# Chapter 6
# Bioremediation Approaches for Degradation and Detoxification of Polycyclic Aromatic Hydrocarbons

**Pavan Kumar Agrawal, Rahul Shrivastava, and Jyoti Verma**

**Abstract** Waste from industry is a noteworthy risk to the earth as it contains different poisonous, mutagenic and cancer-causing substances including polycyclic aromatic hydrocarbons (PAHs). PAHs are a class of different organic compounds with two or more intertwined benzene rings in a linear, angular or cluster array. Eviction of PAHs is crucial as these are persevering toxins with ubiquitous event and adverse natural impacts. There are several remedial techniques, which are productive and financially savvy in elimination of PAHs from the affected environment. These removal approaches are not just eco-friendly; they additionally display an emerging and new strategy in mitigating the ability of PAHs to cause potential risk to living beings. Accessible physical and synthetic techniques are neither eco-accommodating nor financially viable in this way. Natural strategies such as bioremediation techniques are most appropriate for biodegradation of PAHs. Such techniques require less chemicals, less time and less contribution of energy and are cost-effective and eco-accommodating. The lethal PAH mixes can be changed into non-harmful and more straightforward ones utilizing normally occurring microorganisms like algae, bacteria and fungi in a procedure called biodegradation. This chapter mainly focuses on the enhancement in biodegradation of hazardous PAHs by using bioremedial approaches.

**Keywords** Polycyclic aromatic hydrocarbons · Enzymatic approach · Biodegradation · Bioremediation

---

P. K. Agrawal (✉) · J. Verma
Department of Biotechnology, G.B. Pant Engineering College, Ghurdauri, Pauri, Garhwal, Uttarakhand, India

R. Shrivastava
Department of Biotechnology & Bioinformatics, Jaypee University of Information Technology, Waknaghat, Solan, Himachal Pradesh, India



R. N. Bharagava, P. Chowdhary (eds.), *Emerging and Eco-Friendly Approaches for Waste Management*, https://doi.org/10.1007/978-981-10-8669-4_6

## 1 Introduction

PAHs are effective environmental toxicants that consist of fused aromatic rings. PAHs are originated in unrefined petroleum, coal tar and blacktop (Ukiwe et al. 2013). PAHs are included in the US Environmental Protection Agency (EPA) and European Community (EC) Contaminant Candidate List. EPA currently regulates 16 PAH compounds as priority pollutants in water and as 'aggregate PAHs' in defiled soil and sediments (Hadibarata et al. 2009). PAHs are of big concern to humans and animals as contaminant, some even recognized as cancer causing, mutagenic or teratogenic.

PAH compounds have two- to seven-membered benzene rings. They are lacking water affinity mixes with aqueous solubility declining almost linearly with increases in molecular mass (Parrish et al. 2004). Physicochemical properties and molecular weight of PAHs vary with the number of rings in the atom. Increment in subatomic weight of PAHs leads to decrease in chemical reactivity, aqueous solubility and volatility of PAHs. High-molecular-weight PAHs have high resonance energies because of the thick mists of pi-electrons surrounding the aromatic rings making them steady in the earth and recalcitrant to degradation. The recalcitrant nature of compounds may be attributed to their low water solubility and high soil sorption (Parrish et al. 2004).

PAH degradation is controlled by several physicochemical as well as biological processes, which vary their fate and transport in the surface environment. Biodegradation of hydrocarbons is achieved either by microorganism such as bacteria (Hamamura et al. 2013), fungus (Cerniglia and Sutherland 2010) or algae (Chan et al. 2006) or by enzymatic approaches. Fungi are considered as a productive competitor for effective degradation of PAHs. In any case, filamentous growth has capacity to develop on wide spectrum of substrates by secreting extracellular hydrolytic enzymes, even equipped for growing under non-ambient environment (Juhasz and Naidu 2000). Bioremediation includes either indigenous or exogenous microbial population, which is known as not proficient degraders in contaminated site (Yadav et al. 2017; Bharagava et al. 2017). Fungi have advantages over bacteria because of their fungal hyphae and potent hydrolytic enzymes, which can enter and corrupt the hydrocarbons affected environment (Venkatesagowda et al. 2012). Fungal enzymes especially oxidoreductases, laccase and peroxidases have noticeable application in removal of PAH contaminants either in fresh, marine water or terrestrial. Nevertheless, interest on growths gets an impressive consideration for bioremediation of hydrocarbon contaminated sites associated fungi for enzyme discharge (to expel hydrocarbons from nature). The persistence, toxicity and carcinogenicity of PAH molecules draw public concern to decontaminate PAH-polluted sites.

## 2 Sources of Polycyclic Aromatic Hydrocarbons in the Environment

For the most part, PAH contamination happens by unprocessed and processed oil, which comes from tanker accidents, refinery effluents, metropolitan and modern release from pipelines and seaward productions and waste oil from two-wheeler and four-wheeler administration stations, which causes contamination (Uzoamaka et al. 2009). Polycyclic aromatic hydrocarbon compounds are formed, what's more, discharged into the earth through both natural and anthropogenic sources. Natural sources of PAHs include their development as exudates from trees woods and rangeland fires, fungi and bacteria (Fig. 6.1). In nature, PAHs remain prevalently distributed as parts of plant oils, cuticles of insects, components of surface waxes of leaves and lipids of microorganisms. PAHs are formed naturally during thermal geologic reactions associated with fossil fuel and mineral generation.

Anthropogenic sources like fuel ignition, vehicles, spillage of petroleum products, electric fuel generation, internal ignition motors and waste incinerators are critical sources of PAHs into the environment (Arulazhagan and Vasudevan 2011). Anthropogenic wellsprings of PAHs are the real reason for natural contamination and, hence, the focus of a lot of bioremediation programmes. PAHs remain saved in the environment through generally scattered sources covering significantly the land surface area. At such sources PAHs are observed to be consumed strongly to soil particles.

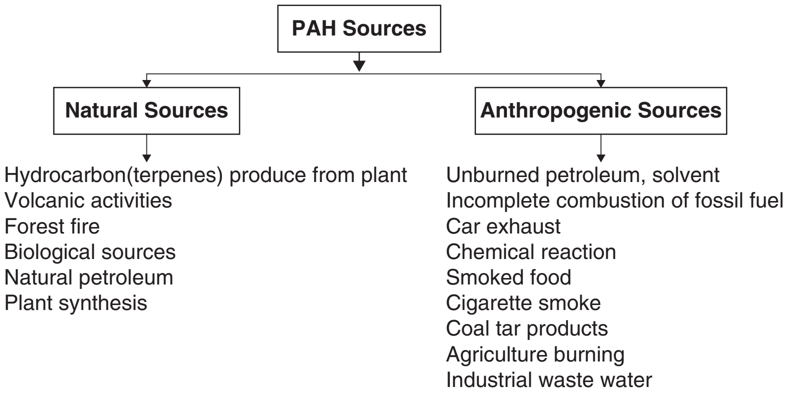


**Fig. 6.1** Natural and anthropogenic sources of polycyclic aromatic hydrocarbons (PAHs)

# 3 Environmental Problems of PAHs

Increasing knowledge of potent adverse effects of toxicant on human health and environment has led to enhanced attention and measures for remediation and renovation of environment contaminants. Polycyclic aromatic hydrocarbons (PAHs) are omnipresent organic pollutants with serious environmental concerns. PAHs are distributed in various ecosystems and are pollutants of severe concern due to their potential toxicity, mutagenicity and carcinogenicity.

PAHs have an innate property for bioaccumulation in food chains, which makes their presence in any ecosystem alarming for human health (Morehead et al. 1986; Xue and Warshawsky 2005). Accumulation of PAHs in plants poses hazardous effect to human health because of their position in the food chain. Experimental studies have also demonstrated interference with plant carbon allocation and root symbioses by PAHs, which ultimately affect plant growth and the environment. Because of their concentration and toxicity, 16 PAHs have been enlisted as priority environmental pollutants by the US Environmental Protection Agency (US EPA).

# 4 Various Techniques for PAH Degradation

## *4.1 Chemical Degradation*

The availability of PAHs in anaerobic conditions relies on certain components, which incorporate substrate interaction, pH and redox conditions (Chang et al. 2002). The most oxidation reactions in the environment are initiated by oxidants such as peroxides ($H_2O_2$), ozone ($O_3$) and hydroxyl radicals generated by photochemical processes. The degradation pathways are such that the oxidation reactions involving hydroxyl radicals or $O_3$ react with aromatic compounds such as PAHs at near diffusion-controlled rates by abstracting hydrogen atoms or by expansion to twofold bonds (Ukiwe et al. 2013). The reaction proceeds with complex pathways creating various intermediates. In such reactions, the last response products include a mixture of ketones, quinones, aldehydes, phenols and carboxylic acids for both oxidants (Reisen and Arey 2002). During chemical reaction PAHs are transformed into other polyaromatic hydrocarbons (they do not lose their aromatic character). Their aromaticity is conserved since considerable amounts of energy are required to change an aromatic compound into a non-aromatic compound. PAHs could be degraded through fermentative digestion system, while some studies have demonstrated that PAH degradation in anaerobic environment is much slower than in aerobic environment (Ambrosoli et al. 2005). Effects of carbon (C) and nitrogen (N) on PAH degradation have also been investigated by several authors (Quan et al. 2009). The efficiency of PAH chemical degradation is limited by their low aqueous solubility and vapour pressure (Fernando et al. 2009). However, surfactants enhance the solubility of hydrophobic compounds (Ukiwe et al. 2013). Several reports have been focused on the significance of surfactants to expand the solubility of PAHs by decreasing the interfacial surface tension amongst PAH and the dirt/water interphase (Li and Chen 2009).

### 4.2 Phytodegradation

It is characterized as the utilization of plants to expel contaminations from the earth to render them nontoxic (Table 6.1). Plants can take the toxicant up and accrue them in their tissues. It is an in situ, solar energy-regulated technique, which minimizes environmental disturbance and reduces costs (Haritash and Kaushik 2009). Researchers have indicated that various grasses and leguminous plants are potential candidates for phytodegradation of organics (Newman and Reynolds 2004; Ukiwe et al. 2013). Some tropical plants have also been reported to show effective degradation tendency due to inherent properties such as deep fibrous root system and tolerance to high hydrocarbon and low nutrient availability (Dzantor et al. 2000; Chandra et al. 2012). Many species of grass such as *Agropyron smithii*, *Bouteloua gracilis*, *Cynodon dactylon*, *Elymus canadensis*, *Festuca arundinacea*, *Festuca rubra*, *Melilotus officinalis*, etc. are known to degrade PAHs (McCutcheon and Schnoor 2003). Researchers are also investigating that grasses and legumes induce the removal of PAHs from affected soil. Plants also play an indirect role in the removal of PAHs by releasing of enzymes by roots. These enzymes are capable of transforming organic contaminants by catalysing chemical reactions in soil (Ndimele et al. 2010). Plant enzymes also act as causative agents in the transformation of contaminants mixed with sediment and soil. The identified enzyme systems included dehalogenase, nitroreductase, peroxidase and laccase (Thomson and Ndimele 2010). Rasmussen and Olsen studied the efficiency of orchard grass (*Dactylis glomerata*) towards PAH removal. The study reported that a soil/sand mixture vegetated with orchard grass exhibited high treatment efficiency with an input from the microbial catabolic degradation by plant exudates (Parish et al. 2004).

**Table 6.1** Plants useful in phytodegradation of PAHs

| S.No. | Isolate name | Compound | % Removal | Incubation | References |
|---|---|---|---|---|---|
| 1. | *Festuca arundinacea* | Pyrene | 38% | 190 days | Chen et al. (2003) |
| 2. | *Pannicum virgatum* | Pyrene | 38% | 190 days | Chen et al. (2003) |
| 3. | *E. crassipes solani* | Naphthalene | 45% | 7 days | Nesterenko et al. (2012) |
| 4. | *Scirpus Grossus* | Petroleum hydrocarbons | 81.5% | 72 days | Al-Baldawi et al. (2015) |

### 4.3 Biodegradation

Biodegradation is a reasonable technique for degradation of natural contaminations. It is the use of microorganisms to degrade or detoxify environmental pollutants (Bamforth and Singleto 2005; Saxena and Bharagava 2017). Several additional factors affecting rate of degradation incorporate pH, temperature, nearness of oxygen and supplement accessibility. The concentration of nutrients and the state of the nutrients (organic, inorganic) are important for biodegradation. The biodegradation of PAHs is financially savvy, eco-friendly (as it prevents environmental damage during transportation of contaminants). Biological degradation is an approach that presents the possibility to remove organic pollutants with the help of natural biological activity available in the substrate (Zeyaullah et al. 2009; Bharagava and Chandra 2010). The microorganisms used for biodegradation could be indigenous to the contaminated region or site (Das and Chandran 2011). The complete mineralization products of the pollutant by biodegradation process include $CO_2$, $H_2O$ and cell biomass (Gratia et al. 2006). During biodegradation process optimization involves many factors such as microbial consortia capable of degrading the pollutant, bioavailability of the pollutants to microbial attack and soil type, temperature, soil pH, oxygen level of soil, electron acceptor agents and nutrient content of soil (environmental factors) contributing to microbial growth (Gratia et al. 2006; Epelde et al. 2009; Mulla et al. 2017; Bharagava and Chandra 2010). Complete degradation of PAHs to $CO_2$, water, microbial carbon and other inorganic compound is the ultimate goal. Haeseler et al. (2001) showed enhanced, but incomplete, degradation of PAH compounds in a field study. When remediation was complete, final toxicity was very less because the metabolites tended to be less stable and more soluble than the parent compounds, making them more available to degraders (Haeseler et al. 2001). Microbial degradation is the most suitable alternative and effective method of removal of those toxic chemicals. Microbes (including bacteria, fungi and algae) can biologically degrade PAH compounds during direct microbial metabolism of carbon energy sources or by co-metabolism while consuming another substrate (Lundstedt et al. 2006) (Table 6.2).

## 5 Role of Various Microorganisms in Bioremediation

The problem linked with the PAHs can be mitigating by the use of conventional approaches, which involve degradation, modification or isolation of the toxicant. These approaches involve excavation of contaminate and its incineration or containment. These technologies are expensive and in many cases transfer the pollutant from one phase to another. On the other hand, bioremediation is the tool to transform the compounds to less hazardous/nonhazardous forms with less input of chemicals, energy and time (Ward et al. 2003; Yuan et al. 2001; Chandra et al. 2011). Microorganisms are known to be their catabolic activity in biological remediation

**Table 6.2** Movement and fate of organic chemicals, such as PAHs, in the environment

| S.No. | Degradation process | Consequence | Factors | Advantages/ disadvantages |
|---|---|---|---|---|
| 1. | Chemical degradation | Alteration of PAHs by chemical processes such as photochemical (i.e., UV light) and oxidation-reduction reactions | High and low pH, structure of PAH, intensity and duration of sunlight, exposure to sunlight and same factors as for microbial degradation | Limited effectiveness and can be expensive |
| 2. | Phytodegradation | Breakdown of contaminants or pesticides through metabolic processes within the plant | Molecular weight of PAHs, sunlight, enzymes | Low-cost methods for cleaning the environment |
| 3. | Biodegradation | Degradation of PAHs by microorganisms, biodegradation and co-metabolism | Environmental factors (pH, moisture, temperature, oxygen), nutrient status, organic matter content, PAH bioavailability, microbial community present, molecular weight of PAH (LMW or HMW) | Cost-effectiveness and complete cleanup, catabolic versatility of microorganisms, less labour-intensive, relying on solar energy, have a lower carbon footprint and have a high level of public acceptance |

(Bharagava et al. 2009), but changes in microbial communities are still unpredictable, and the microbial community is still termed as a ‘black box’ (Dua et al. 2002). The PAH-degrading microorganism could be algae, bacteria or fungi. It involves the breakdown of organic compounds either usually by microorganism in to less complex metabolites or through mineralization into inorganic minerals, $H_2O$, $CO_2$ (aerobic) or $CH_4$ (anaerobic). The extent and rate of contaminant degradation depend on many factors including pH, temperature, $O_2$, microbial population, degree of acclimation, convenience of nutrients, compounds chemical structure, properties of cellular transport and chemical partitioning in the growth medium (Singh and Ward 2004).

## *5.1 Biodegradation of PAHS by Fungi*

Fungus is known to have the properties of degradation of persistent organic pollutants (Table 6.3). Distinct properties differentiate filamentous fungus from other life forms to decide why they are potent biodegraders agents. First, the mycelial growth habit gives a competitive benefit over single cells such as bacteria and yeasts, especially with respect to the colonization of insoluble substrates (Bennet et al. 2002). The isolates identified as *Deuteromycetes* belonging to the genera *Cladophialophora*, *Exophiala* and *Leptodontium* and the ascomycete *Pseudeurotium zonatum* are

**Table 6.3** Fungal isolates involve in degradation of PAHs

| S.No | Isolate name | Compound | % Removal | Incubation | References |
|---|---|---|---|---|---|
| 1. | *Phomopsis liquidambari* | Indole | 41.7% | 6 days | Chen et al. (2013) |
| 2. | *Fusarium verticillioides* | Naphthalene | 87.78% | 8 days | Mohamed et al. (2012) |
| 3. | *Fusarium solani* | Anthracene, benz[a]anthracene | 40% and 60%, resp. | 40 days | Wu et al. (2009) |
| 4. | *Aspergillus terreus* | Naphthalene, anthracene | 98.5% and 91%, resp. | 4 weeks | Mohamed et al. (2012) |
| 5. | *Fusarium* sp. | Naphthalene | 42% | 7 days | Ahirwae and Dehariya (2013) |

toluene-degrading fungi; they use toluene as sole carbon and energy source (Francesc et al. 2001). Clemente et al. (2001) reported that degree of degradation of PAH varies with a variation of lignolytic enzymes producing deuteromycete ligninolytic fungal isolates.

Low-molecular-weight PAHs (two to three rings) were found to be degraded most extensively by *Aspergillus sp.*, *Trichocladium canadense* and *Fusarium oxysporum*. For high-molecular-weight PAHs (four to seven rings), maximum degradation has been observed by *T. canadense*, *Aspergillus sp.*, *Verticillium sp.* and *Acremonium sp.* Such studies have found that fungi have a great capability to degrade a broad range of PAHs under low-oxygen conditions. As a large and novel microbial resource, endophytic fungi have been paid more attention in their ecological functions.

The effect of microbes on litter component decomposition (Osono and Hirose 2011) but extended fungal degradation to more recalcitrant carbohydrate (Russell et al. 2011).

PAHs degradation by fungi has mostly focused on white-rot fungi. Their broad-range degradation potential is one reason of PAHs, such as *Irpex lacteus* found with a degradative ability of ANT, phenanthrene, pyrene as well as fluoranthene, and their degradative mechanisms were also investigated (Cajthaml et al. 2002). The other reason is because of their efficient production of ligninolytic enzymes; e.g. *Phanerochaete chrysosporium* could degrade ANT and phenanthrene by producing lignin peroxidase (LiP) and manganese-dependent peroxidise (MnP) (Hammel 1995). *Lentinus* (*Panus*) *tigrinus* showed out the MnP transformation ability after carrying out in vivo and in vitro degradation of PAHs (Covino et al. 2010). *Cunninghamella* sp. and *Aspergillus* sp. were reported for their potential in the transformation of benzo[a]pyrene and the conjugation mechanisms during the degradation (Wu et al. 2009). *Fusarium* spp. have shown their capability to degrade high-molecular-weight organic compounds such as coal cellulose, xylan, pectin, different hydrocarbons (Kang and Buchenauer 2000) as well as PAHs (Chulalaksananukul et al. 2006). Lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase (Lac) have shown to degrade not only lignocellulose but also pollutants such as crude oil wastes, textile effluents, distillery wastewater pollutants, organochloride agrochemicals and pulp effluents which are a cause of serious environmental pollution (Mtui and Nakamura 2004; Chandra and Chowdhary 2015; Chowdhary et al. 2017a, b, 2018).

### *5.2 Biodegradation by Bacterial Isolates*

Several reports refer to degradation ability of different bacterial isolates of environmental pollutants (Table 6.4). Many bacterial spp. are even known to nourish completely on hydrocarbons (Yakimov et al. 2007). Degradation of PAHs can occur under aerobic and anaerobic conditions, as in the case for the nitrate-reducing bacterial strains *Pseudomonas* sp. and *Brevibacillus* sp. isolated from petroleum contaminated soil (Grishchenkov et al. 2000). Several species of microorganisms have been successfully utilized in major hazardous waste cleanup processes (Levinson et al. 1994). Abd et al. (2009) reported that two- to three-ring PAHs (naphthalene, anthracene and phenanthrene) can be degraded using *Pseudomonas geniculata* and *Achromobacter xylosoxidans*. Bacterial isolates capable of chrysene metabolism include *Rhodococcus* sp. strain UW1 (Walter et al. 1991) and *Sphingomonas yanoikuyae* which oxidized chrysene (Boyd et al. 1999), while *Pseudomonas fluorescens* utilized chrysene and benz[a]anthracene as sole carbon sources (Caldini et al. 1995). The microorganisms capable of surviving in such a polluted environment are those that develop specific enzymatic and physiological responses that allow them to use hydrocarbon as a substrate. Kafilzadeh et al. (2011) reported ten genera as follows: *Bacillus*, *Corynebacterium*, *Staphylococcus*, *Streptococcus*, *Shigella*, *Alcaligenes*,

**Table 6.4** Biodegradation of PAHs by various bacterial isolates

| S.No | Isolate name | Compound | % Removal | Incubation | References |
|---|---|---|---|---|---|
| 1. | *Pseudomonas* sp. | Naphthalene, fluorene | 95% | 4 days | Kumar et al. (2010) |
| 2. | *Pseudomonas* sp. | Anthracene | 74.8% | 10 days | Kumar et al. (2010) |
| 3. | *Bacillus* sp. | Anthracene | 82.6% | 72 h | Neelofur et al. (2014) |
| 4. | *Mesoflavibacter zeaxanthinifaciens* | Benzo[a]pyrene | 86% | 42 days | Okai et al. (2015) |
| 5. | *Mycobacterium flavescens* | Pyrene | 89.4% | 2 weeks | Dean-Ross et al. (2002) |
| 6. | *Rhodococcus* sp. | Anthracene | 53.0% | 2 weeks | Ross et al. (2002) |

*Acinetobacter*, *Escherichia*, *Klebsiella* and *Enterobacter* out of 80 bacterial strains. *Bacillus* was the best hydrocarbon-degrading genus. Bacterial strains that are able to degrade aromatic hydrocarbons have been repeatedly isolated, mainly from soil. The bacterial genera *Mycobacterium*, *Corynebacterium*, *Aeromonas*, *Rhodococcus* and *Bacillus* have been also reported for biodegradation pathways (Mrozik et al. 2003).

## 5.3 Biodegradation of PAHs by Algal Isolates

Algae are important microbial members in both aquatic and terrestrial ecosystems; reports are insufficient regarding their concern in hydrocarbon biodegradation (Table 6.5). (Das and Chandran 2011; Walker et al. 1975) isolated an alga, *Prototheca zopfii* which was capable of utilizing crude oil and a mixed hydrocarbon substrate and exhibited extensive degradation of n-alkanes and isoalkanes as well as aromatic hydrocarbons. Cerniglia and Gibson (1977) observed that nine cyanobacteria, five green algae, one red alga, one brown alga and two diatoms could oxidize naphthalene. Some research has demonstrated that certain fresh algae (e.g., *Chlorella vulgaris*, *Scenedesmus platydiscus*, *S. quadricauda* and *S. capricornutum*) are capable of uptaking and degrading PAHs (Wang and Zhao 2007). Warshawsky et al. (2007) found that *Selenastrum capricornutum*, a freshwater green alga, metabolizes BaP to cis-dihydrodiols using a dioxygenase enzyme system as found in heterotrophic prokaryotes. Certain algae have been reported to enhance the removal fluoranthene and pyrene when present with bacteria. Borde et al. (2003) first reported that photosynthesis enhanced degradation of toxic aromatic compounds by algal-bacterial microcosms in a one-stage treatment. *Pseudomonas migulae* and *Sphingomonas yanoikuyae* were studied for phenanthrene degradation. The green alga *Chlorella sorokiniana* was cultivated in the presence of the pollutants at different concentrations, showing increasing inhibitory effects in the order salicylate < phenol

**Table 6.5** Biodegradation of PAHs by various algal isolates

| S.No | Isolate name | Compound | % Removal | Incubation | References |
|---|---|---|---|---|---|
| 1. | *Prototheca zopfii* | Petroleum hydrocarbons | 12–41% | 3 days | Kirk et al. (1999) |
| 2. | *Selenastrum capricornutum* | Benzo[*a*]pyrene | 41% | 4 days | Warshawsky et al. (2007) |
| 3. | *Selenastrum capricornutum* | Fluoranthene | 99% | 7 days | Ke et al. (2010) |
| 4. | *Lyngbyala gerlerimi* | Naphthol | 36.6% | 5 days | Mostafa et al. (2012) |
| 5. | *Nostoc linckia* | Catechol | 56.38% | 5 days | Mostafa et al. (2012) |
| 6. | *Oscillatoria rubescens* | β-naphthol | 3.04% | 7 days | Mostafa et al. (2012) |

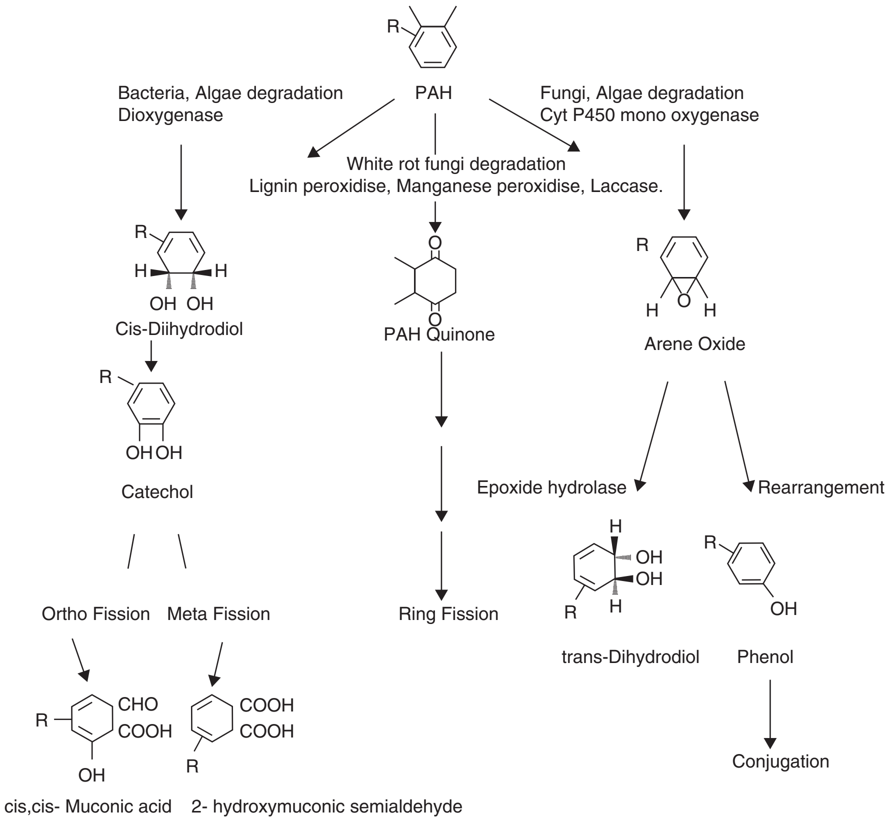


**Fig. 6.2** The three main pathways for polyaromatic hydrocarbon degradation by fungi and bacteria. (Adopted by Muthuswamy et al. 2008)

<phenanthrene (Lei et al. 2007). The study of fluoranthene, pyrene and a mixture of fluoranthene and pyrene by *Chlorella vulgaris*, *Scenedesmus platydiscus*, *Scenedesmus quadricauda* and *Selenastrum capricornutum* has shown that removal is algal species-specific and toxicant-dependent. PAH removal in 7 days of treatment was 78% and 48%, respectively, by *S. capricornutum* and *C. vulgaris*. Hong et al. (2008) studied the accumulation and biodegradation of phenanthrene and fluoranthene by the algae enriched from a mangrove aquatic environment (Fig. 6.2).

## 6 Enzymatic Degradation of PAHs

Major enzymes useful in PAH degradation belong to oxygenase, dehydrogenase and lignolytic categories. Lignolytic enzymes secreted by majority of fungal species are lignin peroxidase, manganese peroxidase and laccase, which are extracellular in nature and are secreted for catabolism of substrate food material (Chandra and Chowdhary 2015). Spent mushroom compost (SMC) is often used as an inoculum source which enhances the rate of PAH degradation. The SMC are high in laccase and Mn-dependent peroxidase, whereas the production of ligninase is reported to be low in SMC (Haritash and Kaushik 2009). In which most of enzymes are active at many temperature and have optimum activity at mesophilic temperatures and it reduces with very high and very low temperatures. Some of the enzymes are reported to be active even at extremes of temperatures (Haritash and Kaushik 2009). Enzymes also show substrate specificity, but ligninolytic enzymes are non-specific in nature, acting on phenolic and non-phenolic organic compounds via the generation of cation radicals after one $e^-$ oxidation (Lau et al. 2003).

## *6.1 Lignin Degrading Enzymes*

Oxidoreductive enzymes play a key role in transformation and degradation of polymeric substances (Table 6.6). The less degraded or oxidized items can without much of a stretch be taken up by microbial cells where they are totally mineralized. A class of oxidoreductive enzymes include lignin-degrading compounds (LDEs) which have practical application in bioremediation of polluted environment (Husain 2006). LDEs belong to two classes, viz., the heme-containing peroxidases and the copper-containing laccases. A progression of redox responses are started by the laccases. LDEs degrade the lignin or lignin-derived pollutants. The LDEs oxidize the aromatic compounds until the aromatic ring structure is cleaved, which is followed by further debasement with different compounds.

**Table 6.6** Biological functions of ligninolytic enzymes

| Enzymes | Applications | References |
|---|---|---|
| Lignin peroxidase | Biodegradation of lignin | Martínez et al. (2005) |
| | Degradation of azo | Stolz (2001) |
| | Mineralization of environmental contaminants | Harms et al. (2011) |
| | Degradation of pharmaceuticals and their metabolites | Marco-Urrea et al. (2009) |
| Manganese peroxidase | Degradation of lignin | Martínez et al. (2005) |
| | PAH degradation | Baborová et al. (2006) |
| | Synthetic dyes, DDT, PCB, TNT | Hernández et al. (2008) |
| | Textile dye degradation and bleaching | Kalyani et al. (2008) |
| Laccase | Spore resistance | Lu et al. (2012) |
| | Rhizomorph formation | Ranocha et al. (2002) |
| | Pathogenesis | Langfelder et al. (2003) |
| | Fruit bodies formation | Nagai et al. (2003) |
| | Pigment synthesis | Eisenman et al. (2007) |

Enzymes involved in the degradation of PAHs are oxygenase, dehydrogenase and ligninolytic enzymes. Fungal ligninolytic enzymes are lignin peroxidase, laccase and manganese peroxidase. They are extracellular and catalyse radical formation by oxidation to destabilize bonds in a molecule (Hofrichhter et al. 1999).

Peroxidases perform heme-containing degradation with other enzymes. Peroxidases are heme-containing enzymes that comprise manganese-dependant peroxidase (MnP), lignin peroxidase (LiP) and versatile peroxidase (VP). They oxidize lignin subunits using extracellular hydrogen peroxide generated by unrelated oxidases as co-substrate. Most mineralization activity of the lignin polymers to $CO_2$ and $H_2O$ in terrestrial ecosystem is performed by fungal species. These fungi produce a wide range of lignin-degrading enzymes (LDEs), which in turn act on lignin and lignin-analogous compounds. PAHs are primarily degraded using extracellular oxidative enzymes, although use of laccases and peroxidases in PAH bioremediation is currently being studied (Harms et al. 2011). The white-rot fungi (WRF) belonging to the basidiomycetes produce various isoforms of extracellular ligninolytic enzymes, laccases (Lac) and different peroxidases, including lignin peroxidase (LiP), manganese peroxidase (MnP) and versatile peroxidase (VP), the latter sharing LiP and MnP catalytic properties (Martínez 2002). The natural substrate of these enzymes (lignocellulose) is degraded in the environment by the WRF, along with various xenobiotic compounds, including dyes (Wesenberg et al. 2003). Some WRF produce all the three lignin-modifying enzymes, while others produce only one or two of them. Lignin-modifying enzymes are produced by WRF during their secondary metabolism since lignin oxidation provides no net energy to the fungus (Eggert et al. 1996).

### 6.1.1 Lignin Peroxidases (EC 1.11.1.14)

LiPs (EC 1.11.1.14) are an extracellular hemeprotein. They are related to the family of oxidoreductases (Higuchi 2004). LiP has high redox potential and low optimum pH (Piontek et al. 2001) and is capable of oxidizing a variety of reducing substrates including polymeric substrates (Oyadomari et al. 2003). Due to their high redox potentials and enlarged substrate range, LiPs have more potential for application in several industrial processes (Erden et al. 2009). Enzymatic activity of LiP is $H_2O_2$ dependent; here $H_2O_2$ gets reduced to $H_2O$ by picking up an electron from LiP (which itself gets oxidized). The oxidized LiP then returns to its native reduced state by picking up an $e^-$ from veratryl alcohol and oxidizing into veratryl aldehyde. Veratryl aldehyde then gets reduced back to veratryl alcohol by picking up an electron from lignin or equivalent structures such as xenobiotic compounds.

### 6.1.2 Manganese Peroxidases (EC 1.11.1.13)

MnP (EC 1.11.1.13) belong to the family of oxidoreductases (Higuchi. 2004). Studies have shown that MnP is distributed in almost all white-rot fungi (Hofrichter 2002). Manganese peroxidases (MnP) seem to be distributed amongst white-rot fungi than LiP (Hammel and Cullen. 2008). MnP oxidizes $Mn^{2+}$ to highly reactive $Mn^{3+}$, which oxidizes phenolic structures to phenoxyl radicals (Hofrichter 1999). The $Mn^{3+}$ forms complex with chelating organic acids resulting in products such as oxalates or malates (Makela et al. 2002). The redox potential of the Mn peroxidase system is lower than that of lignin peroxidase, and it has shown capacity for preferable oxidation of phenolic substrates. On the other hand, studies indicate that contrary to LiP, MnP may oxidize Mn (II) without $H_2O_2$ with decomposition of acids and concomitant production of peroxyl radicals that may affect lignin structure. Versatile peroxidase (VP) enzymes produced by *Pleurotus* spp. are also able to oxidize phenolic compounds and dyes efficiently that are substrates of generic peroxidases and related peroxidases or the well-known horseradish peroxidase (HRP). VP (EC 1.11.1.16) oxidizes $Mn^{2+}$, similar to MnP, and have a high redox potential aromatic as LiP enzymes. Due to these qualities, interest in VP has increased during the last years (Martínez et al. 2009).

### 6.1.3 Laccases (EC 1.10.3.2)

Laccases (EC 1.10.3.2) belong to a multicopper oxidase family (Alcalde 2007), present in bacteria, e.g., *Azospirillum lipoferum*, *Actinomycetes* like *Streptomyces*, fungi, plants and insects (Baldrian 2006; Chandra and Chowdhary 2015). This enzyme had been reported more than a hundred years ago (Desai and Nityanand 2011), but the significance and broad studies over the role of this enzyme in wood degradation have been conducted in the last few decades. However, many laccases were reported from fungi, and most biotechnologically useful laccases also originated from fungus (Kalmis et al. 2008).

## 7 Challenges

In spite of considerable progress made in the study of the biodegradation of PAHs over the past few decades, removal of petroleum hydrocarbons in the environment is a daunting problem of the real world. Advancement in various approaches such as genomics, proteomics and metabolomic study has contributed immensely in understanding the PAH-degrading microorganisms and the biochemistry involved in the degradation pathways; however, challenges are posed by various aspects of PAH bioremediation which are either unknown or insufficient information is available regarding them.

Little or no information is available related to genes, enzymes and molecular mechanism of PAH degradation in high-salt environments or low-oxygen and anaerobic environments. Scarce data and research are there on the transmembrane trafficking of PAHs and their metabolites; no transporter molecule/protein has been characterized till date with specific role in the transport of PAHs into microorganisms.

Thorough understanding of genetic regulation of the pathways involved in PAH degradation by different bacteria and fungi has been used for efficient biotransformation or metabolism of PAH pollutants in recent past; a deeper understanding of the microorganism-mediated mechanisms of PAH catalysis will enable strategizing novel methods to enhance the bioremediation of PAHs in the environment.

The use of genetically modified organism in bioremediation represents a research frontier with broad implications to improve the degradation of hazardous wastes under laboratory conditions. The potential benefits of using genetically modified microorganisms are significant. Combining genetic engineering tools such as gene conversion, gene duplication and mutation, enzyme overexpression and novel strains can be produced with desirable properties for effective bioremediation applications. Ecological and environmental concerns and regulatory constraints pose major obstacles and challenges for testing genetically modified organism in the field. These problems must be solved before a genetically modified organism can provide an effective, safer and more efficient method than the present alternatives for removal process at very low-cost and eco-friendly way.

# 8 Conclusion

The present status of work done on biodegradation of biologically toxic PAHs using different microorganism has been reviewed. The environmental toxicity and persistence of PAHs have resulted in several laboratory-based experiments to change these substances into less unsafe/nondangerous substances with the use of microorganisms in the process called as biodegradation. Removal of PAHs from the affected environment is a tough job. Therefore, it is very essential to understand the mechanism of several degradation processes. Degradation of PAHs remains affected by numerous factors, which need to be addressed and explored. Biological approaches appear be the most efficient, cost-effective and eco-friendly method to mitigate/remove PAHs from affected area.

**Acknowledgement** We gratefully acknowledge TEQIP-II and G.B. Pant Engineering College, Pauri, Garhwal, for financial supports and providing other facilities.

# References

Ahirwae S, Dehariya K (2013) Isolation and characterization of hydrocarbon degrading microorganism from petroleum oil contaminated soil sites. Bull Environ Sci res 2(4):5–10

Al-Baldawi IA, Abdullah SRS, Anuar N et al (2015) Phytodegradation of total petroleum hydrocarbon (TPH) in diesel-contaminated water using Scirpus grossus. Ecol Eng 74:463–473

Alcalde M (2007) Laccase: biological functions, molecular structure and industrial applications. In: Polaina J, Maccabe AP (eds) Industrial enzymes: structure, function and applications, vol 26. Springer, Netherlands, pp 461–476

Ambrosoli R, Petruzzelli L, Luis Minati J et al (2005) Anaerobic PAH degradation in soil by a mixed bacterial consortium under denitrifying conditions. Chemosphere 60(9):1231–1236

Arulazhagan P, Vasudevan N (2011) Role of nutrients in the utilization of polycyclic aromatic hydrocarbons by halotolerant bacterial strain. J Environ Sci 23(2):282–287

Baborová P, Möder M, Baldrian P et al (2006) Purification of a new manganese peroxidase of the white-rot fungus Irpex lacteus, and degradation of polycyclic aromatic hydrocarbons by the enzyme. Res Microbiol 157(3):248–253

Baldrian P (2006) Fungal laccases occurrence and properties. FEMS Microbiol Rev 30:215–242

Bamforth SM, Singleto I (2005) Bioremediation of polycyclic aromatic hydrocarbons: current knowledge and future directions. J Chem Technol Biotechnol 80:723–736

Bennet JW, Wunch KG, Faison BD (2002) Use of fungi biodegradation. Manual of environmental microbiology.2nd edn. ASM Press, Washington, DC, pp 960–971

Bharagava RN, Chandra R (2010) Biodegradation of the major color containing compounds in distillery wastewater by an aerobic bacterial culture and characterization of their metabolites. Biodegradation J 21:703–711

Bharagava RN, Chandra R, Rai V (2009) Isolation and characterization of aerobic bacteria capable of the degradation of synthetic and natural melanoidins from distillery wastewater. World J Microbiol Biotechnol 25:737–744

Bharagava RN, Chowdhary P, Saxena G (2017) Bioremediation: an eco-sustainable green technology, its applications and limitations. In: Bharagava RN (ed) Environmental pollutants and their bioremediation approaches. CRC Press, Taylor & Francis Group, Boca Raton, pp 1–22

Borde X, Guieysse B, Delgado O et al (2003) Synergistic relationships in algal-bacterial microcosms for the treatment of aromatic pollutants. Bioresour Technol 86(3):293–300

Boyd DR, Sharma ND, Hempenstall F et al (1999) Bis-cis-Dihydrodiols: a new class of metabolites from biphenyl dioxygenase catalyzed sequential asymmetric cis-dihydroxylation of polycyclic arenas and heteroarenes. J Organomet Chem 64:4005–4011

Cajthaml T, Moder M, Kacer P et al (2002) Study of fungal degradation products of polycyclic aromatic hydrocarbons using gas chromatography with ion trap mass spectrometry detection. J Chromatogr A 974:213–222

Caldini G, Cenci G, Manenti R et al (1995) The ability of an environmental isolate of Pseudomonas fluorescens to utilize chrysene and other four-ring polynuclear aromatic hydrocarbons. Appl Microbiol Biotechnol 44(1):225–229

Cerniglia CE, Gibson DT (1977) Metabolism of naphthalene by Cunninghamella elegans. Appl Environ Microbiol 34:363–370

Cerniglia CE, Sutherland JB (2010) Degradation of polycyclic aromatic hydrocarbons by fungi. In: Timmis KN (ed) Handbook of hydrocarbon and lipid microbiology. Springer, Berlin, pp 2079–2110

Chan SMN, Luan T, Wong MH et al (2006) Removal and biodegradation of polycyclic aromatic hydrocarbons by Selenastrum capricornutum. Environ Toxicol Chem 25:1772–1779

Chandra R, Chowdhary P (2015) Properties of bacterial laccases and their application in bioremediation of industrial wastes. Environ Sci Process Impacts 17:326–342

Chandra R, Bharagava RN, Kapley A, Purohit JH (2011) Bacterial diversity, organic pollutants and their metabolites in two aeration lagoons of common effluent treatment plant during the degradation and detoxification of tannery wastewater. Bioresour Technol 102:2333–2341

Chandra R, Bharagava RN, Kapley A, Purohit HJ (2012) Characterization of *Phragmites communis* rhizosphere bacterial communities and metabolic products during the two stage sequential treatment of post methanated distillery effluent by bacteria and wetland plants. Bioresour Technol 103:78–86

Chang BV, Shiung LC, Yuan SY (2002) Anaerobic biodegradation of polycyclic aromatic hydrocarbons in soil. Chemosphere 48:717–724

Chen YC, Banks MK, Schwab AP (2003) Pyrene degradation in the rhizosphere of tall fescue (*Festuca arundinacea*) and switch grass (*Panicum virgatum*). Environ Sci Technol 37(24):5778–5782

Chen Y, Xie XG, Ren CG et al (2013) Degradation of N-heterocyclic indole by a novel endophytic fungus Phomopsis liquidambari. Bioresour Technol 129:568–574

Chowdhary P, Yadav A, Kaithwas G, Bharagava RN (2017a) Distillery wastewater: a major source of environmental pollution and its biological treatment for environmental safety. Green technologies and environmental sustainability. Springer International, Cham, pp 409–435

Chowdhary P, More N, Raj A, Bharagava RN (2017b) Characterization and identification of bacterial pathogens from treated tannery wastewater. Microbiol Res Int 5:30–36

Chowdhary P, Raj A, Bharagava RN (2018) Environmental pollution and health hazards from distillery wastewater and treatment approaches to combat the environmental threats: a review. Chemosphere 194:229–246

Chulalaksananukul S, Gadd GM, Sangvanich P et al (2006) Biodegradation of benzo(a) pyrene by a newly isolated Fusarium sp. FEMS Microbiol Lett 262(1):99–106

Clemente AR, Anazawa TA, Durrant LR (2001) Biodegradation of polycyclic aromatic hydrocarbons by soil fungi. Braz J Microbiol 32(4):255–261

Covino S, Svobodova K, Kresinova Z et al (2010) In vivo and in vitro polycyclic aromatic hydrocarbons degradation by Lentinus (Panus) tigrinus CBS 577.79. Bioresour Technol 101(9):3004–3012

Das N, Chandran P (2011) Microbial degradation of petroleum hydrocarbon contaminants: an overview. Biotechnol Res Int 2011 Article ID 941810, pp 13

Dean-Ross D, Moody J, Cerniglia CE (2002) Utilization of mixtures of polycyclic aromatic hydrocarbons by bacteria isolated from contaminated sediment. FEMS Microbiol Ecol 41:1–7

Desai SS, Nityanand C (2011) Microbial laccases and their applications: a review. Asian J Biotechnol 3(2):98–124

Dua M, Singh A, Sethunathan N et al (2002) Biotechnology and bioremediation: successes and limitations. Appl Microbiol Biotechnol 59(2–3):143–152

Dzantor EK, Chekol T, Vough L (2000) Feasibility of using forage grasses and legumes for phytoremediation of organic pollutants. J Environ Sci Health A 35(9):1645–1661

Eggert C, Temp U, Eriksson KE (1996) The ligninolytic system of the white rot fungus Pycnoporus cinnabarinus: purification and characterization of the laccase. Appl Environ Microbiol 62(4):1151–1158

Eisenman HC, Mues M, Weber SE et al (2007) Cryptococcus neoformans laccase catalyses melanin synthesis from both D-and L-DOPA. Microbiology 153(12):3954–3962

El A, Haleem D, Al-Thani RF et al (2009) Isolation and characterization of polyaromatic hydrocarbons-degrading bacteria from different Qatari soils. Afr J Microbiol Res 3:761–766

Epelde L, Mijangos I, Becenil J et al (2009) Soil microbial community as bio-indicator of the recovery of soil functioning derived from metal phytoextraction with sorghum. Soil Biol Biochem 41:1788–1794

Erden E, Ucar CM, Gezer T et al (2009) Screening for ligninolytic enzymes from autochthonous fungi and applications for decolorization of remazole marine blue. Braz J Microbiol 40(2):346–353

Fernando Bautista L, Sanz R, Carmen Molina M et al (2009) Effect of different non-ionic surfactants on the biodegradation of PAHs by diverse aerobic bacteria. Int Biodeterior Biodegrad 63(7):913–922

Francesc X, Boldu P, Kuhn A et al (2001) Isolation and characterization of fungi growing on volatile aromatic hydrocarbons as their sole carbon and energy source. Mycol Res 105(4):477–484

Gratia E, Weekers F, Margesin R et al (2006) Selection of a cold-adopted bacterium for bioremediation of wastewater at low temperature. Extremophiles 13:763–768

Grishchenkov VG, Townsend RT, McDonald TJ et al (2000) Degradation of petroleum hydrocarbons by facultative anaerobic bacteria under aerobic and anaerobic conditions. Process Biochem 35(9):889–896

Hadibarata T, Tachibana S, Itoh K (2009) Biodegradation of chrysene, an aromatic hydrocarbon by Polyporus sp. S133 in liquid medium. J Hazard Mater 164(2–3):911–917

Haeseler F, Blanchet D, Werner P et al (2001) Ecotoxicological characterization of metabolites produced during PAH biodegradation in contaminated soils. In: Magar VS, Johnson G, Ong

SK, Leeson A (eds) Bioremediation of energetics phenolics and polycyclic aromatic hydrocarbons, vol 6(3). Batelle Press, San Diego, pp 227–234, 313 pp
Hamamura N, Ward DM, Inskeep WP (2013) Effects of petroleum mixture types on soil bacterial population dynamics associated with the biodegradation of hydrocarbons in soil environments. FEMS Microbiol Ecol 85:168–178
Hammel KE (1995) Mechanisms for polycyclic aromatic hydrocarbon degradation by lignolytic fungi. Environ Health Perspect 103:41–43
Hammel KE, Cullen D (2008) Role of fungal peroxidases in biological ligninolysis. Curr Opin Plant Biol 11(3):349–355
Haritash AK, Kaushik CP (2009) Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): a review. J Hazard Mater 169(1–3):1–15
Harms H, Schlosser D, Wick LY (2011) Untapped potential: exploiting fungi in bioremediation of hazardous chemicals. Nat Rev Microbiol 9(3):177–192
Hernández RL, González-Franco AC, Crawford DL et al (2008) Review of environmental organopollutants degradation by white-rot basidiomycete mushrooms. Tecnociencia Chihuahua 2(1):32–39
Higuchi T (2004) Microbial degradation of lignin: role of lignin peroxidase, manganese peroxidase, and laccase. Proc Jpn Acad, Ser B 80(5):204–214
Hofrichhter M, Vares T, Kalsi M et al (1999) Production of manganese peroxidase and organic acids and mineralization of 14C-labelled lignin (14C-DHP) during solid state fermentation of wheat straw with the white rot fungus Nematoloma forwardii. Appl Environ Microbiol 65(5):1864–1870
Hofrichter M (2002) Review: lignin conversion by manganese peroxidase (MnP). Enzym Microb Technol 30:454–466
Hong YW, Yuan DX, Lin QM et al (2008) Accumulation and biodegradation of phenanthrene and fluoranthene by the algae enriched from a mangrove aquatic ecosystem. Mar Pollut Bull 56(8):1400–1405
Husain Q (2006) Potential applications of the oxidoreductive enzymes in the decolorization and detoxification of textile and other synthetic dyes from polluted water: a review. Crit Rev Biotechnol 26:201–221
Juhasz AL, Naidu R (2000) Bioremediation of high molecular weight polycyclic aromatic hydrocarbons: a review of the microbial degradation of benzo[a]pyrene. Int Biodeterior Biodegrad 45:57–88
Kafilzadeh F, Sahragard P, Jamali H et al (2011) Isolation and identification of hydrocarbons degrading bacteria in soil around Shiraz Refinery. Afr J Microbiol Res 4(19):3084–3089
Kalmis E, Yasa I, Kalyoncu F et al (2008) Ligninolytic enzyme activities in mycelium of some wild and commercial mushrooms. Afr J Biotechnol 7(23):4314–4320
Kalyani DC, Patil PS, Jadhav JP et al (2008) Biodegradation of reactive textile dye red BLI by an isolated bacterium Pseudomonas sp.SUK1. Bioresour Technol 99(11):4635–4641
Kang Z, Buchenauer H (2000) Ultra structural and cytochemical studies on cellulose, xylan and pectin degradation in wheat spikes infected by Fusarium culmorum. J Phytopathol 148(5):263–275
Ke L, Luo LJ, Wang P, Luan TG, Tam NFY (2010) Effects of metals on biosorption and biodegradation of mixed polycyclic aromatic hydrocarbons by a freshwater green alga Selenastrum capricornutum. Bioresour Technol 101:6950–6961
Kumar G, Singla R, Kumar R (2010) Plasmid associated anthracene degradation by pseudomonas sp. isolated from filling station site. Nat Sci 8(4):89–94
Langfelder K, Streibel M, Jahn B et al (2003) Biosynthesis of fungal melanins and their importance for human pathogenic fungi. Fungal Genet Biol 38(2):143–158
Lau KL, Tsang YY, Chiu SW (2003) Use of spent mushroom compost to bioremediate PAH-contaminated samples. Chemosphere 52(9):1539–1546
Lei AP, Hu ZL, Wong YS et al (2007) Removal of fluoranthene and pyrene by different microalgal species. Bioresour Technol 98(2):273–280

Levinson W, Stormo K, Tao H et al (1994) Hazardous waste clean-up and treatment with encapsulated or entrapped microorganisms. In: Chaudry GR (ed) Biological degradation and bioremediation of toxic chemicals. Chapman and Hall, London, pp 455–469

Li JL, Chen BH (2009) Effects of non-ionic surfactants on biodegradation of phenanthrene by marine bacteria of Neptunomnas naphthovorans. J Hazard Mater 162(1):66–73

Lu L, Zhao M, Wang T (2012) Characterization and dye decolorization ability of an alkaline resistant and organic solvents tolerant laccase from Bacillus licheniformis LS04. Bioresour Technol 115:35–40

Lundstedt S, Persson Y, Oberg LG (2006) Transformation of PAHs during ethanol- Fenton treatment of an aged gasworks soil. Chemosphere 65:1288–1294

Makela M, Galkin S, Hatakka A et al (2002) Production of organic acids and oxalate decarboxylase in lignin-degrading white rot fungi. Enzym Microb Technol 30(4):542–549

Marco-Urrea E, Pérez-Trujillo M, Vicent T et al (2009) Ability of white-rot fungi to remove selected pharmaceuticals and identification of degradation products of ibuprofen by Trametes versicolor. Chemosphere 74(6):765–772

Martínez AT (2002) Molecular biology and structure-function of lignin degrading heme peroxidases. Enzym Microb Technol 30(4):425–444

Martínez AT, Speranza M, Ruiz-Dueñas FJ et al (2005) Biodegradation of lignocellulosics: microbial chemical and enzymatic aspects of the fungal attack of lignin. Int Microbiol 8(3):195–204

Martínez AT, Ruiz-dueñas FJ, Martínez MJ et al (2009) Enzymatic delignification of plant cell wall: from nature to mill. Curr Opin Biotechnol 20(3):348–357

McCutcheon SC, Schnoor JL (2003) Phytoremediation: transformation and control of contaminants. Wiley-Inter Science, Hoboken, p 987

Mohamed I, Ali A, Khalil NM et al (2012) Biodegradation of some polycyclic aromatic hydrocarbons by Aspergillus terreus. Afr J Microbiol Res 6(16):3783–3790

Morehead NR, Eadie BJ, Lake B et al (1986) The sorption of PAH onto dissolved organic matter in Lake Michigan waters. Chemosphere 15:403–412. https://doi.org/10.1016/0045-6535(86)90534-5

Mostafa MES, Ghareib MM, Abou-EL-Souod GW (2012) Biodegradation of phenolic and polycyclic aromatic compounds by some algae and cyanobacteria. J Bioremed Biodegr 3(1):1–9

Mrozik A, Piotrowska-Seget Z, Labuzek S (2003) Bacterial degradation and bioremediation of polycyclic aromatic hydrocarbons. Pol J Environ Stud 12(1):15–25

Mtui G, Nakamura Y (2004) Lignin-degrading enzymes from mycelial cultures of basidiomycete fungi isolated in Tanzania. J Chem Eng Jpn 37(1):113–118

Mulla SI, Ameen F, Tallur PN, Bharagava RN, Bangeppagari M, Eqani SAMAS, Bagewadi ZK, Mahadevan GD, Yu CP, Ninnekar HZ (2017) Aerobic degradation of fenvalerate by a Gram-positive bacterium *Bacillus flexus* strain XJU-4. 3 Biotech 7:320–328

Muthusamy K, Gopalakrishnan S, Ravi TK, Sivachidambaram P (2008) Biosurfactants: properties, commercial production and application. Current Science 94:736–747

Nagai M, Kawata M, Watanabe H et al (2003) Important role of fungal intracellular laccase for melanin synthesis: purification and characterization of an intracellular laccase from Lentinula edodes fruit bodies. Microbiology 149(9):2455–2462

Ndimele PE, Oni AJ, Jibuike CC (2010) Comparative toxicity of crude oil-plus dispersant to Tilapia guineensis. Res J Environ Toxicol 4(1):13–22

Neelofur M, Shyam PV, Mahesh M (2014) Enhance the biodegradation of anthracene by mutation from bacillus species. BioMed Res 1(1)

Nesterenko MA, Kirzhner F, Zimmels Y et al (2012) Eichhornia crassipes capability to remove naphthalene from waste water in the absence of bacteria. Chemosphere 87(10):1186–1191

Newman L, Reynolds C (2004) Phytodegradation of organic compounds. Curr Opin Biotechnol 15:225–230

Okai M, Kihara I, Yokoyama Y et al (2015) Isolation and characterization of benzo[*a*]pyrene degrading bacteria from the Tokyo bay area and Tama river in Japan. FEMS Microbiol Lett 362 fnv143 362(18):1–7

Osono T, Hirose D (2011) Colonization and lignin decomposition of pine needle litter by Lophodermium pinastri. Forest Pathol 41:156–162

Oyadomari M, Shinohara H, Johjima T et al (2003) Electrochemical characterization of lignin peroxidase from the white-rot basidiomycete Phanerochaete chrysosporium. J Mol Catal B Enzym 21(4–6):291–297

Parrish ZD, Banks MK, Schwab AP (2004) Effectiveness of phytoremediation as a secondary treatment for polycyclic aromatic hydrocarbons (PAHs) in composted soil. Int J Phytomediation 6:119–137

Piontek K, Smith AT, Blodig W (2001) Lignin peroxidase structure and function. Biochem Soc Trans 29(2):111–116

Quan X, Tang Q, He M et al (2009) Biodegradation of polycyclic aromatic hydrocarbons in sediments from the Dalian River watershed, China. J Environ Sci 21:865–871

Ranocha P, Chabannes M, Chamayou S et al (2002) Laccase down-regulation causes alterations in phenolic metabolism and cell wall structure in poplar. Plant Physiol 129(1):145–155

Reisen F, Arey J (2002) Reactions of hydroxyl radicals and ozone with acenaphthene and acenaphthylene. Environ Sci Technol 36:4302–4311

Ross DD, Moody J, Cerniglia CE (2002) Utilization of mixtures of polycyclic aromatic hydrocarbons by bacteria isolated from contaminated sediment. FEMS Microbiol Eco 41(1):1–7

Russell JR, Huang J, Anand P et al (2011) Biodegradation of polyester polyurethane by endophytic fungi. Appl Environ Microbiol 77:6076–6084

Saxena G, Bharagava RN (2017). Organic and inorganic pollutants in industrial wastes, their ecotoxicological effects, health hazards and bioremediation approaches, Bharagava RN Environmental pollutants and their bioremediation approaches. CRC Press, Taylor & Francis Group, Boca Raton (9781138628892)

Singh A, Ward OP (2004) Biodegradation and bioremediation. Series: Soil Biology, vol 2. Springer-Verlag, New York, p 310

Stolz A (2001) Basic and applied aspects in the microbial degradation of azo dyes. Appl Microbiol Biotechnol 56(1–2):69–80

Thomson ISI, Ndimele PE (2010) A review on phytoremediation of petroleum hydrocarbon. Pak J Biol Sci 13(15):715–722

Ukiwe LN, Egereonu UU, Njoku PC et al (2013) Polycyclic aromatic hydrocarbons degradation techniques: a review. Int J Chem 5(4):43–45

Uzoamaka GO, Floretta T, Florence MO (2009) Hydrocarbon degradation potentials of indigenous fungal isolates from petroleum contaminated soils. J Phy Nat Sci 3:1–6

Venkatesagowda B, Ponugupaty E, Barbosa AM (2012) Diversity of plant oil seed-associated fungi isolated from seven oil – bearing seeds and their potential for the production of lipolytic enzymes. World J Microbiol Biotechnol 28:71–80

Walker JD, Colwell RR, Vaituzis Z et al (1975) Petroleum-degrading a chlorophyllous algae Prototheca zopfi. Nature 254:423–424

Walter U, Beyer M, Klein J et al (1991) Degradation of pyrene by Rhodococcus sp. UW1. Appl Microbiol Biotechnol 34:671–676

Wang XC, Zhao HM (2007) Uptake and biodegradation of polycyclic aromatic hydrocarbons by marine seaweed. J Coast Res 50:1056–1061

Ward OP, Singh A, Van Hamme J (2003) Accelerated biodegradation of petroleum hydrocarbon waste. J Ind Microbiol Biotechnol 30(5):260–270

Warshawsky D, Radike M, Jayasimhulu K et al (1988) Metabolism of benzo[a]pyrene by a dioxygenase system of freshwater green alga Selenastrum capricornutum. Biochem Biophys Res Commun 152:540–544

Warshawsky D, La Dow K, Schneider J (2007) Enhanced degradation of benzo[*a*]pyrene by *Mycobacterium* sp. in conjunction with green algae. Chemosphere 69(3):500–506

Wesenberg D, Kyriakides I, Aghatos SN (2003) White-rot fungi and their enzymes for the treatment of industrial dye effluents. Biotechnol Adv 22:151–187

Wu YR, He TT, Lun JS et al (2009) Removal of benzo[a]pyrene by a fungus Aspergillus sp. BAP14. World J Microbiol Biotechnol 25(8):1395–1401

Xue W, Warshawsky D (2005) Metabolic activation of polycyclic and heterocyclic aromatic hydrocarbons and DNA damage: a review. Toxicol Appl Pharmacol 206:73–93. https://doi.org/10.1016/j.taap.2004.11.006

Yadav A, Chowdhary P, Kaithwas G, Bharagava RN (2017) Toxic metals in environment, threats on ecosystem and bioremediation approaches in: handbook of metal-microbe interactions and bioremediation. In: Das S, Dash HR (eds). CRC Press, Taylor & Francis Group, Boca Raton, pp 813–841

Yakimov MM, Timmis KN, Golyshin PN (2007) Obligate oil-degrading marine bacteria. Curr Opin Biotechnol 18(3):257–266

Yuan SY, Chang JS, Yen JH et al (2001) Biodegradation of phenanthrene in river sediment. Chemosphere 43(3):273–278

Zeyaullah MD, Atif M, Islam B et al (2009) Bioremediation: a tool for environmental cleaning. Afr J Microbiol Res 36:310–314