroxylation at ortho-position, chlorohydroquinones. The intermediate formed is further catalyzed by either ortho-cleavage, meta-cleavage, hydroxylation, or dehalogenation, followed by the benzene ring structure disruption. Under anaerobic condition, first chlorine is removed from the benzene ring structure giving partial or fully dechlorinated intermediate. So, in the case of the anaerobic condition, dichlorination is done before the benzene ring disruption. In aerobic conditions, dichlorination can be done before or after ring disruption (Arora and Bae 2014a).

7.3.5 Nitroaromatic Compounds

Nitroaromatic compounds are the organic compounds that contain at least one or more nitro group ($-NO_2$) and is the most useful organic compounds in the chemical industry. They have employed a significant part of the synthesis of explosives. Some of the examples of nitroaromatic compounds are nitrobenzene, nitrotoluenes, nitrophenols, etc. Naturally, they are produced as a product of the metabolism of plants, bacteria, and fungi. Also, they are accumulated in the environment by the incomplete combustion of fossil fuels. During the combustion, hydrocarbons released serve as the substrate for the nitroaromatic compounds by nitration. The naturally occurring bacterial genus *Streptomyces*, *antibiotic-producing bacteria*, *produces a wide variety of antibiotics*, *containing* a nitroaromatic component. Chloramphenicol, aureothin, neoaureothin, orinocin, azomycin, dioxapyrrolomycin, thaxtomins, rufomycins are some of the well-known antibiotics produced from the *Streptomyces* sp. (Peres and Agathos 2000; Singh et al. 2015b). *Structurally all these compounds contain one or more nitro groups*, *but a large number of nitroaromatic compounds are produced and used for industrial purposes such as* pesticides, dyes, explosives, polyurethane foams, elastomers, and industrial solvents. The recent exponential growth of industry and substantial use of explosives has given rise to the production of synthetic nitroaromatic compounds. Due to the unregulated use of pesticides and ineffective effluent treatment of chemical industry waste, these harmful nitroaromatic compounds find their ways to nature, such as water bodies, fertile lands, and dumping zones. Studies have proved that some of these compounds have ecotoxicity, immunotoxicity, carcinogenicity, and mutagenicity to humans and microorganisms (Peres and Agathos 2000; Singh et al. 2015b). There is a huge requirement for the degradation study of these compounds to humans' well-being and the safekeeping of nature. In the following section, some of the mechanisms for the degradation of nitroaromatic compounds are briefly explained.

Generally, aerobic degradation of nitroaromatic compounds in bacteria is a three-step procedure (Fig. 7.6). The first step involves changes in the nitroaromatic substrate's substituent group due to monooxygenases or dioxygenases enzyme. Monooxygenase adds one oxygen to the benzene ring, causing the release of the nitro group. While in the case of dioxygenase, the addition of two hydroxyl groups

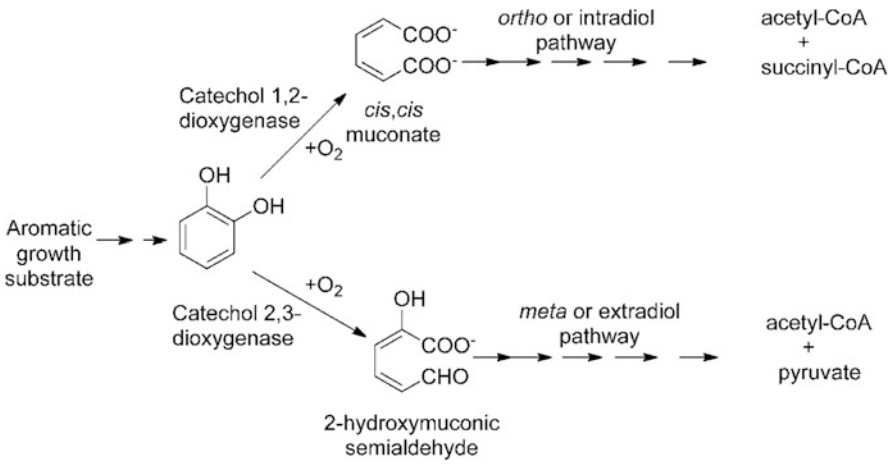

Fig. 7.6 The general pathway for the degradation of the nitroaromatic compounds by aerobic bacteria (Creative Commons Attribution License© 2015, Intech Open.) (Singh et al. 2015b)

causes the release of two nitro groups in the form of nitrate from the benzene ring. Hydroxyl group is induced by the enzymes to give dihydroxy aromatic metabolites. Usually, dihydroxybenzenes or catechols (1,2-dihydroxybenzenes) are metabolites produced in the first step and is the substrate for the second step of catabolism. The carbon–carbon bond in the ring structure is disrupted by the dioxygenases enzymes producing an unsaturated aliphatic acid. As there are two types of dioxygenases enzymes, two courses of action can happen during the ring opening. One is the intradiol (or *ortho)* dioxygenases (Fe^{3+} requiring enzyme) produces *cis*, *cis*muconic acid, or a derivative, and second is the extradiol (or *meta)* dioxygenases (Fe^{2+} requiring enzyme) produce 2-hydroxymuconic semialdehyde or a derivative. Finally, the ring cleavage derivatives produced in the second step are converted to small aliphatic compounds directed toward the central metabolism. Ring cleavage and the following metabolic steps for intradiol cleavage are referred to as the/3-ketoadipate (or *ortho)*pathway and for extradiol cleavage as the *meta* pathway (Singh et al. 2015b; Kulkarni and Chaudhari 2007).

Nitrobenzene is one of the essential classes of chemicals in the nitroaromatic compounds family. The substitution of the nitro group and chlorine group gives more structural stability to the compounds. Nitrobenzene and its derivatives are primarily used in the synthesis of aniline, the principal precursor for synthesizing azo dyes, pesticides, urethane polymers, rubber, explosives, and pharmaceutical products. Although compounds have significant advantages for the industrial processes, their extensive and accumulation in the environment, they present a substantial threat to the living organisms. Nitrobenzene and its derivatives are considered to be toxic and easily absorbed through the skin. It can damage the eyes, central nervous system, kidney/liver dysfunction, fatigue, weakness, headache, and acute methemoglobinemia (Kumar et al. 2017; Arora and Bae 2014b). Various

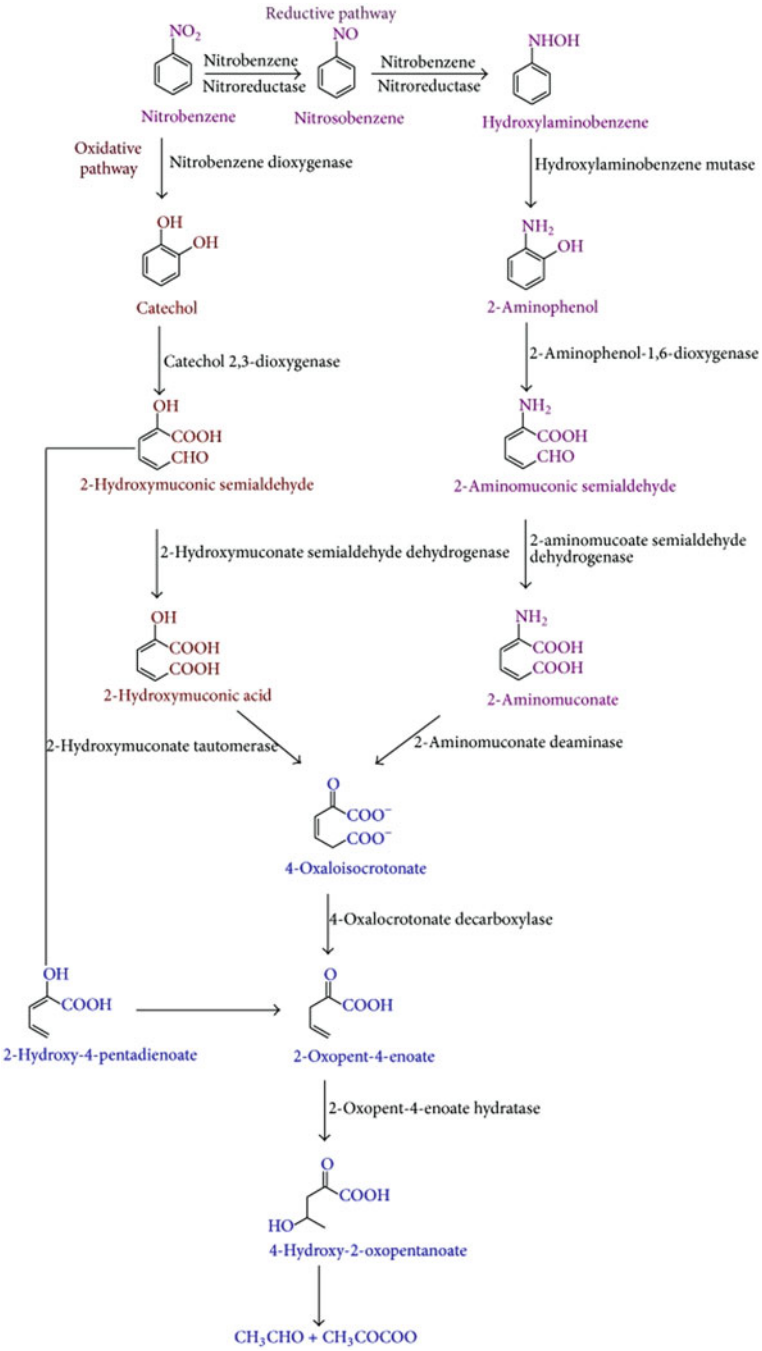

Fig. 7.7 The reductive pathway or oxygenated pathway for Nitrobenzene (Creative Commons Attribution License© 2014, Hindwi) (Arora and Bae 2014b).

microorganisms are found in nature, which has the capability of using nitrobenzene and its derivatives as the sole carbon source employing the co-metabolism system. Nitrobenzene is degraded by either a reductive pathway or an oxygenated pathway in the microbial system (Fig. 7.7) (Arora and Bae 2014b). In the oxygenase pathway, first nitrobenzene dioxygenase adds one hydroxyl group (-OH) to the benzene ring producing catechol. Further, catechol 2,3-dioxygenase (Fe^{2+} requiring enzyme) disrupts the benzene ring structure to give 2-hydroxymuconic semialdehyde and ultimately reduced to acetaldehyde and pyruvic acid by a series of the enzyme. By following the reductive pathway, initially, nitrobenzene is converted to hydroxylaminobenzene by nitroreductase. Then hydroxyl aminobenzene mutase adds one hydroxyl group (-OH) to a benzene ring, and di-oxygenase opens up the ring structure to give 2-aminomuconic semialdehyde. Gradually 2-aminomuconic semialdehyde is degraded to acetaldehyde and pyruvic acid by series of enzymes (Arora and Bae 2014b). *Pseudomonas pseudoalcaligenes*, *Pseudomonas pseudoalcaligenes* JS45, *Pseudomonas putida* HS12 produce the enzyme required for the benzene degradation. *Pseudomonas pseudoalcaligenes* were found to be using nitrobenzene as the sole carbon source. Studies show that *Pseudomonas mendocina* KR-1 converted nitrobenzene to a mixture of 3- and 4-nitrophenol, *Pseudomonas pickettii* PKO1 degrades nitrobenzene to 3- and 4-nitrocatechol via 3- and 4-nitrophenol. *Pseudomonas stutzeri ZWLR2-1 uses the* 2-bromonitrobenzene as sole carbon source and energy source (Singh et al. 2015b; Somerville et al. 1995). In another investigation, *Comamonas testosteroni* and *Acidovorax delafieldii* strains were isolated from the municipal activated sludge. These microorganisms were found to degrade nitrobenzene by catechol pathway. *Comamonas* sp. strain CNB-1 utilizes 4-chloronitrobenzene (4-CNB) and nitrobenzene as sole carbon sources (Zhao and Ward 1999). ZhiquanLiu et al. reported species of cyanobacteria *Microcystis aeruginosa can absorb nitrobenzene and reduce to* aniline with the help of nitrobenzene reductase (Liu et al. 2014). Chunli et al. isolated strain from the nitrobenzene-contaminated sludge. The isolated strain was completely capable of remineralizing nitrobenzene and using it as sole energy and carbon source. The strain was identified as *Rhodotorula mucilaginosa Z1 (*Zheng et al. 2009*)*.

During the anaerobic degradation, nitroaromatic compounds were reduced to aromatic amines through electron transport. The reduction is facilitated by the nitroreductase enzyme, which transforms the nitro group into nitroso derivatives, hydroxylamines, and amines. As microorganisms completely degrading, nitroaromatic compounds are rare; thus, anaerobic microorganism works in symbiotic with aerobic microorganisms (Singh et al. 2015b). 2,4,6-Trinitrotoluene, commonly called as TNT, is one of the primarily used military explosives around the world. Due to wars and war-like activities worldwide, large areas of fertile lands, forest lands, and water bodies get contaminated. After the explosion, only ash, crystalline debris, flakes, and yellowish powder remains, and studies have proved that these contents can be toxic to rodents, fish, plants, and algae. Not only the explosion but also the synthesis of TNT itself is a hazardous and poisonous method. The precursors required for the TNT synthesis are toxic, mutagenic, and

carcinogenic (Singh et al. 2015b). Degradation of TNT through microorganisms is an extensively well-researched area (Rieger et al. 2002; Serrano-González et al. 2018). Various species of *Clostridium*, *Desulfovibrio*, and archaebacteria as *Methanococcus* sp. can completely degrade TNT under anaerobic conditions. Various *Clostridium* sp. are capable of completely degrading TNT under anaerobic conditions. TNT is reduced to 2,4,6-triaminotoluene. *Clostridium acetobutylicum*, *Clostridium bifermentans* CYS-1, *Clostridium bifermentans* LJP-1, Clostridium pasterianum, *Clostridium thermoaceticum* are some of the *Clostridium* sp., which degrades TNT. *Desulfovibrio* sp. was found to use 2,4,6-trinitrotoluene (TNT) as a sole nitrogen source(Rieger et al. 2002). *Methanococcus spand*, *Veillonella alkalescens* are some of the microorganisms that degrade TNT anaerobically (Rieger et al. 2002).

7.3.6 *S*-Triazine

S-Triazine is an organic compound with a six-membered heterocyclic structure with alternating carbon and nitrogen atoms joined by a double bond and represented by the general formula $(HCN)_3$ (Rehan 2016). *S*-Triazine is a herbicide and is usually used to control grassy weeds and broadleaf in crops like macadamia nuts, sugarcane, pineapple, sorghum, and corn (Singh et al. 2016). Also, *S*-triazine herbicides are used on the golf course and residential lawns (Rehan 2016). Atrazine (2-chloro-4-ethyl amino-6-isopropyl amino-1,3,5-triazine) is the most widely used s-Triazine–based herbicides. Simazine (6-chloro-*N*,*N*′-diethyl-1, 3, 5-triazine-2, and 4-diamine) has also been used as herbicides to control annual grasses and weeds in the crop field (Jiang et al. 2020). These herbicides are highly persistent in the soil and accumulate due to their prolonged degradation rate (Jiang et al. 2020; Almeida Lage et al. 2019; Fuscaldo et al. 1999). Atrazine causes damage to the immunomodulatory and reproductive systems of reptiles, crustaceans, mammals, and amphibians, while for humans, it is a carcinogen and endocrine disrupter (Jiang et al. 2020; Hayes et al. 2002).

The degradation of s-triazine has been studied using several bacterial species such as *Agrobacterium*, *Nocardiodes*, *Pseudomonas*, *Pseudominobacter*, *Stenptrophomonas*, *Rhodococcus*, *Chelatobacter*, and *Arthrobacter*, etc. The mechanisms of degradation of *s*-triazine are well discussed and reported in several pieces of literature (Phale et al. 2019). The bacterial pathway includes atrazine degradation into carbon dioxide and ammonia via different routes, as noted earlier (Phale et al. 2019; Singh and Jauhari 2017). The first pathway involves the atrazine chlorohydrolase catalyzed dechlorination of atrazine into hydroxyatrazine, followed by cyanuric acid metabolism to yield biuret. Further, the generated biuret is metabolized by biuret hydrolase to get allophante followed by allophanate hydrolase catalyzed conversion to produce CO_2 and NH_3. Other pathways include the atrazine monooxygenase, or *N*-dealkylase catalyzed synthesis of deisopropylatrazine or deethylatrazine via oxidative removal of isopropyl or ethyl group, respectively. As discussed above, the formed products are further converted into ammonia and

carbon dioxide via cyanuric acid metabolism. Atrazine's biodegradation involves the different individual species or microbial consortia to prevent atrazine's effective degradation. Xu et al. have studied and proposed a 16 different microorganism combination to get effective degradation of atrazine (Xu et al. 2019).

Several fungal strains, such as *Phanerochaete chrysosporium*, *Aspergillus flavipes*, *Hymenoscyphus ericae* 1318, *A. fumigatus*, *Trichoderma viride*, *Pleurotus pulmonarius*, etc., have been used for the degradation of atrazine (Singh and Jauhari 2017; Marco-Urrea and Reddy 2012). Fungal degradation of atrazine involves the formation of deisopropylatraine and deethylatrazine via N-dealkylation. The finished product then can be further converted into the CO_2 and NH_3 using microbe consortia, as discussed earlier. Recently, Lopes et al. have shown the efficient degradation of atrazine (82%) using the fungal strain *Pleurotus ostreatus* INCQS 40310 over 22 days of fungal incubation (Lopes et al. 2020).

7.3.7 Organic Sulfonic Acids

Organic sulfonic acids or organic sulfonates-based xenobiotics compounds have found applications in the various industries, such as a surfactant, hydrotropic, or optical brightener in detergents, dyes industries, personal care, food, wetting agents, dispersants, and pharmaceuticals, etc.(Nicolella et al. 2005; Cook et al. 1998; Merrettig-Bruns and Jelen 2009). Some of the common sulfonates are *p*-toluenesulfonic acid, *m*-nitrobenzenesulfonate, naphthalenesulfonates, alachlor-ethanesulfonate, saccharine, alkylbenzenesulfonate, amino benzene sulfonates, amino naphthyl sulfonates, etc. (Cook et al. 1998). These compounds are derived either by direct sulfonation of starting reactants or as a by-product of chemical and process industries. The microbial degradation of sulfonates depends on their molecular structures as the benzene, and mono substitute naphthalene sulfonates are readily biodegradable, whereas the complex molecules like nitro, amino, and hydroxy group substituted sulfonates are difficult to degrade (Nicolella et al. 2005). Further, these compounds are resistant to biodegradation by bacteria using normal hydrocarbons due to highly specific transport enzymes' requirement to facilitate their entry in the cells (Singh et al. 2012). The degradation of sulfonates mainly occurs via desulfonation reaction using several anaerobic and aerobic microorganisms that can utilize these substrates as a source of carbon and energy (Cook et al. 1998).

The dissimilating anaerobic degradation of sulfonated compounds are well studied using several bacterial strains like *Desulfovibrio* sp. RZACYSA, *Bilophila wadsworthia* RZATAU DSM 11045, *Syntrophomonad* GRZTAU DSM 11270, *Desulfovibrio desulfuricans* IC1, *Desulfovibrio* sp. RZACYSA, *Desulfomicrobium baculatus* DSM 1741, *Desulfovibrio* sp. GRZCYSA DSM 11493, *Desulfobacterium autotrophicans* DSM 3382, *Alcaligenes* sp. NKNTAU DSM 11046,.*Paracoccus denitri¢cans* NKNCYSA, *Desulfovibrio desulfuricans* ATCC 29577, *Alcaligenes* sp. NKNTAU, etc. Similarly, the bacterial strains for assimilating sulfonates" degradation are also studied using *Klebsiella* sp., *Clostridium pasteurianum*,

Clostridium beijerinckii EV4, *Clostridium* sp. strain *like* KNNDS, RZES, RZHS, etc. (Cook et al. 1998; Merrettig-Bruns and Jelen 2009; Linder 2018). *Clostridium pasteurianum* DSM 12136 has been used for the assimilating type of degradation for several aromatic sulfonates such as 4-xylene-2-sulfonic acid,1,3-benzene-disulfonic acid, 4- aminobenzenesulfonic acid, 4-toluenesulfonic acid, and 4-xylene-2-sulfonic acid (Chien 2005). The substituted sulfonates are challenging to degrade, and very few reports are published showing a partial degradation of these compounds. González-Gutiérrez et al. have reported partial degradation of amino-naphthyl sulfonates via a series of reactions carried out by enzymes using anaerobic sludge along with dextrose and yeast extract as a source of carbon and nitrogen, respectively (González-Gutiérrez et al. 2009).

Aerobic degradation using bacterial consortia and pure cultures is reported for the degradation of sulfonated compounds, where these organisms utilize the sulfonates as sources of carbon and energy. Several pure and consortial bacterial strains such as *Alcaligenes* sp, *Hydrogenophaga palleroni*, *Agrobacterium radiobacter*, ,*Pseudomonas pseudoalcaligenes*, *Pseudomonas citronellolis*, *Pseudomonas testosterone*, *Sphingomonas* sp. ICX, *Comamonas testosteroni* A3,*Sphingomonas xenophaga* BN6,*Pesudomonas* sp. BN9, *Pseudaminobacter salicylatoxidans* BN12, *Methylosulfonomonas methylovora*, are used for aerobic degradation of sulfonated compounds (Cook et al. 1998; Linder 2018; Ruff et al. 1999). Song et al. have reported the efficient use of *Arthrobacter* sp. 2AC and *Comamonas* sp. 4BC strains for naphthalene-2-sulfonic acid biodegradation (Song et al. 2005).

Several fungal strains, such as *Ganoderma* sp. En3, *Saccharomyces cerevisiae*, *Lipomyces starkeyi*, *R. toruloides*, *Trametes versicolor*, *Coriolopsis gallica*, *Pleurotus ostreatus*, *Cunninghamella polymorpha and Penicillium chrysogenum* have also been reported for the assimilation type desulfonation of sulfonates (Wesenberg 2003). Song and Burns have studied fungus, i.e., *Cunninghamella polymorpha*, *to suppress naphthalene-2-sulfonic acid condensation products efficiently (*Song and Burns 2005*)*. Coasta et al. have shown high efficiency of *Penicillium chrysogenum for biodegradation of linear alkyl sulfonates*, i.e., sodium dodecylbenzene sulfonate (Costa et al. 2020).

7.4 Conclusion

Various national and international research efforts have been encouraged in recent years, given the extent of pollution led by xenobiotics in multiple environments. Understanding of the mechanisms of biodegradation of xenobiotic compounds is still in its infancy. Although certain mechanisms have been observed in laboratory conditions, their study in a natural environment remains largely unexplored. The molecular mechanisms of biodegradation and biomineralization of xenobiotics are yet to be fully understood. The diversity of microorganisms associated with these different stages of biodegradation is also in a primitive stage. Understanding these processes is especially important in defining the biodegradation rates of xenobiotics and better predicting the xenobiotic-contaminated environment's future. The

primary site of plastic xenobiotics is the landfills, which ultimately pushes it into the water bodies. Although spreading awareness can limit xenobiotics to some extent, it cannot be wholly relied upon, and a significant step towards excluding it from the environment needs to be taken. Replacing conventional xenobiotics with bio-based and biodegradable alternatives seems to be the only option to tackle this global environmental crisis. Finally, it can be concluded that for environmental safety, we have to restrict or reduce the use of xenobiotics while simultaneously encouraging the development of more efficient biodegradable compounds that can replace the traditional xenobiotics to make the planet earth a sustainable biosphere again.

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