Chapter 16
Microbial Bioremediation: A Cutting-Edge Technology for Xenobiotic Removal

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Contents

Abstract Industrialization, urbanization, and the use of modern technology in agriculture have its pros and cons. On one hand, they improve the standard of living but impact the structure and function of different ecosystems drastically. In a broad sense, a decline in crop productivity, impairment in activity of soil microbes, death of aquatic fauna, as well as carcinogenicity and mutagenicity in humans and animals are some of the ill effects due to xenobiotic presence in the environment. It is thus imperative to develop certain strategies that can notably ensure the perspective of development without compromising the health of the ecosystem. Among the various physical and chemical methods for xenobiotic degradation, bioremediation using microorganisms is unequivocally an economical and ecologically sound approach. This chapter emphasizes the applicability of the bioremediation process for the effective degradation of different classes of xenobiotic compounds like pesticides, dyes, phenols, pharmaceuticals, etc. The up-to-date information about the

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involvement of two major microbes, namely, bacteria and fungi as well as enzymes from different sources, are described in the context of xenobiotic degradation and detoxification. Last but not the least, the various factors that come into play for significant removal of a xenobiotic are also explained in a well-defined manner.

Keywords Xenobiotics · Pesticides · Bioremediation process · Dye degradation · Enzymes

16.1 Introduction

Due to the industrial revolution together with urbanization and the introduction of contemporary agricultural practices, a remarkable advancement of economic growth has been apparent during the past few years. As a consequence, a plethora of environment-unfriendly xenobiotic compounds are generated daily. The term xenobiotics signifies those compounds which are considered as foreign to the biological system (Loredana et al. 2017). More precisely, these include a wide range of man-made chemical substances which are manufactured in the laboratory, for instance, pharmaceuticals, pesticides, hydrocarbons, artificial sweeteners, plastics, lignin, aromatics, and certain solvents like phenol and carcinogens as well (Rieger et al. 2002). The prevalence of certain elements has been experienced in the structure of xenobiotics which are not generally exist in natural environments. Examples of such elements include aromatic sulfonic acids (present in dyes), diazo bond, and polychlorination of either an alkane or aromatic compound (Knapp and Bromley-Challoner 2003).

Although xenobiotics are present in very low concentrations (ng/L to μg/L) in the environment but the existence of such toxic compounds are regarded as major havoc to environmental integrity. Being recalcitrant, they accumulate in the hydrosphere, lithosphere, and atmosphere where they are known to negatively impact the flora, fauna, and humans (Gangola et al. 2018). When xenobiotic-contaminated effluents from industries like oil refineries, food industries, paint, pesticides, and textile are discharged into the surrounding water bodies, the dissolved oxygen gets significantly depleted (Garg and Tripathi 2017; Lellis et al. 2019). On top of that, the colored effluent from the textile industries blocks the sunlight's entry into the waterways, thus impairing the photosynthesis in aquatic plants (Imran et al. 2015; Hassan and Carr 2018). On the other hand, the spray of pesticides and herbicides causes the introduction of these toxic compounds into the agricultural lands. Interminable pesticide consumption is responsible for the impairment in soil properties and reduction in the population of soil microbes (El-Ghany and Masmali 2016). Moreover, they also flow into lakes and rivers through surface runoff, thus making the water infected (Casara et al. 2012; Pande et al. 2020). These xenobiotics persist in the environment for longer durations which ultimately enter into the food chain,

hence causing biomagnification. It has been observed that long-term exposure towards xenobiotics can induce various tumorigenic, neurotoxic, genotoxic, immunotoxic, and mutagenic effects in humans and animals (Terry 2012). The possible effects of xenobiotic presence in the environment on various communities are delineated in Fig. 16.1.

Environmental restoration is the prime necessity in the present scenario. To achieve this motive, it is essential to tranquilize the deleterious effects of xenobiotics on air, water, and soil. For reducing the toxicity content of xenobiotics, many physical and chemical treatment processes such as photolysis, advanced oxidation, ozonation, hydrolysis, membrane filtration, electrocoagulation, adsorption, and flocculation are put into practice (Kaneco et al. 2006; Foo and Hameed 2010; Behera et al. 2011; Plakas and Karabelas 2012; Adak et al. 2019). But considering the problems of sludge generation, high operational cost, and generation of toxic degradation products associated with the physicochemical methods, the bioremediation method is of particular interest for eco-friendly and cost-effective treatment of xenobiotics (Lopez et al. 2004; Linley et al. 2012; Youssef et al. 2016). Furthermore, the problem of toxic degradation product generation is not associated with the bioremediation method. Many microorganisms, namely, bacteria and fungi as well as microbial enzymes, are the key players in the bioremediation process.

However, the conditions under which the microbes and enzymes can reveal maximum degradation capability are an important point in question. In this chapter, comprehensive knowledge concerning the sources of xenobiotics is provided. An attempt is made to evaluate the various microbes responsible for carrying out the degradation of various xenobiotic compounds, for example, pesticides, pharmaceuticals, dyes, phenols, etc. Finally, the different nutrient and physicochemical conditions underpinning the bioabsorption, transformation, and mineralization of xenobiotics by microbes are extensively addressed.

16.2 Classification and Sources of Xenobiotics

Every day huge quantities of several hazardous substances (the xenobiotics) are released into the environment (Fig. 16.2). The potential sources of different types of xenobiotics are documented in Table 16.1. Many industries, namely, pharmaceuticals, paper and pulp bleaching, petrochemical, coal refineries, and textile industries, play an important role in the introduction of these toxic recalcitrant substances. One of such toxic compound is dyes that are increasingly being used by industries during several industrial processes like for coloration of fabrics in textile industries, for hair, nails, lip coloring, eye and facial makeup in cosmetic industries, as well as for photosensitization in the photographic industry. Dyes can persist in the environment for longer durations (Waller et al. 2000; Guerra et al. 2018; Lellis et al. 2019). Some of the most commonly used dyes in these industries comprise of azo-type dyes during the industrial processes. Around 80% of azo dyes used in textile industries are incapable of binding to the fabric and are released as such in the effluent (Rehman

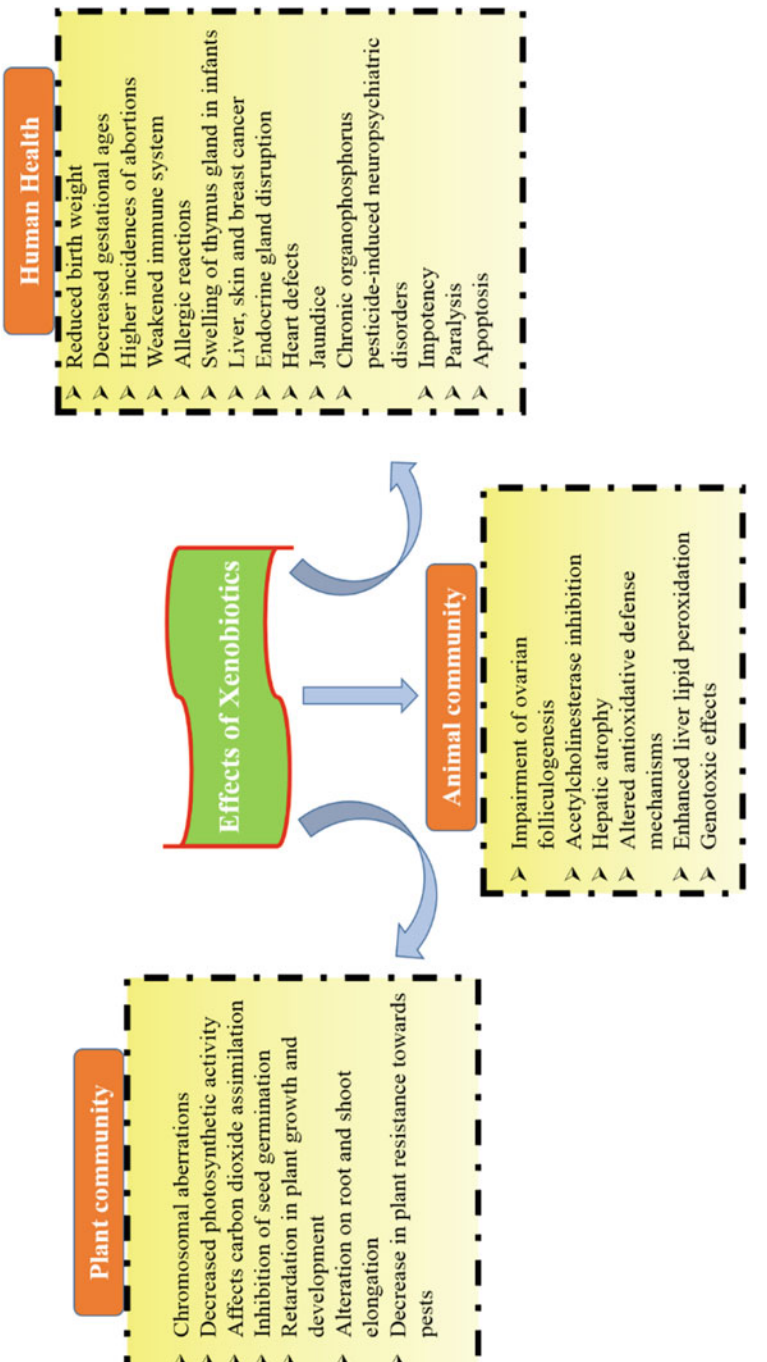

Fig. 16.1 Effects of xenobiotics on the biotic communities

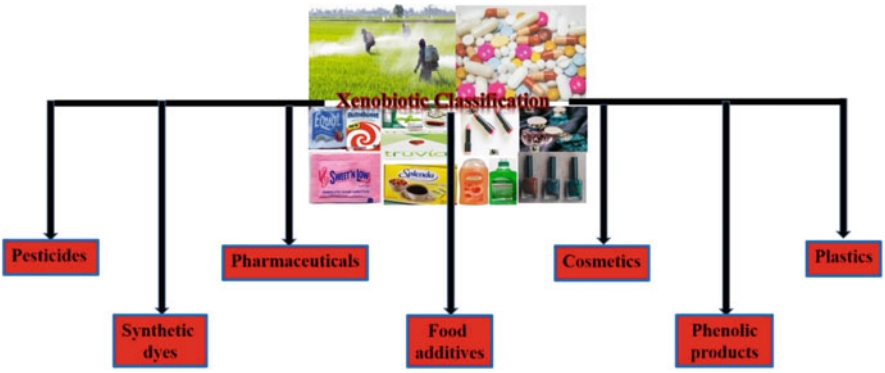

Fig. 16.2 Classification of xenobiotic

et al. 2018). As per reports, around 280,000 tons of textile dyes are lost in the wastewater globally every year (Choudhary et al. 2020).

Likewise, some other industries like petrochemical and pesticide industries are also responsible for the release of an extensive list of xenobiotics which due to their recalcitrant property tends to concentrate in the environment. For example, the petrochemical industry which is considered as one of the fastest-growing industrial sectors generates hazardous wastes such as polycyclic aromatic hydrocarbons (anthracene and naphthalene). The effluents from petrochemical industries such as oil refineries as well as plastic industries and olive processing plants are also rich in polyphenolics (Aggelis et al. 2002). Another class of xenobiotics, that is, pharmaceutical drugs, including human and veterinary antibiotics, hormones, analgesics, anticonvulsants, antihistamine, antidepressants, and β-blockers, are persistently discharged into the terrestrial and aquatic ecosystems by households, landfills and from effluents released by hospitals, sewage treatment plants, and municipal and industrial facilities (Barnes et al. 2004; Watkinson et al. 2009; Fatta-Kassinos et al. 2011; Chakraborty et al. 2020). Overall, it has been estimated that these industrial plants are responsible for the deposition of approximately 300–400 million tons of toxic sludge, solvents, and heavy metals into the environmental surroundings (Xiao et al. 2015).

Besides the industrial sectors, the contribution of agriculture in deteriorating the environment through the release of xenobiotics is not a matter to be ignored. It has been estimated that by 2025, the world population will reach 8 billion. The continuous ever-increasing population exerts tremendous pressure on the agriculture sector to employ different methods to produce food on a large scale. As a result, the farmers are using pesticides—the chemicals to kill pests of crops which can be weeds, insects, rodents, fungi, etc., to increase the agricultural productivity. A wide variety of pesticides like organophosphates, organochlorines, carbamate, and morpholine successfully control the pest establishment, but due to their broad-spectrum activity, certain nontarget organisms get severely affected.

Table 16.1 Sources involved in the generation of various xenobiotics

Source	Type of xenobiotic released into the environment	Examples of xenobiotics	References
Paper and pulp bleaching	Chlorinated organic compounds	di-, tri-, tetra-, and pentachlorophenols, tetrachloroguaiacols, and tetrachlorocatechols	Tana (1988), Singh (2017)
Sewage treatment plant	Pharmaceuticals	Ofloxacin, ciprofloxacin, erythromycin, diclofenac, ibuprofen, carbamazepine, bezafibrate, atenolol, acetaminophen, oxytetracycline, tylosin, sulfamethoxazole, amoxicillin, diazepam, trimethoprim, clindamycin, lincomycin	Rosal et al. (2010), Zuccato et al. (2010), Subedi et al. (2017)
Hospital wastewater treatment plant	Pharmaceuticals	Acetaminophen, ciprofloxacin, norfloxacin, tetracycline, atenolol, ketoprofen, ibuprofen, estrone, estriol	Kanama et al. (2018)
Municipal wastewater treatment plant	Pharmaceuticals	Fenbendazole	Sim et al. (2013)
Intensive agriculture	Herbicides	Pendimethalin, butachlor, bensulfuron-methyl, pretilachlor	Das et al. (2011), Pinto et al. (2012), Singh (2017), Mohanty and Jena (2019)
	Pesticides and insecticides	Benzimidazoles, methyl parathion, morpholine, chlorpyrifos, aldrin	Singh (2017), Rayu et al. (2017), Doolotkeldieva et al. (2018)
Paper mill	Phenol	–	Sachan et al. (2019)
Chemical and pharmaceutical industry	Synthetic polymers, phenols	–	Singh (2017), Varsha et al. (2011)
Petrochemical industry	Phenol	–	Liu et al. (2016)
Plastic industry	Phenol	–	Liu et al. (2016)
Paint industry	Organic solvents	Toluene, xylene, styrene, ethylbenzene	Moro et al. (2010)
Textile industry	Azo dyes	Black B, Turq Blue GN, Tectilon Yellow 2G, Yellow HEM, Red HEFB, and Navy HER	Tufekci et al. (2007), Acuner and Dilek (2004)
Textile industry	Heavy metals	Ni, Cu, Cr, Pb, Cd, Zn	Khan and Malik (2018)

16.3 Xenobiotic Bioremediation Utilizing Microbes

Bioremediation technology nowadays has achieved stupendous attention for the removal of recalcitrant compounds from soil and water. In this technology, a myriad of microorganisms which are either inhabitant of xenobiotic contaminated sites or genetically modified microbes with amplified biodegradability potential are exploited. Usually, the bioremediation processes involve various reactions including oxidation-reduction, hydrolysis, hydroxylation, conjugation, sulfation, and methylation for the degradation or biotransformation of xenobiotics. Varied microorganisms are likely to degrade diverse xenobiotics present in different sources across the world which are given in detail in the following sections.

16.3.1 Role of Bacteria for Xenobiotic Removal

The role of microbes in bioremediation and their resistance to xenobiotic toxicity has been well documented by various researchers. Table 16.2 summarizes the various studies on the use of bacteria for the removal of a wide range of xenobiotics. Heterogeneous bacteria are known to make use of phenol as a carbon and energy source. Most widely recognized among the bacterial population are *Acinetobacter calcoaceticus*, *Bacillus*, *Pseudomonas*, and *Rhodococcus* (Liu et al. 2016; Mohanty and Jena 2017; Maniyam et al. 2020). From several different environments which most often include agricultural wastewater, natural river biofilms, agricultural soil and sugarcane farm soil, etc. (El-Helow et al. 2013; Tien et al. 2017; Fareed et al. 2017), pesticide degraders have been frequently isolated.

The hydrolysis of labile methylcarbamine linkage with the production of metabolites such as carbofuran-7-phenol and methylamine occurred during the degradation of pesticide carbofuran by bacteria (Yan et al. 2007). The bacteria *Sphingomonas* sp. can degrade carbofuran into various metabolites, for example, 2-hydroxy-3-(3-methylpropan-2-ol) phenol and red intermediates (Park et al. 2006).

The *Pseudomonas aeruginosa* that is isolated from desert soil can simultaneously degrade cadmium (Cd) and Reactive Black 5 (RB5) which are the common xenobiotics found in the industrial effluent (Louati et al. 2020). It has been seen that the Cd is removed by bacteria via biosorption mechanism wherein the metal binds on the microbial surface through processes like electrostatic interaction, complex formation, ion exchange, and precipitation (Hansda et al. 2016; Ayangbenro and Babalola 2017). Apart from that, the extracellular polymers synthesized by *Pseudomonas* sp. are involved in metal chelation (Gupta and Diwan 2017). As per the study by Giovanella et al. (2017), the reduction, biosorption, production of siderophore, and biofilm development are the main mechanisms responsible for metal removal by *Pseudomonas* sp.

The xenobiotic removal by free cells nonetheless is a prime concern due to the complications of activity loss, cell separation, and problem in isolating strain having

Table 16.2 List of various microbes with xenobiotic degrading potential

Type of microbes	Microorganisms degrading xenobiotic	Target xenobiotic compound	Site of isolation	Culture conditions	Percent removal	References
Bacteria	*Gordonia* sp. JAAS1	Chlorpyrifos	Paddy soil	• Temperature—28 °C • Incubation time—24 h • Pesticide concentration—110 mg/L	100%	Abraham et al. (2013)
	Sphingobacterium sp. JAS3	Chlorpyrifos	Paddy soil	• Temperature—30 ± 2 °C • Incubation time—24 h • Pesticide concentration—300 mg/L	100%	Abraham and Silambarasan (2013)
	Bacillus subtilis Y242	Chlorpyrifos	Agricultural wastewater	• Temperature—30 °C • Incubation time—48 h • Pesticide concentration—150 mg/L	95.1%	El-Helow et al. (2013)
	Mesorhizobium sp. HN3	Chlorpyrifos	Soil	• Temperature—37 °C • pH—7 • Incubation time—5–7 days • Pesticide concentration—100 mg/L	100%	Jabeen et al. (2015)

	Acinetobacter calcoaceticus PA	Phenol	Oil refinery wastewater	• Temperature—30 °C • Incubation time—48 h • Agitation speed—150 rpm • Phenol concentration—800 mg/L	91.6%	Liu et al. (2016)
	Pseudomonas japonica I-15	Reactive Black 5 dye	Activated sludge	• Temperature—28 °C • Daily light integral—240 μmol/m^2/s^1 • Relative humidity—61% • Incubation time—48 h • Dye concentration—150 mg/L	81%	Shafqat et al. (2017)
	Bacillus sp. SR-2-1/1	Azo dyes	Rhizosphere samples of sorghum plants grown at textile wastewater-contaminated soil	• Temperature—30 °C • pH—7 • Incubation time—48 h • Dye concentration—150 mg/L	80–90%	Mahmood et al. (2017)
	Sulfitobacter dubius	Lindane	Demosponge *Hymeniacidon perlevis* associated	• Temperature—22 °C • Incubation time—12 days • Pesticide concentration—0.05 mg/L	97%	Loredana et al. (2017)

(continued)

Table 16.2 (continued)

Type of microbes	Microorganisms degrading xenobiotic	Target xenobiotic compound	Site of isolation	Culture conditions	Percent removal	References
	Alteromonas australica	Lindane	Demosponge *Hymeniacidon perlevis* associated	• Temperature—22 °C • Incubation time—12 days • Pesticide concentration—0.05 mg/L	97%	Loredana et al. (2017)
	Pseudovibrio ascidiaceicola	Lindane	Demosponge *Hymeniacidon perlevis* associated	• Temperature—22 °C • Incubation time—12 days • Pesticide concentration—0.05 mg/L	95%	Loredana et al. (2017)
	Xanthomonas sp. 4R3-M1	Chlorpyrifos	Sugarcane farm soils	• Temperature—28 °C • Incubation time—6 days • Pesticide concentration—10 mg/L	80%	Rayu et al. (2017)
	Pseudomonas sp. 4H1-M3	Chlorpyrifos	Sugarcane farm soils	• Temperature—28 °C • Incubation time—6 days • Pesticide concentration—10 mg/L	90%	Rayu et al. (2017)

	Rhizobium sp. 4H1-M1	Chlorpyrifos	Sugarcane farm soils	• Temperature—28 °C • Incubation time—6 days • Insecticide concentration—10 mg/L	75%	Rayu et al. (2017)
	Pseudomonas sp.	Phenol	Sewage and wastewater discharged site	• Temperature—30 to 32 °C • pH—6.8 to 7.2 • Incubation time—168 h • Agitation speed—150 rpm • Phenol concentration—1000 mg/L	>98%	Mohanty and Jena (2017)
	Ochrobactrum sp.	Erythromycin	Soil sample	• Temperature—32 °C • pH—6.5 • Incubation time—72 h • Erythromycin concentration—100 mg/L	97%	Zhang et al. (2017)
	Sphingobacterium multivorum	Carbofuran	Natural river biofilms	• Temperature—25 °C • pH—7 • Incubation time—7 days • Pesticide concentration—50 mg/L	73.1%	Tien et al. (2017)

(continued)

Table 16.2 (continued)

Type of microbes	Microorganisms degrading xenobiotic	Target xenobiotic compound	Site of isolation	Culture conditions	Percent removal	References
	Thermus thermophilus	Ciprofloxacin	Pharmaceutical sludge	• Temperature—65 to 80 °C • pH—6.5 • Incubation time—72 h • Antibiotic concentration—5 mg/L	>55%	Pan et al. (2018)
	Bradyrhizobium sp. GLC_01	Ciprofloxacin	Activated sludge	• Temperature—25 °C • pH—6.5 • Incubation time—8 days • Agitation speed—150 rpm • Antibiotic concentration—0.05 mg/L	70.4%	Nguyen et al. (2018)
	Arthrobacter soli BS5	Reactive Black 5	Native of soil irrigated with textile industry wastewater	• Temperature—37 °C • pH—5 to 9 • Incubation time—120 h • Dye concentration—50 µg/mL	98%	Khan and Malik (2018)
	Serratia liquefaciens	Azure B	Soil sample	• Temperature—30 °C • Incubation time—48 h • Dye concentration—100 mg/L	>90%	Haq and Raj (2018)

	Neisseria sp. (EK-5)	Novacron Orange FN-R	Dyeing effluent	• Temperature—37 °C • pH—7 • Incubation time—6 days • Dye concentration—100 mg/L	19%	Karim et al. (2018)
	Neisseria sp. (EK-5)	Novacron Brilliant Blue FN-R	Dyeing effluent	• Temperature—37 °C • pH—7 • Incubation time—6 days • Dye concentration—100 mg/L	43% decolorization	Karim et al. (2018)
	Neisseria sp. (EK-5)	Novacron Super Black G	Dyeing effluent	• Temperature—37 °C • pH—7 • Incubation time—6 days • Dye concentration—100 mg/L	65% decolorization	Karim et al. (2018)
	Neisseria sp. (EK-5)	Bezema Yellow S8-G	Dyeing effluent	• Temperature—37 °C • pH—7 • Incubation time—6 days • Dye concentration—100 mg/L	30% decolorization	Karim et al. (2018)
	Vibrio sp. (EK-6)	Novacron Orange FN-R	Dyeing effluent	• Temperature—37 °C • pH—7 • Incubation time—6 days	40% decolorization	Karim et al. (2018)

(continued)

Table 16.2 (continued)

Type of microbes	Microorganisms degrading xenobiotic	Target xenobiotic compound	Site of isolation	Culture conditions	Percent removal	References
				• Dye concentration—100 mg/L		
	Vibrio sp. (EK-6)	Novacron Brilliant Blue FN-R	Dyeing effluent	• Temperature—37 °C • pH—7 • Incubation time—6 days • Dye concentration—100 mg/L	67% decolorization	Karim et al. (2018)
	Vibrio sp. (EK-6)	Novacron Super Black G	Dyeing effluent	• Temperature—37 °C • pH—7 • Incubation time—6 days • Dye concentration—100 mg/L	65% decolorization	Karim et al. (2018)
	Vibrio sp. (EK-6)	Bezema Yellow S8-G	Dyeing effluent	• Temperature—37 °C • pH—7 • Incubation time—6 days • Dye concentration—100 mg/L	50% decolorization	Karim et al. (2018)
	Vibrio sp. (EK-6)	Bezema Red S2-B	Dyeing effluent	• Temperature—37 °C • pH—7 • Incubation time—6 days	42% decolorization	Karim et al. (2018)

				• Dye concentration—100 mg/L		
	Bacillus sp. (EK-7)	Novacron Orange FN-R	Dyeing effluent	• Temperature—37 °C • pH—7 • Incubation time—6 days • Dye concentration—100 mg/L	25% decolorization	Karim et al. (2018)
	Bacillus sp. (EK-7)	Novacron Brilliant Blue FN-R	Dyeing effluent	• Temperature—37 °C • pH—7 • Incubation time—6 days • Dye concentration—100 mg/L	83% decolorization	Karim et al. (2018)
	Bacillus sp. (EK-7)	Novacron Super Black G	Dyeing effluent	• Temperature—37 °C • pH—7 • Incubation time—6 days • Dye concentration—100 mg/L	35% decolorization	Karim et al. (2018)
	Bacillus sp. (EK-7)	Bezema Yellow S8-G	Dyeing effluent	• Temperature—37 °C • pH—7 • Incubation time—6 days • Dye concentration—100 mg/L	55% decolorization	Karim et al. (2018)
	Bacillus sp. (EK-7)	Bezema Red S2-B	Dyeing effluent	• Temperature—37 °C • pH—7	41% decolorization	Karim et al. (2018)

(continued)

Table 16.2 (continued)

Type of microbes	Microorganisms degrading xenobiotic	Target xenobiotic compound	Site of isolation	Culture conditions	Percent removal	References
				• Incubation time—6 days • Dye concentration—100 mg/L		
	Bacillus sp. (EK-9)	Novacron Orange FN-R	Dyeing effluent	• Temperature—37 °C • pH—7 • Incubation time—6 days • Dye concentration—100 mg/L	39% decolorization	Karim et al. (2018)
	Bacillus sp. (EK-9)	Novacron Super Black G	Dyeing effluent	• Temperature—37 °C • pH—7 • Incubation time—6 days • Dye concentration—100 mg/L.	40% decolorization	Karim et al. (2018)
	Bacillus sp. (EK-9)	Bezema Yellow S8-G	Dyeing effluent	• Temperature—37 °C • pH—7 • Incubation time—6 days • Dye concentration—100 mg/L	47% decolorization	Karim et al. (2018)
	Aeromonas sp. (EK-13)	Novacron Orange FN-R	Dyeing effluent	• Temperature—37 °C • pH—7	27% decolorization	Karim et al. (2018)

				• Incubation time—6 days • Dye concentration—100 mg/L		
	Aeromonas sp. (EK-13)	Novacron Brilliant Blue FN-R	Dyeing effluent	• Temperature—37 °C • pH—7 • Incubation time—6 days • Dye concentration—100 mg/L	90% decolorization	Karim et al. (2018)
	Aeromonas sp. (EK-13)	Novacron Super Black G	Dyeing effluent	• Temperature—37 °C • pH—7 • Incubation time—6 days • Dye concentration—100 mg/L	23% decolorization	Karim et al. (2018)
	Aeromonas sp. (EK-13)	Bezema Yellow S8-G	Dyeing effluent	• Temperature—37 °C • pH—7 • Incubation time—6 days • Dye concentration—100 mg/L	38% decolorization	Karim et al. (2018)
	Aeromonas sp. (EK-13)	Bezema Red S2-B	Dyeing effluent	• Temperature—37 °C • pH—7 • Incubation time—6 days • Dye concentration—100 mg/L	41% decolorization	Karim et al. (2018)

(continued)

Table 16.2 (continued)

Type of microbes	Microorganisms degrading xenobiotic	Target xenobiotic compound	Site of isolation	Culture conditions	Percent removal	References
	Bacillus polymyxa	Aldrin	Soil	• Temperature—25 °C • pH—7.2 • Incubation time—12 days • Insecticide concentration—0.2 mg/mL	48.2%	Doolotkeldieva et al. (2018)
	Pseudomonas fluorescens	Aldrin	Soil	• Temperature—25 °C • pH—7.2 • Incubation time—12 days • Insecticide concentration—0.2 mg/mL	43.2%	Doolotkeldieva et al. (2018)
	Serratia ureilytica strain AS1	Butachlor	Herbicide-contaminated soil	• Temperature—32.5 °C • pH—7.5 • Incubation time—10 days • Herbicide concentration—500 mg/L	100%	Mohanty and Jena (2019)
	Rhodococcus UKMP-5M	Phenol	Petroleum-contaminated soil	• Temperature—30 °C • pH—7.4 • Incubation time—24 h	89%	Suhaila et al. (2019)

				• Phenol concentration—0.5 g/L		
	Rhodococcus strain UCC 0016	Methyl red dye	Palm oil mill effluent	• Temperature—30 °C • pH—7 • Incubation time—24 h • Incubation at static condition • Dye concentration—0.5 g/L	100%	Maniyam et al. (2020)
	Enterobacter sp.	Carbofuran	Agricultural soil	• Temperature—37 °C • Incubation time—68 h • Insecticide concentration—100 mg/L	100%	Mustapha et al. (2020a)
Fungi	*Trametes versicolor*	Norfloxacin	–	• Temperature—30 °C • Incubation time—7 days • Agitation speed—150 rpm • Antibiotic concentration—2 mg/L	>90%	Prieto et al. (2011)
	Trametes versicolor	Ciprofloxacin	–	• Temperature—30 °C • Incubation time—7 days • Agitation speed—150 rpm	>90%	Prieto et al. (2011)

(continued)

Table 16.2 (continued)

Type of microbes	Microorganisms degrading xenobiotic	Target xenobiotic compound	Site of isolation	Culture conditions	Percent removal	References
				• Antibiotic concentration—2 mg/L		
	Lentinula edodes	Pendimethalin	Loamy sand soil and a biomixture	• Temperature—25 °C • Incubation time—10 days • Agitation speed—150 rpm • Pesticide concentration—25 mg/L	~ 96%	Pinto et al. (2012)
	Penicillium brevicompactum	Terbuthylazine	Loamy sand soil and a biomixture	• Temperature—25 °C • Incubation time—10 days • Agitation speed—150 rpm • Pesticide concentration—25 mg/L	99.5%	Pinto et al. (2012)
	Trametes versicolor	Flumequine	–	• Temperature—28 °C • Incubation time—4 days • Antibiotic concentration—12 μg/mL	100%	Cvancarova et al. (2013)
	Irpex lacteus	Flumequine	–	• Temperature—28 °C • Incubation time—4 days	90%	Cvancarova et al. (2013)

				• Antibiotic concentration—12 μg/mL		
	Dichomitus squalens	Flumequine	–	• Temperature—28 °C • Incubation time—4 days • Antibiotic concentration—12 μg/mL	90%	Cvancarova et al. (2013)
	Pestalotiopsis sp. NG007	Reactive Green 19	Textile industry wastewater	• Temperature—30 °C • pH—3 to 12 • Salinity—0 to 10% w/v • Incubation time—24 h	94% decolorization	Yanto et al. (2014)
	Pestalotiopsis sp. NG007	Reactive Orange 64	Textile industry wastewater	• Temperature—30 °C • pH—3 to 12 • Salinity—0 to 10% w/v • Incubation time—24 h	54% decolorization	Yanto et al. (2014)
	Pestalotiopsis sp. NG007	Reactive Red 4	Textile industry wastewater	• Temperature—30 °C • pH—3 to 12 • Salinity—0 to 10% w/v • Incubation time—24 h	47% decolorization	Yanto et al. (2014)
	Phanerochaete chrysosporium	Amido Black 10B	–	• Temperature—37 °C • pH—3 to 7 • Incubation time—	98% decolorization	Senthilkumar et al. (2014)

(continued)

Table 16.2 (continued)

Type of microbes	Microorganisms degrading xenobiotic	Target xenobiotic compound	Site of isolation	Culture conditions	Percent removal	References
				3 days • Agitation speed—150 rpm		
	Penicillium citrinum	Chlorfenvinphos	Untreated surface water	• Temperature—27 °C • Incubation time—82 days • Agitation speed—100 rpm • Pesticide concentration—250 µg/L	100%	Oliveira et al. (2015)
	Penicillium ochrochloron AMDB-12	Reactive Blue 13	Acidic mine drainage	• Temperature—40 °C • pH—2 • Contact time—120 min • Agitation speed—150 rpm • Dye concentration—50 ppm	55%	Aytar et al. (2016)
	Penicillium ochrochloron AMDB-12	Reactive Blue 72	Acidic mine drainage	• Temperature—40 °C • pH—2 • Contact time—120 min • Agitation speed—150 rpm • Dye concentration—50 ppm	61%	Aytar et al. (2016)

	Aspergillus fumigatus A23	Simulated textile effluent	–	• Temperature—40 °C • pH—4 • Incubation time—7 days • Agitation speed—100 rpm	86%	Dharajiya et al. (2016)
	Phanerochaete chrysosporium	Simulated textile effluent	–	• Temperature—30 °C • pH—5 • Incubation time—7 days • Agitation speed—100 rpm	62% decolorization	Dharajiya et al. (2016)
	Trichoderma harzianum	Malathion	Soil samples	• Temperature—20 to 40 °C • Incubation time—20 days • Insecticide concentration—10 to 40 mg/L	>90%	El-Ghany and Masmali (2016)
	Metarhizium anisopliae	Profenofos	Soil samples	• Temperature—20 to 40 °C • Incubation time—20 days • Insecticide concentration—40 mg/L	63.6%	El-Ghany and Masmali (2016)
		Diazinon	Soil samples	• Temperature—20 to 40 °C • Incubation time—20 days • Insecticide	85.6%	El-Ghany and Masmali (2016)

(continued)

Table 16.2 (continued)

Type of microbes	Microorganisms degrading xenobiotic	Target xenobiotic compound	Site of isolation	Culture conditions	Percent removal	References
				concentration—10 mg/L		
		Malathion	Soil samples	• Temperature—20 to 40 °C • Incubation time—20 days • Insecticide concentration—20 mg/L	>90%	El-Ghany and Masmali (2016)
	Thamnidium elegans	Reactive Yellow 2	–	• pH—2 • Contact time—39.4 min • Dye concentration—100 mg/L	95%	Akar et al. (2017)
	Pleurotus ostreatus	Ciprofloxacin	–	• Temperature—25 °C • Incubation time—14 days • Antibiotic concentration—500 ppm	95%	Singh et al. (2017)
	Aspergillus sp. TS-A CGMCC 12964	Mordant Yellow 1	Activated sludge of a textile factory	• Temperature—30 °C • pH—6 • Incubation time—1 h • Agitation speed—160 rpm • Dye concentration—50 mg/L	98.6%	Kang et al. (2018)

the ability to withstand high toxic concentrations of xenobiotics (Kathiravan et al. 2010; Fareed et al. 2017). To overcome this limitation, many researchers immobilized the free bacterial cells into the numerous organic materials as well as inorganic materials. Mustapha et al. (2020a) reported efficient degradation of carbofuran even at 250 mg/L concentration, whereas the free cells are unable to tolerate such a high concentration of carbofuran. Similarly, several other pesticides, for example, carbofuran, carbamates, pendimethalin, profenofos, atrazine, and cypermethrin, are found to be competently degraded by immobilized cells of bacteria contrary to free bacterial cells (Kadakol et al. 2011; Tallur et al. 2015; More et al. 2015; Talwar and Ninnekar 2015; Kumar et al. 2017; Fareed et al. 2017). Besides, Wang et al. (2017) confirmed the degradation of di-n-butyl phthalate using *Acinetobacter* species strain LMB-5 coated with magnetic nanoparticles.

16.3.2 Role of Fungi in Xenobiotic Removal

The fungi as a probable candidate for xenobiotic removal are attracting profound attention in contemporary times. The specific activity and growth morphology make fungal species a proficient degrader of xenobiotics (Mollea et al. 2005). *Aspergillus*, *Galactomyces geotrichum*, *Podoscypha elegans*, and *Scheffersomyces spartinae* are routinely used for detoxification of recalcitrant compounds (Ali et al. 2008; Waghmode et al. 2011; Tan et al. 2016; Dharajiya et al. 2016; Chaudhry et al. 2014; Pramanik and Chaudhuri 2018). Fungi such as *Aspergillus niger* cleave the carbon-phosphorus bond of organophosphonates, thereby releasing phosphate ions (Adelowo et al. 2015). Carbon-phosphorus bond cleavage is indicated to be the first step during the degradation of organophosphonate pesticides.

Among the fungal population, the white-rot fungi outclass as a major xenobiotic degrader. Many xenobiotic compounds, namely, pharmaceuticals, dyes, pesticides, phenols, lignin, etc., are converted into nontoxic metabolites using different white-rot fungi such as *Trametes versicolor*, *Trametes polyzona*, *Pleurotus ostreatus*, *Phanerochaete chrysosporium*, *Bjerkandera adusta*, and *Cerrena unicolor* (Singh et al. 2010; Prieto et al. 2011; Asgher et al. 2016; Singh et al. 2017; Zhang et al. 2018; Bouacem et al. 2018; Lueangjaroenkit et al. 2019). The xenobiotic degradative ability of varied fungal species is evident from Table 16.2.

16.4 Bioremediation with Microbial Enzymes

Microbial enzymes have drawn profound attention when it comes to xenobiotic degradation. Reductase, laccase, peroxidase, oxidase, and hydrolase are some of the enzymes isolated from either bacteria or fungi which are relevantly functional in the degradation of a wide variety of substrates, for example, dyes, benzene, pharmaceuticals, pesticides, phenolic compounds, and polycyclic aromatic hydrocarbons

Table 16.3 Microbial enzymes employed for xenobiotic degradation

Enzyme used	Source of enzyme	Type of xenobiotic degraded	References
Alkaline phosphatase	*Spirulina platensis*	Chlorpyrifos	Thengodkar and Sivakami (2010)
Laccase	*Trametes versicolor*	Norfloxacin and ciprofloxacin	Prieto et al. (2011)
Laccase	*Trametes versicolor* and *Phanerochaete chrysosporium*	Benzo[a]pyrene	Qian and Chen (2012)
Laccase	*Trametes versicolor*	Acid Violet 7, Acid Red 1, Allura Red AC, Orange G, and Sunset Yellow FCF	Legerska et al. (2018)
Manganese peroxidase	*Phanerochaete chrysosporium*	Sulfamethoxazole	Gao et al. (2018)
Laccase	*Pycnoporus sanguineus*	Sulfamethoxazole, ciprofloxacin, norfloxacin	Gao et al. (2018)
Manganese peroxidase	*Aspergillus* sp. TS-A CGMCC 12964	Mordant Yellow 1	Kang et al. (2018)
Lignin peroxidase	*Bjerkandera adusta* strain CX-9	Acid Blue 158, Cibacet Brilliant Blue BG, Polymeric dye R, Remazol Brilliant Blue Reactif, Remazol Brilliant Violet 5R, Indigo Carmine, and Methyl Green	Bouacem et al. (2018)
Manganese peroxidase	*Bjerkandera adusta* strain CX-9	Acid Blue 158, Cibacet Brilliant Blue BG, Polymeric dye R, Remazol Brilliant Blue Reactif, Remazol Brilliant Violet 5R, Indigo Carmine, and Methyl Green	Bouacem et al. (2018)
Laccase	*Trametes* sp. MA-X01	Direct Blue 53, Direct Blue 14, Acid Orange 10, Acid Red 18, Acid chrome blue K, and Janus green B	Wang et al. (2018)
Laccase	*Kluyveromyces dobzhanskii*, *Pichia manshurica*	Malachite green and methyl red	Wakil et al. (2019)
Laccase	*Pseudomonas mendocina*	Mixed azo dye (reactive red, reactive brown, and reactive black)	Sridharan et al. (2019)
Laccase	*Trametes polyzona* KU-RNW027	Remazol brilliant blue, Remazol navy blue, and Remazol brilliant yellow, Remazol red, tetracycline, doxycycline, amoxicillin, and ciprofloxacin	Lueangjaroenkit et al. (2019)
Manganese peroxidase	*Trametes polyzona* KU-RNW027	Remazol brilliant blue, Remazol navy blue, and Remazol brilliant yellow, tetracycline, doxycycline, amoxicillin, and ciprofloxacin	Lueangjaroenkit et al. (2019)

(Weng et al. 2013; Zafra and Cortes-Espinosa 2015; Lellis et al. 2019). Table 16.3 enlists the studies concerning the use of bacterial and fungal enzymes for xenobiotic degradation or detoxification. The function of manganese peroxidase is to oxidize

Mn^{2+} to Mn^{3+} in the presence of H_2O_2 which thereby causes oxidation of various toxic compounds (Chen et al. 2015b; Bilal et al. 2016). The enzyme lignin peroxidase brought about the oxidation of aromatic compounds through the generation of free radicals in the presence of H_2O_2 (Bilal et al. 2017). Another vital enzyme laccase is a member of blue copper oxidases containing four copper atoms and is mainly found in fungi and higher plants. At the enzyme active site, copper atoms of four different types, that is, type I, type II, and two type III, are present which accounts for the catalytic activity of laccase (Wang et al. 2018). The type I copper atom of laccase oxidized the substrate which in turn loses electron that is accepted by type I.

From type I, the electrons are transported to types II and III where molecular oxygen is reduced to two molecules of water and substrate produces free radicals (Zucca et al. 2016; Wang et al. 2018; Lellis et al. 2019). Laccase is a efficient xenobiotic degrader unlike other enzymes as the requisite of cofactor, namely, hydrogen peroxide for substrate oxidation is not there (Liebminger et al. 2009; Bayramoğlu et al. 2010). Along with it, they have specificity for many substrates and hence can be increasingly useful for oxidizing diverse toxic pollutants (Saito et al. 2003; Aslam et al. 2012).

Immobilization of enzymes is a predominant research focus during the last decades. Enzymes when immobilized have better stability and activity as well as recovered from the suspension without difficulty (Shah and D'Mello 2007; Hebert and Rochefort 2008; Roman-Gusetu et al. 2009). A variety of carriers are available for immobilization of enzymes including alginate beads, glass beads, mesoporous silica, porous poly(GMA/EGDMA) beads, chitosan beads, poly(ethyleneimine) microcapsule, and nanoparticles (Dominguez et al. 2007; Mureseanu et al. 2009; Arica et al. 2009; Roman-Gusetu et al. 2009; Bilal et al. 2016; Sridharan et al. 2019). The nano-sized materials as a support material for immobilization are usually preferred nowadays as they provide large surface area for attachment and also ensure easy separation of enzymes in a magnetic field (Sridharan et al. 2019). Bayramoğlu et al. (2010) immobilized *Trametes versicolor* laccase onto the poly(4-vinylpyridine) grafted and Cu(II) chelated magnetic beads. The immobilized enzyme showed activity at a broader pH and temperature. Besides, it is possible to reuse immobilized enzymes for five cycles for dye degradation which is not possible in the case of free enzymes.

16.5 Factors Influencing the Biodegradation Ability of Microbes

The microbes need specific conditions for optimum growth and efficacious xenobiotic removal. The behavior of microbes varies depending upon different environmental factors such as pH, temperature, nutrients availability, and physicochemical conditions for xenobiotic degradation (Nagase et al. 2006; Tien et al. 2017). For

example, the carbofuran pesticide degrading ability of *Sphingobacterium multivorum* was found to be maximum at pH 7 and a temperature of 25 °C (Tien et al. 2017). The bacterial genus *Enterobacter* efficiently removes 92.5 mg/L carbofuran at pH 6 and temperature 27.5 °C and nitrogen source of 0.45 g/L (Mustapha et al. 2020b). Likewise, the laccase enzyme secreted by bacteria *Pseudomonas mendocina* revealed better degradation of azo dyes at pH 5.8 and temperature 20 °C (Sridharan et al. 2019). The laccase enzyme from macro fungus *Podoscypha elegans* can carry out an effective degradation of azo dyes (Congo Red, Orange G, Direct Blue 15, Direct Yellow 27, and Rose Bengal) at a broad pH range of 5.5–7 (Pramanik and Chaudhuri 2018). Other enzymes, that is, manganese peroxidase, isolated from white-rot fungi, *Trametes polyzona* KU-RNW027, have exhibited optimal activity at pH 4.5 (Lueangjaroenkit et al. 2019).

The availability of nutrients, namely, glucose, iron, manganese, magnesium, phosphorus, and other trace elements, ameliorates the xenobiotic degradation activity of microbes. Bacterial species such as *Sphingobacterium* sp. strain D6 and *Sphingomonas* exhibited higher degradation of DDT and methomyl in a media enriched by glucose than that of glucose devoid media (Fang et al. 2010; Chen et al. 2015a). Conversely, no significant difference was observed during the degradation of carbofuran by *Sphingobacterium multivorum* cultured in a medium with or without sugar (Tien et al. 2017). Furthermore, the presence of phosphorus is considered to be an essential requirement for better biodegradation of antibiotics and oil/hydrocarbons (Abu and Atu 2008; Nnenna et al. 2011). In fungi, *Phanerochaete chrysosporium*, the availability of nitrogen is considered to be favorable for the effective degradation of polycyclic aromatic hydrocarbons (Mollea et al. 2005). The activity of fungal enzymes such as manganese peroxidases and lignin peroxidase increases in the presence of manganese and hydrogen peroxide at a concentration of less than 1 mM during dye degradation (Kang et al. 2018). Also, surplus substrates enhance the activity of manganese peroxidases to a greater extent. The exhaustive list of these substrates includes 2,6-dimethoxyphenol, *o*-dianisidine, veratryl alcohol, 2,4-dichlorophenol, commercial humic acid, levodopa, signayl alcohol, 2,6-dichlorophenol, 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid), coniferyl alcohol, 2,4,5-trichlorophenol, 2,4,6-trichlorophenol, guaiacol, and pyrogallol (Bouacem et al. 2018). Other important nutrients like magnesium, copper, manganese, and zinc stimulate the activity of laccase enzyme isolated from the genus *Trametes*. On the other hand, laccase works best in the absence of certain elements like lead, potassium, sodium, and calcium as well as protein reductants including β-mercaptoethanol, L-cysteine, dithiothreitol, and sodium azide (Aslam et al. 2012; Wang et al. 2018).

Also, the presence of heavy metals might either have a positive or negative effect on the microbial degradation of xenobiotics. In a study by Mustapha et al. (2020a), most severe effects are noticed when the media is supplemented with mercury and copper at a concentration of 1 mg/L during degradation of carbofuran by *Enterobacter* sp. However, another heavy metal, that is, cadmium, exhibited a negligible toxic effect at this concentration. It has been reported that the low

concentration of around 1mM of silver have increased the activity of laccase enzyme (Lueangjaroenkit et al. 2019).

In some cases, the preservation of microbes has proved to be worthwhile during pesticide degradation. Tien et al. (2017) reported increased carbofuran degradation activity of microbial consortia (*Comamonas jiangduensis*, *Pseudomonas fulva*, *Stenotrophomonas* sp., and *Thermolithobacter* sp.) after preservation at 25 °C for 1 month. Despite that, some bacteria like *Sphingobacterium* sp., *Sphingomonas* sp., and *Pseudomonas* sp. showed a decrease in methomyl pesticide degradation after preservation (Chen et al. 2015a).

16.6 Conclusion

Development particularly in terms of industrialization compromises environmental integrity on a large scale. The entry of xenobiotics into the environment is one of the deleterious consequences of industrialization. Hence, it is obligatory to devise certain techniques through which the toxicity of xenobiotics can be controlled effectively. Bioremediation by microorganisms and microbial enzymes is considered to be one such cost-effective technique that removes a vast number of environmental pollutants. Being environment friendly, this field is flourishing day by day. With the advent of genetic engineering technology, it has also become possible to improve the xenobiotic degradation efficiency of microorganisms. The success of bioremediation, however, depends upon the various environmental conditions and nutrients requirement which must be taken care of.

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