

Chapter 12

Marine-Derived Fungi: Promising Candidates for Enhanced Bioremediation



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12.1 Introduction

Extensive use and need for the more novel materials for rapidly increasing industrialization and population explosion in the world has resulted in accelerated degradation of environmental components on a large-scale (Deshmukh et al. 2016). Humans have synthesized various novel components which are proving to be contaminants/pollutants of the natural environment and are becoming a major cause of concern today. Various environmental compartments are being loaded with a large quantum of contaminants and recalcitrant compounds including plastics, petroleum oil products, materials containing heavy metals, etc. These compounds are major pollutants

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found in the marine environments. Marine environment also represents relatively less explored niche with context to fungi which have potential to degrade or transform these pollutants (Prasad 2017, 2018).

Fungi comprise a group of heterotrophic microorganisms which have evolved biologically more precisely biochemically in a diverse manner which has resulted in tremendous metabolic potential of these organisms towards an array of recalcitrant compounds. Fungi have some distinct characteristic features that enable them to be potential degraders (Bennet et al. 2002). Marine fungi have the ability to adapt to high saline conditions and pH extremes compared to terrestrial fungi. The mycelial growth pattern of filamentous fungi benefits them with respect to invasion and colonization of insoluble substrates. Fungi produce a battery of extracellular degradative enzymes that can digest diverse substances enabling easy penetration of the mycelia. The filamentous mycelial growth form provides mechanical adjunct. High surface to cell ratio of filaments of fungi provides more wide contact to the substrate. As most of the fungal degradative enzymes are extracellular, insoluble substrates which cannot cross the cell membrane also can be easily utilized by fungi compared to bacteria. Majority of the fungal degradative enzymes are non-specific or can act upon diverse substrate, thus enabling fungi to metabolize diverse substances. Besides, fungi also play an important role in the treatment of different pollutants by being an important component of biofilm-forming microbial communities.

In addition to their application in hydrocarbons and heavy metals bioremediation, the potential of marine fungi in production of secondary metabolites, biosurfactants, novel enzymes, polysaccharides and polyunsaturated fatty acids has also been well documented (Damare et al. 2012). The pivotal role of marine fungi in biodegradation of various contaminants has been well described in literature by numerous researchers (Bonugli-Santos et al. 2012; Gao et al. 2013; Naranjo-Briceno et al. 2013). The present chapter focuses on the potentials of marine-derived fungi relevant to environmental clean-up including biofilm formation and degradation/transformation of prime hazardous pollutants such as plastics, synthetic and textile dyes, petroleum oil and heavy metal(oid)s.

12.2 Diverse Potentials of Marine-Derived Fungi Relevant to Bioremediation

12.2.1 Marine-Derived Fungi in Biofilm Formation

Fungi do occur ubiquitously in marine environment and even the harshest habitats can be inhabited by many fungal species (Sole et al. 2008; Salamone et al. 2016). Fungi are also an important component of biofilm-forming microbial communities, and they could be potential candidates as bioindicators of ecosystem disturbance (Sole et al. 2008; Salamone et al. 2016).

With adhesion and subsequent differential gene expression as intrinsic and fundamental processes, fungi are regular biofilm-forming biota (Gutiérrez-Correa and Villena 2003; Mitra et al. 2013). Higher transcription of exoglucanase (*exo*) and xylanase (*xynB*) genes in the biofilm cultures than that in the submerged cultures of *Aspergillus niger* ATCC 10864 (Villena et al. 2009; Mitra et al. 2013) suggests significant role of biofilm-forming organisms in bioremediation processes.

Mycobiota in the marine environment may function as secondary producers; they help sustain many of the primary consumers supporting even the artificial reef habitats. Looking to the role of mycobiota in processing of organic matter, it has been suggested that the current marine microbial loop model should consider their important role (Gutierrez et al. 2011; Salamone et al. 2016).

Recently, Salamone et al. (2016) characterized marine biofilm-forming fungal communities associated with artificial reef. From the artificial reef biofilm samples, 295 isolates, belonging to 36 fungal genera, could be cultured. *Aspergillus*, *Aureobasidium*, *Cladosporium*, *Penicillium* and *Trichoderma* were among the most commonly occurring genera in the biofilms. The authors claim the work to be the first ever report on fungal biofilm communities from in situ artificial reef substrates.

Yarrowia lipolytica has been reported to be biotechnologically relevant and to play an important role in the treatment of effluents and hydrocarbons, and also it metabolizes explosives. Biofilm formation by tropical marine *Yarrowia lipolytica* NCIM 3589 on various materials have been examined (Dusane et al. 2008). With different carbon sources, the test isolate exhibited variation in morphology. The authors suggested possible occurrence of highly structured biofilms in diverse ecological niches that are the source for the yeast.

According to Zinjarde et al. (2014), the biofilm-forming capability of *Y. lipolytica* could play an important role under field conditions and in the course of treatment of different pollutants. During bioremediation and in waste treatment processes, occurrence of microbes as biofilms has intrinsic benefits over their planktonic counterparts. The biofilm mode allows cells to be shielded within extracellular polymeric substance leading to better chance of adaptation and endurance during stress period (Singh et al. 2006). For application of yeast for bioremediation, understanding of their morphological traits in presence of hydrocarbons is very important. For field application, the choice of strains entirely growing in yeast form or those developing biofilms may be very useful (Zinjarde et al. 2014).

Mitra et al. (2013) investigated biotransformation of polycyclic aromatic hydrocarbon fluoranthene by intertidally derived fungus *Cunninghamella elegans* under biofilm-based and niche-mimicking conditions. Increased level of cytochrome P450 (CYP) monooxygenase mRNA in the biofilm under the niche-mimicking condition was observed compared to that under submerged conditions that were observed. The most suitable condition for biotransformation of fluoranthene was formation of biofilm on a hydrophobic surface with alternating immersion and emersion in the fluid medium.

12.2.2 *Marine-Derived Fungi in Heavy Metal(oid) Removal*

In natural as well as synthetic environments, microorganisms interact with metals and minerals. Such interactions are important to microbial biomineralization processes (Gadd 2010).

In aquatic environment, heavy metal(oid)s get absorbed by aquatic biota, and such metals are likely to be accumulated in humans via food chain. The consequences of metal(oid) exposure to human vary according to type and concentration of metal(oid)s. For example, health implications including minor headache, allergy, respiratory tract disorder, various cancers, etc. are among the common consequences of metal exposure (Kaufman 1970; Katz and Salem 1993; Costa 2003; Park et al. 2005; Kotas and Stasicka 2000; Vala and Dave 2017; Panseriya et al. 2018). Looking to the devastating effects that metal(oid) may exert, it is mandatory to treat the metal-contaminated waste water before its release into the environment. The treatment methods conventionally being used (e.g. chemical precipitation, electro dialysis, evaporation, ion exchange, liquid extraction, membrane process and reverse osmosis) suffer from one or the other limitations, viz. high-energy requirement, inefficient removal and generation of toxic sludge; besides they are not eco-friendly. Many of them despite being effective are not practicable when used for large-scale applications (Akcil et al. 2015; Deshmukh et al. 2016). Hence, bioremediation has been considered as a favourable alternative (Eccles 1999; Paul 2017; Vala and Dave 2017). As fungi possess unique characteristics including greater growth capability, capacity to propagate in diverse habitats, reach owing to mycelial network, capability to produce a battery of enzymes and accumulate metals, etc., they hold prime importance as promising candidates for bioremediation among microbes (Vala et al. 2004; Deshmukh et al. 2016; Vala and Dave 2017).

Whether having biological role or otherwise, metals in bioavailable form above a threshold concentrations impart toxicity in a number of ways (Gadd 1993, 2007). Most of the mycobiota, however, are tolerant towards metal owing to diverse mechanisms which support their survival and growth even in sites extremely contaminated with metal (Gadd 2007). In contaminated soils, shift in population from unicellular bacteria to streptomycetes to fungal biota has been observed (Chander et al. 2001a, b; Khan and Scullion 2000; Gadd 2007; Vala and Dave 2017). Figure 12.1 portrays various fungal mechanisms involved in tolerance to and detoxification of metals.

Studies pertaining to metal removal from terrestrial environment have been advanced; however, their counterparts from marine environment have comparatively been less explored (Taboski et al. 2005; Vala 2010; Vala and Dave 2017). Fungi, the key components of the inshore microorganisms, routinely encounter ions and complexes of metals in large harbours. Explorations on these microorganisms for removal of metal contaminants are a fascinating area of research (Millward et al. 2001; Hyde et al. 1998; Newell and Barlocher 1993; Vala et al. 2004; Vala 2010; Vala and Dave 2017).

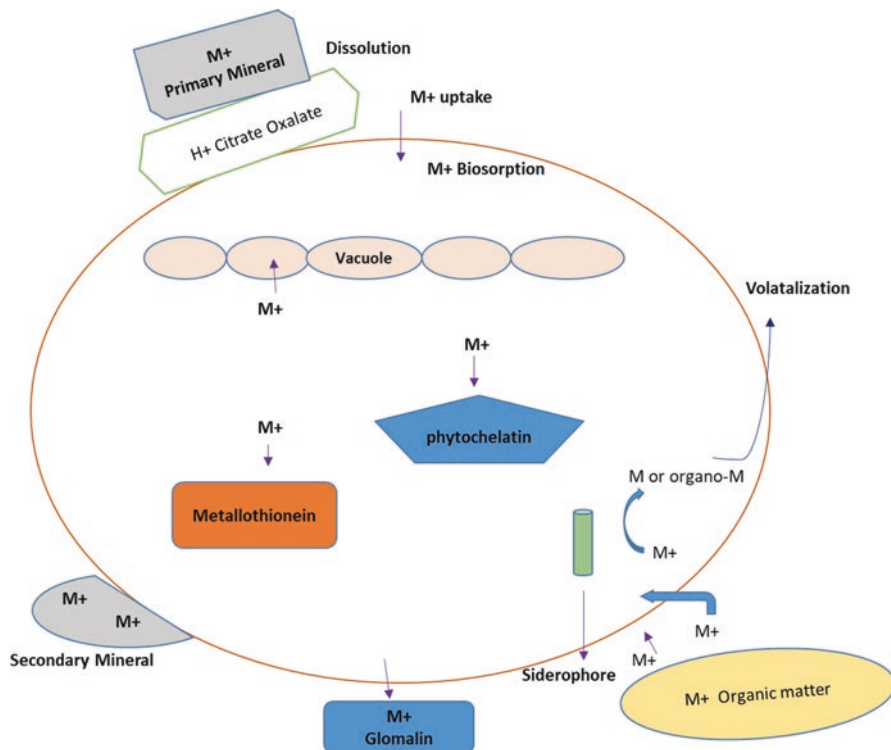


Fig. 12.1 Diverse means of fungal-metal interactions. (Adapted from Harms et al. 2011, Nat Rev. Microbiol 9: 177–192)

Though comparatively less explored, the reports so far available have established the noteworthy role of marine-derived fungi in heavy metal(oid) removal and, hence, substantiate their role as potential bioremediation agents. Marine-derived fungi, viz. *Aspergillus flavus*, *A. niger*, *A. terreus*, *A. wentii* and *Trichoderma viride*, have been observed to tolerate and successfully remove hexavalent chromium, a carcinogen (Vala et al. 2004; El-Kassas and El-Taher 2009; Khambhaty et al. 2009).

Fungi in the marine environment play a noteworthy role in reducing the toxicity of arsenic, a Group A Category 1 human carcinogen (Beldowski et al. 2013; US EPA 1997; International Association for Research on cancer IARC 2004; Vala 2010; Bahar et al. 2013). Marine fungi have been observed to be more tolerant to arsenic than their non-marine counterparts (Irvine and Jones 1975). Marine-derived aspergilli have been envisaged to be significant contributors in tackling the arsenic pollution (Vala 2010, 2018; Vala et al. 2010, 2011; Vala and Patel 2011; Vala and Sutariya 2012). Fungi have been reported playing role in As biosequestration even at seafloor hydrothermal fields (Dekov et al. 2013).

Yeasts from marine environment have also been observed to have bioremediation potential. Strains of marine yeast *Yarrowia lipolytica* have been reported as potential

candidates for chromium removal (Bankar et al. 2009; Rao et al. 2013; Imandi et al. 2014). Among marine yeasts, *Rhodotorula rubra* has been studied in detail for arsenic metabolism (Button et al. 1973; Vidal and Vidal 1980; Cullen and Reimer 1989; Maher and Butler 1988). However, there are no recent reports on arsenic-marine yeast interactions (Vala and Dave 2017). Resting as well as growing cells of mercury resistant *Yarrowia* sp. have been reported to be applicable as a bioremediation agent (Oyetibo et al. 2015). Deep-sea psychrotolerant yeast *Cryptococcus* sp. was observed to tolerate and remove higher concentrations of cadmium, copper, lead and zinc (Singh et al. 2013).

12.2.3 Marine-Derived Fungi and Treatment of Synthetic Dyes and Textile-Dye Effluent

Nearly 10,000 dyes are available commercially (Przystas et al. 2015). Dyeing and succeeding washing processes ultimately lead to release of 10–15% unused dyes into waste water. Such dye-laden waste water upon mixing with large water body weakens primary productivity, impairs diffusion of gases and influences man health besides imparting aesthetically unacceptable coloration (Baughman and Weber 1994; Ciullini et al. 2008; Rodriguez et al. 2015). Various health implications of dye exposure include nausea, ulceration of the skin and mucous membranes, haemorrhage and even severe damage to the kidney, reproductive system, brain, liver and central nervous system. Several synthetic dyes are carcinogenic, toxic and mutagenic (Vala and Dave 2017). Various physico-chemical processes like adsorption, coagulation, flocculation, flotation, precipitation, oxidation and reduction, membrane separation and ozonation are used for treatment of coloured effluents. However, they involve high cost and have drawbacks (Azmi et al. 1998; Robinson et al. 2001). Activated sludge process also is used widely; however, most of the dyes are poorly removed, and when mixed and treated together with sewage, it is inefficient in decolorizing textile effluents (Przystas et al. 2015).

Looking to various health risks due to dye exposure and limitations of most of the conventional treatment methods, it is very important to have a more-efficient and cost-effective alternative for treatment of dye waste water. Biodegradation (mineralization or biotransformation) and adsorption on biomass are the main means of dye removal. Among microbes mycobiota could be an excellent candidate for removal of dye (Przystas et al. 2015). Dye decolorization can be achieved by employing fungal metabolic potential; by oxidative reactions, fungi are able to generate non-toxic derivatives from toxic dyes (Ciullini et al. 2008). Among the battery of enzymes produced by fungi, lignin-degrading enzymes play a very important role in textile dye degradation due to their non-specific nature and efficiency to attack aromatic compounds sharing little similarity with lignin (Field et al. 1992; Arun et al. 2008; Vala and Dave 2017).

Marine-derived fungi due to their adaptability to extreme conditions are better suited for treatment of coloured effluents than their counterparts in the terrestrial environment (Raghukumar et al. 2004; Bonugli-Santos et al. 2015; Vala and Dave 2017). Efficient production of non-specific enzymes like lignin peroxidase, manganese peroxidase and laccase plays an important role in employing such fungi for dye decolorization.

Several marine-derived fungi have been reported to bring about dye degradation successfully, among them basidiomycetous fungi have been observed to be dominating the list. The use of marine-derived fungi for dye degradation involves the use of growing culture, immobilized cells and biosorbent prepared from cultures. *Cerrena unicolor* (D'Souza-Ticlo et al. 2009), *Penicillium janthinellum* (Wang et al. 2015), *Aspergillus niger* (Lu et al. 2016; Joshi et al. 2012), *Flavodon flavus* (Raghukumar et al. 1999; Mtui and Nakamura 2008), *Alternaria tenuissima* (El Aty et al. 2017), *Peniophora* sp. (Bonugli-Santos et al. 2016), *Tinctoporellus* sp. (CBMAI 1061), *Marasmiellus* sp. (CBMAI 1062), and *Peniophora* sp. (CBMAI 1063) (Bonugli-Santos et al. 2012) *Phialophora* sp. (MF 6), *Penicillium* sp. (MF 49), and *Cladosporium* sp. (Torres et al. 2011) are some of the examples of potential dye degraders.

Lalitha et al. (2011) examined bioremediation of synthetic, paper mill and colour photographic dyes using marine *Aspergillus flavus* isolated from Bay of Bengal and observed 80% and 90% removal of synthetic dyes and 100% removal in colour photographic effluents within 3–7 days and 8 days, respectively. During the study, the authors observed a correlation between biomass, sugar used and quality of protein produced. Dye decolorization is affected by availability of nutrients and physical parameters (Singh et al. 2013); dye structure also has been observed to govern the mechanism involving laccase (D'Souza et al. 2006; Bonugli-Santos et al. 2015).

Rapid decolorization and detoxification of an anthraquinone dye Reactive Blue 4 were achieved by the development of a two-step technique involving enzymatic oxidation followed by sorption of degraded products on marine-derived fungal biomass (Verma et al. 2012). Development of such processes would help in reducing time required for decolorization.

12.2.4 Marine-Derived Fungi and Plastic Degradation

Plastics are basically synthetic organic polymers with high molecular mass, and generally these polymers include many types of aliphatic polyesters, including polyhydroxyalkanoates (PHAs), poly(ϵ -caprolactone) (PCL), polyethylene (PE) and poly(l-lactide) (PLA). Some variants of plastics also contain hydrocarbons derived from petroleum feedstock (Law and Thompson 2014). The versatility, durability, low cost and ease of manufacturing of these materials have increased their use over the past three decades and have led to their involvement in almost all aspects of everyday life (Derraik 2002). Our dependence on plastics in our everyday life has also resulted in its ubiquitous presence as waste in various environmental

compartments. Plastic can enter into the marine environment through various anthropogenic sources such as discharge of municipality waste, industrial raw materials, fragments of fishing nets and many more. Plastic pollution in aquatic environment has become a future research priority as it is extremely persistent in marine environment and it has been now recognized as an emerging global threat for social and environmental point of view.

Plastics in marine environment may pose hazardous impacts not only on the marine biota but on the human activities related to marine environment. Plastic debris may either float on the sea surface or may settle down to the seafloor posing deleterious effects on the marine environment. Figure 12.2 shows the potential hazardous impact of plastics on marine biota and humans.

Bacterial degradation of plastic polymers has been well documented; however, there are very few reports on the fungal degradation of plastic polymers and their relative hydrolytic enzymes.

12.2.4.1 Polyhydroxyalkanoates (PHAs)

Polyhydroxyalkanoates (PHAs) are natural polyesters produced intracellularly by numerous bacteria. PHAs are generally divided into two major groups based on the length of the carbon chain as short chain length (SCL)-PHAs having (R)-hydroxyalkanoates of C_3 – C_5 and medium chain length (MCL)-PHAs having aliphatic and/or aromatic (R)-hydroxyalkanoates of C_6 – C_{14} (Kim and Rhee 2003). Many filamentous fungi and yeasts are reported to be the potential degraders of poly(3-hydroxybutyrate) PHB. A list of 95 different genera of PHAs degrading fungi has been listed by Neumeier (1994) which were isolated from soil and marine environments. Out of these 95 genera, majority (97%) was comprised of *Basidiomycotina*, *Deuteromycotina* and *Ascomycotina*. Species of *Aspergillus* and



Fig. 12.2 Impact of plastic pollution on marine biota and sea-based human activities

Penicillium have found to be contributing considerably in PHA degradation (Kim et al. 2000; Sang et al. 2002).

BIOPOL™ is one of the copolymers of PHAs and is produced on a large scale by Imperial Chemical Industries, England, today Zeneca, and is being used as packaging material. Gonda et al. (2000) have studied fungal degradation of BIOPOL™ by a filamentous fungus *Aspergillus ustus* and a yeast *Rhodospiridium sphaerocarpum* under simulated deep-sea environmental conditions. Matavulj and Molitoris (2009) have studied degradation of BIOPOL™ by 134 marine fungal strains. Out of 134 strains, *Debaryomyces hansenii* M-111 and *Fusarium merismoides* M-46 degraded BIOPOL™ when it is supplemented as sole source of carbon and energy. *Asteromyces cruciatus* strain M-1 demonstrated degradation of BIOPOL™ and PHB. However, *Candida guilliermondii* strain M-122 was able to depolymerise only PHB. *Nia vibrissa* strain M-167, fungus belonging to *Basidiomycota*, was able to depolymerize PHB. They have also concluded that the degrading activities are not evenly distributed among the different groups of marine fungi investigated, and their results indicated that the PHA-degrading activity in marine fungi is higher among the *Deuteromycotina* than in other groups.

PHA depolymerases are a class of serine hydrolases enzymes which are produced extracellularly by various microorganisms and are responsible for the degradation of PHAs. Based on the substrate specificity, these enzymes are divided into two major groups as SCL-PHA depolymerases which are able to degrade only PHB and its copolyesters and MCL-PHA depolymerases which act upon the aliphatic and aromatic PHAs consisting of 3-hydroxyalkanoates of C6–C14. PHA depolymerases from fungal origin have some distinct characteristics from bacterial PHA depolymerases. Fungal depolymerases have an acid to neutral pI values, and they are mostly glycoproteic in nature. Fungal PHB depolymerases do not hydrolyse tributyrin, olive oil or p-nitrophenyl palmitate (Kim and Rhee 2003).

12.2.4.2 Polyethylenes (PE)

Polyethylene (PE), comprising of long chains of the monomer ethylene, is a thermoplastic synthetic polymer. The major grades of PE are low-density polyethylene (LDPE) and high-density polyethylene (HDPE). The prime reason behind the resistance of PE to biodegradation is its high hydrophobicity and chemical inertness, and thus the biodegradation of PE is a very slow process. It also lacks functional groups in its structure to be recognizable by microbial enzymatic systems (Hamid 2000).

Kathiresan (2003) has isolated fungi *Aspergillus glaucus* and *Aspergillus niger* from the mangrove soil having potential to degrade polyethylene materials among which *Aspergillus glaucus* degraded 28.80% of polythene and 7.26% of plastics in 1-month period. Pramila and Ramesh (2011) have isolated *Aspergillus versicolor* and *Aspergillus* sp. from seawater and have studied degradation of LDPE in powdered form by these fungal isolates.

As plastic makes its entry into marine environment, various chemical, physical and biological processes result in the formation of microplastic fragments (Caruso

2015; Paço et al. 2017). These microplastics are more harmful than the original macroform because microplastics are mistaken as food by the smaller organisms such as zooplanktons, seabirds, fish, etc. and more readily ingested by them (Caruso 2015). Microplastics are hazardous, not only because they cause physical harm but also due to the adherence and absorbance of contaminants and pollutants on it (da Costa et al. 2016). Barata (2006) has isolated the fungus *Zalerion maritimum* from Mira river salt marsh in Portugal. This fungus was studied for its potential to degrade polyethylene microplastics by Paço et al. (2017). Their results indicated that naturally occurring fungus may actively contribute to the biodegradation of microplastics, requiring minimum nutrients.

Today, the worldwide utilization of plastic has increased greatly, and the production of plastics to satisfy the need of plastic also has increased. Thus, more intensive studies on the fungal degradation of plastics are expected. This will emphasize not only the ecological significance of fungi, but also on the development of more and more sustainable plastic biodegradation strategies.

12.2.5 Marine-Derived Fungi in Petroleum Oil Degradation

Petroleum oil is a complex natural mixture of about 20,000 hydrocarbon and non-hydrocarbon compounds (Fig. 12.3) which, at appropriate concentration, possesses a measurable toxicity towards living systems (Marshall and Rodgers 2004; Al-Nasrawi 2012). These compounds are derived from crude oil during refining process and mainly contain hydrocarbons. Marine oil pollution is among the most portentous problem that the world is brawling today. Microbial degradation is the foremost route for the removal of oil products from contaminated marine environments in the nature especially fungi which have shown higher tolerance to the toxicity of hydrocarbons due to their physiology and adaptation to such variations in the marine environment. Majority of alkanes, asphaltanes and resins are degraded easily by microorganisms, while the hydrocarbon contents are highly recalcitrant and resistant to biodegradation. Compositional heterogeneity among different crude oils

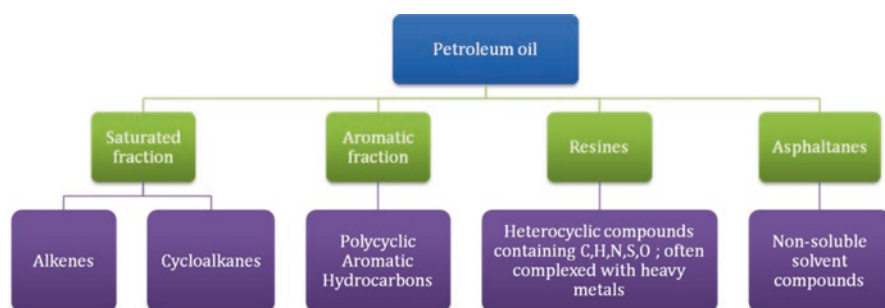


Fig. 12.3 Major components of petroleum oil

and refined products influences the overall rate of biodegradation both of the oil and of its component fractions.

Different components of petroleum oil have different susceptibilities to biodegradation (Goodwin et al. 1983). During oil degradation, the early stages of oil biodegradation includes the loss of n-paraffins (n-alkanes or normal alkanes) followed by the amputation of acyclic isoprenoids. Other components like highly branched and cyclic saturated hydrocarbons as well as aromatic compounds are more resistant to biodegradation compared to other constituents of petroleum.

As most of the hydrocarbon constituents of petroleum oil are hydrophobic, they tend to get adsorb to the marine sediment particles. Thus, marine sediments are one of the desired ecological niches for fungi which can utilize oil hydrocarbons as carbon source. The pivotal role of fungi in degradation of petroleum products has been well reported by many researchers. Commonly studied fungi for petroleum biodegradation belonged to the genera *Alternaria*, *Aspergillus*, *Candida*, *Cephalosporium*, *Cladosporium*, *Fusarium*, *Geotrichum*, *Gliocladium*, *Mucor*, *Paecilomyces*, *Penicillium*, *Pleurotus*, *Polyporus*, *Rhizopus*, *Rhodotorula*, *Saccharomyces*, *Talaromyces* and *Torulopsis* (Saraswathy and Hallberg 2002; Atagana et al. 2006; Adekunle and Adebambo 2007; Gesinde et al. 2008; Husaini et al. 2008; Obire and Anyanwu 2009; Hadibarata and Tachibana 2009a, b; Romero et al. 2010).

Among the hydrocarbon components, polycyclic aromatic hydrocarbons (PAHs) are the major concerned component of petroleum oil which is presently becoming one of the major pollutants of marine environment. Thus, in this chapter, the major focus is given on the petroleum PAH-degrading fungi. PAHs are a group of numerous chemically related organic compounds consisting of two or more than two fused aromatic rings arranged in various structural configurations. These compounds have some unusual physico-chemical properties like high molecular weight, high water/octanol partitioning coefficient ($\log K_{ow}$), high photo-sensitivity, etc., which make these compounds highly persistent in marine environment. Owing to these unusual characteristics, PAHs are proved to be potential carcinogens, mutagens and teratogens to the living organisms (Sachaniya et al. 2018).

Aureobasidium, *Candida*, *Rhodotorula* and *Sporobolomyces* are some of the most common marine isolates which can degrade petroleum oil (Leahy and Colwell 1990). Kirk and Gordon (1988) have studied true marine fungal genera *Corollospora*, *Dendryphiella*, *Lulworthia* and *Varicosporina* for the biodegradation of petroleum oil hydrocarbons. Walker and Colwell (1973) have studied petroleum oil degradation by the fungus *Cladosporium resinae* isolated from Chesapeake Bay and found that this fungus was responsible for 20–40% degradation of petroleum. Petroleum oil hydrocarbon-degrading fungi have also been reported to be important inhabitants of specialized niches like submerged wood (Kirk and Gordon 1988), the surface seawater, decomposing algae and the tarball surface (Ahearn and Crow 1986). Elshafie et al. (2007) have isolated marine fungi from tar balls collected from the beaches of Oman and have found *Aspergillus niger*, *A. terreus* and *Penicillium chrysogenum* to be efficient degraders of n-alkanes and crude oil.

Petroleum hydrocarbon-degrading marine fungi can be broadly divided into two categories as ligninolytic and non-ligninolytic fungi. Both of the categories utilize petroleum hydrocarbon specifically PAHs by their characteristic mechanisms (Fig. 12.4). Ligninolytic fungi are capable of producing enzymes collectively known as lignin-modifying enzymes (LMEs) which include lignin peroxidase (LP), manganese-dependent peroxidase (MnP), laccase, etc. Being non-specific, these enzymes can also be used for the degradation of PAHs. As LMEs are extracellular enzymes, they can degrade PAHs with low bioavailability. The aromatic benzene rings of PAHs are oxidized due to the generation of free hydroxyl radicals by LMEs. This, in turn, results in the formation of PAHs-quinones which by successive ring fission ultimately form CO_2 (Sutherland et al. 1995). PAHs degradation by ligninolytic fungi particularly white rot fungi has been studied intensively during past few years (Hofrichter et al. 1998; Cajthaml et al. 2008). Numerous experiments with LMEs have confirmed the ability of these enzymes to degrade PAHs (Hofrichter et al. 1998). The extent of PAHs degradation varies with variation in LMEs (Clemente et al. 2001). The degradation system of these fungi is inducible. Some of

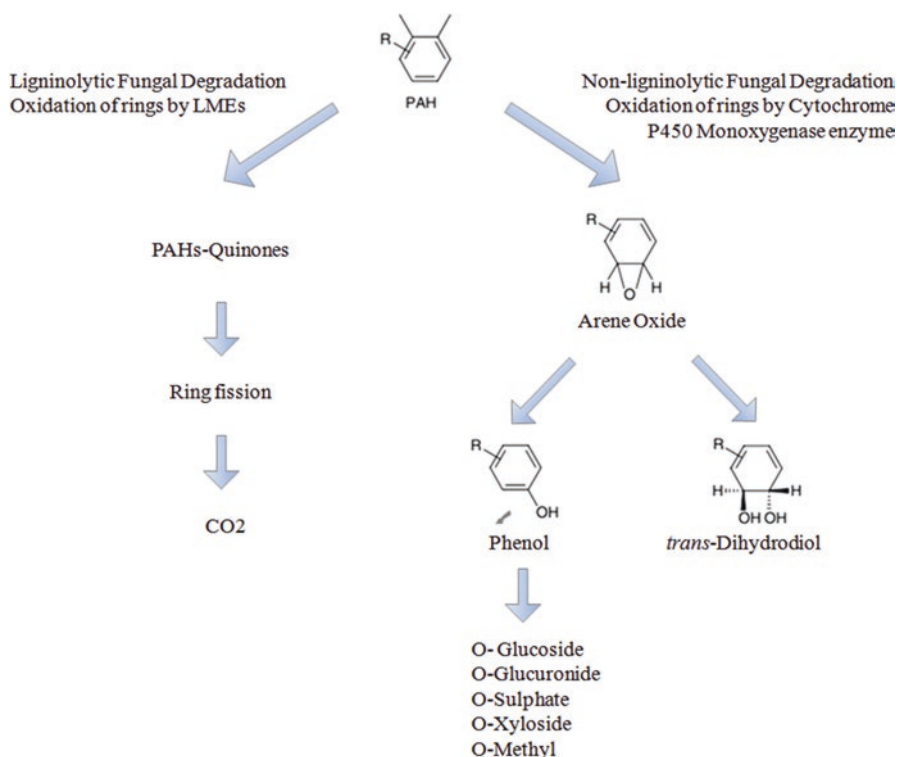


Fig. 12.4 Mechanisms of fungal biodegradation of petroleum PAHs. (Adapted and modified from Bamforth and Singleton 2005)

the most studied ligninolytic fungi for PAHs degradation are *Aspergillus*, *Fusarium*, *Trichoderma*, *Phanerochaete*, *Pleurotus*, *Chlorella*, etc.

Most of the non-ligninolytic fungi cannot completely mineralize PAHs but the transformation products formed by these fungi are comparatively less toxic than parent PAHs. The enzyme, cytochrome P₄₅₀ monooxygenase, first oxidizes the aromatic benzene ring by incorporating only one oxygen atom in the ring to produce arene oxide. This mechanism is similar to the mammalian metabolism of PAHs. The subsequent reaction is then catalysed by epoxide hydrolase to form a *trans*-dihydrodiol (Jerina 1983). Phenol derivatives may also be produced through non-enzymatic rearrangements of the arene oxides. *Chrysosporium pannorum*, *Cunninghamella elegans*, *Aspergillus niger*, etc. are some examples of non-ligninolytic fungi that can degrade PAHs (Bamforth and Singleton 2005). Recently, from our laboratory, Bhatt et al. (2014) have isolated *Cochliobolus lunatus* strain CHR4D, a marine-derived ascomycete fungus from historically contaminated crude oil-polluted shoreline of Alang-Sosiya ship-breaking yard, at Bhavnagar coast, Gujarat. The fungus was capable of efficiently degrading chrysene, a four-ringed high molecular weight (HMW) PAH.

Marine yeasts have also proved to be efficient candidates for petroleum PAHs degradation. Ahearn et al. (1971) have isolated several yeasts from oil-polluted habitats and studied their ability to use hydrocarbons as sole source of carbon. *Trichosporon* species was found to emulsify the oil in their studies. Ahearn and Meyers (1972) have investigated the responses of yeast population to oil pollution in salt marsh in Louisiana. In their study, they have reported that in oil-polluted conditions, the normal predominated species of yeasts were replaced by hydrocarbonoclastic species of yeast mainly including *Pichia ohmeri* and *Trichosporon* sp. *Candida tropicalis* strains 7Y and 15Y are found to be efficient oil degraders by Palittapongarnpim et al. (1998). Zinjarde and Pant (2002) have studied petroleum oil degradation and found that yeasts isolated from marine mud and water around Mumbai were important degraders of the aliphatic fraction of crude oil and all the isolates belonged to the genus *Candida*.

Prior exposure of the marine microbial communities to the petroleum pollution is important in determining the rapidity of biodegradation of the subsequent petroleum component input. This is termed as the adaptation of that particular microbial community towards petroleum pollution (Spain et al. 1980). There are major three interrelated mechanisms for adaptation: (i) induction and/or depression of specific enzymes, (ii) genetic changes which result in new metabolic capabilities and (iii) selective enrichment of organisms able to transform the compound or compounds of interest (Leahy and Colwell 1990).

12.3 Conclusion

Marine-derived fungi exhibiting unique traits, enzymatic features and adaptations to extreme environments form an integral component of marine microbial diversity. Even in artificial reef, they have been observed to be important colonizing community. Marine-derived fungi have been observed to be able to interact with a variety of pollutants including highly recalcitrant ones. By various means including biofilm formation, production of degradative enzymes, etc., these organisms are playing important role in getting rid of hazardous and recalcitrant pollutants. Though several laboratories worldwide have been focusing on these groups of organisms and their involvement in enhanced bioremediation of pollutants, potentials of marine-derived fungi have been comparatively untapped, and they demand attention.

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