Chapter 15
The Role of Fungi and Genes for the Removal of Environmental Contaminants from Water/Wastewater Treatment Plants

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15.1 Fungal Classification

Globally fungi are generally categorized as microfungi and macrofungi according to their fruiting bodies size. The fruiting bodies of macrofungi are visible to the naked eye, due to its diameter of 1 mm (e.g., mushrooms). Furthermore the microfungi cannot be seen with naked eye due to its microscopic fruiting bodies (e.g., *Penicillium*). Reproduction takes place through spore formation. Most fungal spores are different in color and shape, and their size ranges from 2 to 20 lm [22]. Fungi are more basically categorized by their type of reproduction (both asexual and sexual) and the nature of their multicellular or multinucleate hyphal filaments. Traditionally, true fungi are categorized into five taxonomic divisions. The characteristics of each division are given in Table 15.1.

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Table 15.1 Physical characteristics of the most important fungal divisions

Sl. no.	Physical characteristics	Division
1	Common basidiomycetes include mushrooms, puffballs, and toadstools. Visible features of the fungi are the propagative structures. Sexual reproduction includes the basidiospore development on club-shaped cells recognize as basidia	*Basidiomycota*
2	Comprise more than 32,000 species of unicellular (yeasts) to multicellular fungi. Asexual reproduction in yeasts takes place by budding while sexual by forming a sac/ascus	*Ascomycota*
3	Fungi in this division produce zoospores proficient for movement via a fluid medium by means of simple flagella	*Chytridiomycota*
4	Fungi included in this group also lack the main stage (i.e., sexual reproduction) or whose perfect stage is as yet undiscovered. Reproduction most frequently takes place by conidia or conidia-like spores. Various forms of this division are pathogenic in nature affecting humans, wildlife, or floras	*Deuteromycota*
5	In *Zygomycota* the hyphae have long multinucleate cell rather than one nucleus per cell, haploid hyphae that consist of their mycelia. Asexual reproduction takes place by means of spores produced in stalked sporangia	*Zygomycota*

15.2 Water/Wastewater Contamination

The continuous seeking of human toward industrialization and comfort life is leading to the environmental contamination and consequently deterioration of human health.

15.3 Role of Fungi in Wastewater Treatment

Diverse group of microorganisms are present in wastewaters, which become scattered in different phases of the sludge and wastewater management procedure at wastewater treatment plants (WWTPs) (Teixeira et al. 2013). Among the microorganisms, filamentous fungi were detected in sewage and air at WWTPs formerly (Korzeniewskaa et al. 2009). Fungi are eukaryotic organisms which are different in shape and size (Gravesen et al. 1994). They are unicellular like yeast and most of them are filamentous. Some of these microorganisms live as a saprophyte and grow on dead organic materials, and others are either facultative or obligate parasite. Because fungi are heterotrophic microorganisms, they have the ability to break down organic matters by secreting some degradative enzymes (Tran et al. 2013; Haritash and Kaushik 2009). These enzymes can be produced extracellularly or intracellularly to enhance the absorption of organic molecules into the fungal cell. These organic molecules can be used as both energy and carbon sources for the

growth and division of fungal cells (Sankaran et al. 2010). The degradative enzymes are regulated by various groups of catabolic genes.

Among the major problems that faced the bacterial wastewater treatment plant is the generation of large quantities of sludge which is mainly bacterial biomass (Sankaran et al. 2010). The generated sludge is characterized by low value and it requires high-cost for treatment before disposal. The recent strategy suggested to overcome this problem is to cultivate fungi as a source of different valuable biochemicals. So, integration of valuable sources recovery with wastewater remediation may lead to an economically workable solution for wastes management. From this point of view, the use of fungi in wastewater treatment can be an attractive strategy as fungi can utilize the organic waste as a feed to generate economic fungal byproduct with concomitant wastewater remediation (Sankaran et al. 2010). Filamentous fungi offer a diverse prospect. In food industries several filamentous fungi are often cultivated as a source of valuable products such as biochemical and protein with relatively costly substrates such as molasses or starch (Barbesgaard et al. 1992). Mycological cultivation plays an important role in the conversion of wastewater organic materials into readily harvestable mycological biomass, which is further used as a source of animal food and possibly human diet (Guest and Smith 2002). So the use of filamentous fungi is an effective stategy for treatment of highly contaminated wastewater.

Removal and detoxification of pollutants can be achieved by physical, chemical, or biological means (Ryan et al. 2005). However, a biotechnological approach is widely adopted due to its cost-effectiveness, higher efficiency, and generation of nontoxic value-added products. Thus, the fungal process not only offers a solution to wastewater remediation but also provides an opportunity for byproduct recovery (Fountoulakis et al. 2002). One of the several advantages of fungal process is the enzyme-mediated activity that provides solution to the treatment of waste streams containing hazardous or xenobiotic organic pollutants. The enzymes are produced during all phases of the fungal life cycle and are present even at low pollutant concentrations (Ryan et al. 2005). Fungal biomass secretes specific and nonspecific extracellular enzymes that have attracted the attention of researchers working on degradation of complex high-molecular-mass organic pollutants.

15.4 Heavy Metal/Metal Ion Bioremediation

The main source of heavy metal pollution is industrial wastewaters during metal processing as well as other pollutant routes. Any industrial activity using metals has a metal disposal problem (Das et al., 2008) [1]. Nature of heavy metals is persistent and non-biodegradable; therefore, it is very difficult to purify the environmental compartments (soil and water body) from these toxic pollutants.

Heavy metals can be divided into essential metals such as zinc, iron, manganese, and copper and nonessential metals such as lead, mercury, nickel, and cadmium (Grąz et al., 2011) [2]. Cadmium and lead are included among the major pollutants

because of their high toxicity (Salinas et al., 2000, Blaudez et al., 2000, Carrillo-González and González-Chávez, 2012, Jaeckel et al., 2005) [3–6]. The main reasons for release of cadmium to the environment are mine tailing, effluents from textile, tannery, leather, galvanizing and electroplating industries, as well as cadmium batteries. Biomagnification of cadmium in nature and its migration through drinking water, food, and air to human body cause severe health effects like kidney damage, bronchitis, and cancer (Salinas et al., 2000) [3]. So, there is an urgent need to remove these toxic pollutants from the environmental compartments by using effective remediation methods.

Conventional treatment systems have many disadvantages including insufficient metal sequestration, high reagents and/or energy requirements, high costs, and generation of toxic sludge or other waste products that require disposal. Restoring metals in an efficient and economical procedure has necessitated the use of different options in metal-separating methods. Literatures showed that bioaccumulation of metals by organisms has been successful to some extent (Salinas et al. 2000) [3]. Bioremediation of heavy metals from aqueous environment by bacteria, fungi, algae, and plants is the most promising method for complete and safe removal; it is also a cost-effective strategy.

Fungal cell has a great ability to entrap heavy metal ions into its cell structure and subsequently adsorb it on the binding site that located in the fungal cells structure (Brierley 1990; Gadd 1988). This mode of heavy metal uptake is independent on the viability of fungal cells or biological metabolic cycle and is called biosorption or passive uptake. On the other hand, the process of which the heavy metals pass through the cell via cell membrane and participate in the metabolic cycle is so called active uptake or bioaccumulation (Dönmez and Aksu 1999; Malik 2004). The passive uptake (biosorption) of heavy metals is noneffective method for wastewater treatment because they are limited by many factors that decrease the potential of biosorption such as pH, temperature, and the complete saturation of active sites and functional groups of fungal cell structure with heavy metals. So that, the biosorption mode does not achieve any detoxification of heavy metals (Malik 2004). Under such situation, the application of viable microbial cells is a more favorable option for heavy metal removal due to continuous metabolic uptake of metals after physical absorption. After that, the metals diffused into the cells during detoxification get bound to chelating agents or intracellular proteins before being incorporated into vacuoles and other intracellular sites (Malik 2004; Saunders et al. 2001). These processes are often irreversible and ensure less risk of releasing metal back to the environment (Gekeler et al. 1988). The major limitations of active uptake of heavy metal by fungal cells are the requirement of carbon and energy sources, external supplementation, and sensitivity of some fungal strains to high metal/salt concentration. Table 15.2 summarizes the bioremediation of heavy metals by different fungi species.

The walls of fungal biomasses are composed of macromolecules (chitosan, chitin, glucan, phospholipids, lipid), which comprise amino groups (R_2NH, $R\text{-}NH_2$), carboxyl groups (RCOOH), phosphates, melanin, lipids, hydroxides (OH-), and sulfates ($R\text{-}OSO_3$-) (Kapoor et al. 1999). Those functional groups are metal sorption

Table 15.2 Fungi used for heavy metal removal from wastewater and aqueous solution

Heavy metals biosorption/removal	Fungi	References
Cadmium, nickel	*Trichoderma atroviride* strain F6	Babich and Stotzky (1977)
Copper, lead	*Aspergillus niger*, *Trichoderma asperellum*, *Penicillium simplicissimum*	Iskandar et al. (2011)
Cobalt	*Trichoderma*, *Aspergillus*, *Mortierella*, *Paecilomyces*, *Penicillium*, *Pythium*, and *Rhizopus*	Ross and Townsley (1986)
Zinc, lead	*T. harzianum*, *F. phyllophilum*	Ozsoyet al (2008)
Arsenic	*Trichoderma* (FA-06)	Ashida (1965)
Copper, zinc, cadmium	*Trichoderma atroviride*	Tsekova and Todorova (2002)
Zinc	*Trichoderma atroviride*	Yazdani et al. (2010)
Zinc, barium, iron	*Trichoderma atroviride*, *Mortierella exigua*	Karcprzak and Malina (2005)
Copper	*Trichoderma viride*, *Aspergillus niger*, *Penicillium spinulosum*	Delgado et al. (1998), Anand et al. (2006)
Copper, zinc, cadmium	*Trichoderma atroviride*	López Errasquín and Vázquez (2003)
Lead and cadmium	*Aspergillus niger*, *Penicillium*, *Alternaria*, *Rhizopus*, *Monilia*, *Trichoderma*	Zafar et al. (2007)
Chromium (VI)	*Rhizopus arrhizus*	Niyogi et al. (1998)
Copper and zinc	*Aspergillus niger*	Price et al. (2001)
Chromium (VI) and iron (III)	*C. vulgaris* and *R. arrhizus*	Sag et al. (1998)
Lead	*Rhizopus nigricans*	Zhang et al. (1998)
Zinc	*Rhizopus arrhizus*	Zhou (1999)
Copper	*Phanerochaete chrysosporium*	Sing and Yu (1998)
Copper	*Aspergillus niger*	Modak et al. (1996)
Lead	*Aspergillus niger* (strain 4)	Meyer and Wallis (1997)
Lead (II)	*Phellinus badius*	Matheickal and Yu (1997)
Heavy metals	*Aspergillus niger*	Kapoor and Viraraghavan (1998a, b)
Gold and silver	*Aspergillus niger*	Gomes et al. (1998)

sites (Kapoor and Viraraghavan 1997; Javanbakht et al., 2014). Moreover fungi eliminate metals principally by chemisorption (ion exchange), adsorption, complexation, micro-precipitation, chelation, physical adsorption, and coordination (Kapoor and Viraraghavan 1997; Long et al., 2019).

In some filamentous fungi, there are many strategies to overcome the toxicity of heavy metals:

1. Production of oxalate by brown-rot and white-rot fungi. The oxalate secretion process is stimulated under Cu(II) and Cd(II) stress (Clausen and Green 2003; Jarosz-Wilkolazka and Gadd 2003) and leads to the formation of insoluble

metal-oxalate crystals that is thought to prevent toxic metal ions from entering fungal cells (Jarosz-Wilkolazka and Gadd 2003).

2. Production of extracellular mucilaginous materials (ECMM) or bio-emulsifiers with high metal-binding capabilities. Cu(II), Pb(II), and Zn(II) could trigger ECMM production by *Curvularia lunata* (Paraszkiewicz et al. 2009, 2010). Importantly, the pullulan production by *Aureobasidium pullulans* was stimulated by Ni(II) and Cd(II) exposures (Breierová et al. 2004). Additionally, the ratio of ECMM in the *Trametes versicolor* and *Gloeophyllum trabeum* biomass increased when they are exposed to Cu(II) (Vesentini et al. 2006).
3. Production of a soil glycoprotein called glomalin by the arbuscular mycorrhizal fungi *Glomus* and *Gigaspora* species (Wright et al. 1996), which possess a remarkable capability to sequester Cu(II) (Gonzalez-Chavez et al. 2004). Also, chitin and melanin can also take part in metal biosorption (Gonzalez-Chavez et al. 2004).

Glutathione GSH plays a critical role in fungal heavy metal tolerance and oxidative stress defense as well (Jozefczak et al. 2012; Bellion et al. 2006); however, the overexpression of GSH increases the toxic metal/metalloid tolerance (Pócsi 2011). The transgenic plants *Arabidopsis thaliana* could accumulate and tolerate Cd and As when they are stimulated by yeast γ-glutamylcysteine synthetase GSH1 and garlic phytochelatin synthase AsPCS1 (Guo et al. 2008). It is worth noting that recombinant GSH overproducing yeast strains have also been engineered using self-cloning modules containing an intracellular expression vector with GSH1 and GSH2 biosynthesis genes in *Pichia pastoris* (Fei et al. 2009) or the GSH1 gene in *Saccharomyces cerevisiae* (Pócsi 2011; Wang et al. 2009). This approach may be limited particularly with Hg because the overexpression of Hgt1p GSH transporter in *S. cerevisiae* leads to elevation of the intracellular GSH level and subsequently results in the induction of cell toxicity (Pócsi 2011).

The stimulation and overexpression of phytochelatin into the fungal cell could enhance the fungal strain to be more tolerance to metal ion. In addition the heterologous expression of *Saccharomyces pombe*, red alga, higher plant, or nematode phytochelatin synthases provides Cu(II), Cd(II), Sb(III), and As(III) resistance to *S. cerevisiae* (Osaki et al. 2008).

It was mentioned that the low molecular mass metal chelator proteins that are termed metallothioneins exhibit a great affinity toward Zn(II), Cd(II), as well as Cu(II) (Wysocki and Tamás 2010). Furthermore, the overexpression of metallothionein PiMT1 gene in *Piciai volutus* complemented the Cd(II) and Cu(II) hypersensitivity of metallothionein-deficient yeast strains and even enhance the Cu(II) tolerance of the ectomycorrhizal fungus *Hebeloma cylindrosporum* (Bellion et al. 2007).

Cu/Zn-superoxide dismutase (Cu/Zn-SOD) plays an important role in the improvement of metal and oxidative stress tolerance of fungi. This enzyme is mainly expressed by Sod1p gene with co-expressed with the Cu(II)-chaperone Ccs1p (Ferreira et al. 2014). In the absence of either Ccs1p overexpression or high-dose Cu(II) supplementation, the cells showed symptoms of emerging oxidative stress

and shortened chronological life span (Harris et al. 2005). The genetically engineered deep-sea yeast *Cryptococcus liquefaciens* strain N6 exhibited fourfold higher activity compared to Sod1p of baker's yeast *S. cerevisiae* when it was cloned with Cu/Zn-superoxide dismutase (Kanamasa et al. 2007).

In filamentous fungi, intracellular siderophores play a critical role in keeping excess of iron in a thermodynamically inert state (Pócsi 2011). In baker's yeast, iron homeostasis is regulated by the Aft1p and Aft2p transcriptional activators (Pócsi 2011; Johnson 2008).

It was supposed that the major metal/metalloid stress response regulator of baker's yeast when it was exposed to Cd(II), As(III), Sb(III), Se(III), and Hg is the bZIP-typ transcriptional factor Yap1p as well as Yap2p (Wysocki and Tamás 2010; Hirata et al. 1994). According to Azevedo et al. (2007), Yap1p and Yap2p transcription factors share a common Cd(II)-sensing domain. Considering other bZIPs, Yap5p is involved in the regulation of the Fe homeostasis via the regulation of CCC1 encoding the vacuolar iron transporter Ccc1p (Li et al. 2008), and Yap8p plays a pivotal role in the regulation of As(III) detoxification (Haugen et al. 2004; Wysocki et al. 2004).

15.5 Hydrocarbon Bioremediation

Petroleum is a natural resource confined in large deposits in the Earth crust. Accidental petroleum spills alter the impacted environment and trigger the development and implementation of remediation strategies for cleaning up the polluted sites. Oil spills became an international concern in 1967, when ~120,000 tons of crude oil was released by the Torrey Canyon supertanker into the English Channel. This first large-scale oil spill forced UNO's International Maritime Organization to create in 1973 the International Convention for the Prevention of Pollution from Ships MARPOL with the aim of designing emergency protocols and strategies toward oil spills. Since then, there have been a number of significant marine oil spills, even only the emblematic spills usually alert the public opinion. Oil spills are difficult to avoid during petroleum processing and delivery. So, the contamination of water with petroleum hydrocarbon became a serious problem that threaten the biological live.

Petroleum is composed by three main hydrocarbon fractions. Paraffin is usually the most abundant fraction and contains linear and branched aliphatic hydrocarbons. Naphthenes are alicyclic hydrocarbons composed by one or more saturated rings with or without lateral aliphatic branches. The aromatic fraction is composed by hydrocarbons containing at least one aromatic ring. Hydrocarbons can possess from few up to >60 carbons. A higher molecule size correlates with a higher boiling point. Petroleum-derived products are obtained by fractional distillation, by which different fractions are enriched according to its boiling range (Speight 2015).

Many fungal species are known to have the ability to degrade persistent pollutants (Hesham et al. 2017). The majority of studies have been focused on the on

biodegradation of hydrocarbons by white-rot fungi (Hesham et al. 2017; Lee et al. 2014). Fungi have a diversity of tactics to counter with numerous contaminated composites such as persistent organic pollutants (POPs) including polycyclic aromatic hydrocarbons (PAHs) and pesticides (Herzig et al., 2019). These procedures comprise enzymatic practices such biomineralization and bioadsorption as well as biodegradation and biotransformation facilitated by enzymatic structures (Nunes and Malmlöf, 2018). The specific structure of the cell wall such as chitosan or chitin is mediated by bioadsorption (Gadd 2009). In some species of fungi, including *Phoma* sp. UHH 5-1-03, biosorption addicted to fungal mycelia plays a significant role in the removal of 17α-ethinylestradiol, bisphenol A, and triclosan, until reach to the equilibrium (Hofmann and Schlosser 2016).

Polycyclic aromatic hydrocarbons (PAHs) are organic compounds composed of two or more fused benzene rings. These compounds are widely distributed in the environment and formed during the combustion of organic molecules (Kim et al. 2013). PAHs discharged from various activities, such as combustion of fossil fuels including coal, shipping, use and disposal of petroleum products, agricultural burning, and use of wood-preserving products, are persistently hazardous pollutants to the environment (Hadibarata and Teh 2014). Through the industrial wastewater discharges, PAHs are distributed in the marine environment and finally bind to particulate matter of the sediments (Abdel-Shafy and Mansour 2016). Low-molecular-weight (LMW) PAHs (composed by two to three aromatic rings) are predominant in petrogenic sources and can be introduced into water through municipal and urban runoff and oil spills, discharge from tanker operations, etc. High-molecular-weight (HMW) PAHs (composed by four to six aromatic rings), such as pyrene and BaP, are most important in pyrogenic sources and are introduced to the aquatic environment mainly in the form of exhaust and solid residues. These two types of PAH compounds are classified as priority pollutants according to the US Environmental Protection Agency (EPA) and have been accumulating in sediments due to their limited water solubility and high affinity for particulate matter (Cai et al. 2009; Kim et al. 2013).

Once petroleum hydrocarbons reach the aquatic environment, damage can be the result of several causes. Primary biological impact is due to the blocking effect of oil layer to water, nutrients, oxygen, and light access that lead to death of aquatic flora and fauna. Cytotoxic and mutagenic effects of hydrocarbons are behind the long-term pollution consequences (Baboshin and Golovleva 2012).

The main hydrocarbon degraders, are microorganisms which include bacteria, filamentous fungi, and yeasts (van Beilen and Funshoff 2007; Wentzel et al. 2007), are used to overcome low bioavailability of PAHs.

Microorganisms possess evolved mechanisms to activate hydrocarbons, generating metabolic intermediates that funnel to central metabolic pathways. By oxidizing these substrates, microorganisms can take advantage in nutrient-limited niches. Addition of one or two hydroxyl groups to the hydrocarbon skeleton seems to be the ubiquitous first step during aerobic catabolism (Fig. 15.1). So, microbial degradation of hydrocarbons by bacteria, filamentous fungi, and yeasts can be considered as an attractive biotechnological alternative for achieving possible mineralization of

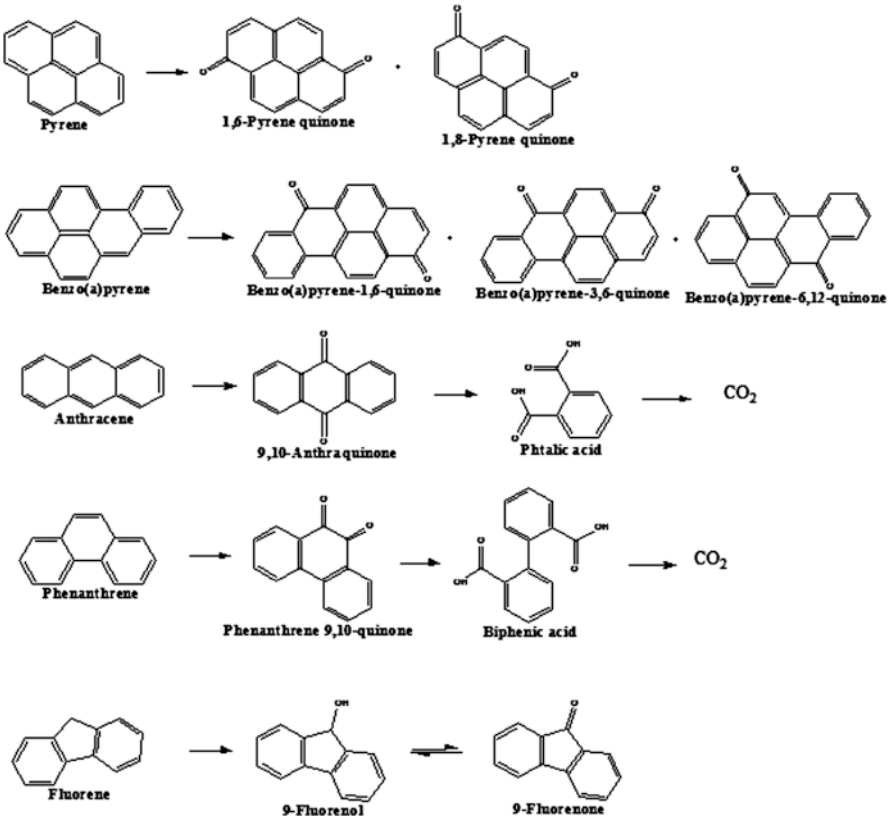

Fig. 15.1 Degradation of polycyclic aromatic hydrocarbons by ligninolytic fungi (Kadri et al. 2017)

pollutant 12 and its transformation into less toxic products with greater solubility in water (Lee et al. 2014; Fuentes et al. 2014).

Many fungi metabolize polycyclic aromatic hydrocarbons with enzymes that include lignin peroxidase, manganese peroxidase, laccase, cytochrome P450, and epoxide hydrolase. The products include trans-dihydrodiols, phenols, quinones, dihydrodiol epoxides, and tetraols, which may be conjugated to form glucuronides, glucosides, xylosides, and sulfates. The fungal metabolites generally are less toxic than the parent hydrocarbons. Cultures of fungi that degrade polycyclic aromatic hydrocarbons may be useful for bioremediation of contaminated soils, sediments, and waters. The following white-rot fungi are the most extensively studied PAH degraders: *Phanerochaete chrysosporium* (Barclay et al. 1995; Brodkorb and Legge 1992), *Pleurotus ostreatus* (Vyas et al. 1994), and *Trametes versicolor* (Vyas et al. 1994; Boonchan et al. 2000). These fungi are able to degrade some five-benzene-ring PAHs and detoxify PAH-polluted soils and sediments due to the production of extracellular lignin-degrading enzymes. Figure 15.1 shows Oxidation of polycyclic

aromatic hydrocarbons by ligninolytic fungi. Nonlignolytic fungi, such as *Cunninghamella elegans*, *Penicillium janthinellum*, and *Syncephalastrum* sp., can transform a variety of PAHs, including pyrene, chrysene, and benzo[a]pyrene, to polar metabolites (Kadri et al. 2017).

The key enzymes in hydrocarbon degradation pathways are oxygenases, which catalyze the addition of molecular oxygen to the substrate (Fuentes et al. 2014). Monooxygenase- and dioxygenase-encoding genes play a key role in hydrocarbon biodegradation by fungi and bacteria. These genes are characterized by their wide phylogenetic distribution as well as high sequence divergence (Iwai et al. 2011; Fuentes et al. 2014); these genes become overexpressed after input of hydrocarbons in the medium or environment. The expression of different ring hydroxylating dioxygenase (RHD) genes increased in the presence of polycyclic aromatic hydrocarbons (PAHs) (Fuentes et al. 2014).

Levels of RHD genes changed significantly in environment during bioremediation and after addition of aromatic hydrocarbons. Additionally, alkane monooxygenase (alk)-encoding genes showed different dynamics at soil and seawater (Yergeau et al. 2012) where alkB gene copy number increased up to 100-fold in less than 1 week after pollution (Sei et al. 2003). Genes encoding enzymes catalyzing downstream reactions seem to behave in a similar way as RHD genes (Hesham et al. 2014). For example, levels of catechol 2,3-dioxygenase *xylE* gene from (methyl) toluene degradation pathway correlate with degradation rates in hydrocarbon-polluted environment. A positive correlation between hydrocarbon degradation rate and functional *alkB*, *xylE*, and *nahAc* gene abundance was observed (Salminen et al. 2008). Therefore, catabolic gene quantification can be an adequate approach for monitoring bioremediation processes (Fuentes et al. 2014).

PAHs could be degraded by fungi with cytochrome P450 (P450) monooxygenases and with soluble extracellular enzymes such as manganese peroxidase, lignin peroxidase, and laccase (Peng et al. 2008). Of these, the CYP52, CYP53, and CYP504 P450s, known to be involved in hydrocarbon degradation (Črešnar and Petrič 2011), had higher relative abundances in cliff sample than river water one. Fungal manganese peroxidases and laccases were detected with similar abundances in both metagenomes (Črešnar and Petrič 2011). Under anoxic conditions aromatic compounds are metabolized through alternate pathways, including fumarate addition,O_2-independent hydroxylation, and carboxylation. Genes for benzylsuccinate synthase (bssABCDE), ethylbenzene dehydrogenase (EB_dh), ATP-dependent class I benzoyl coenzyme A (CoA) reductase (brcABCD), and ATP-independent class II benzoyl-CoA reductase (bamBCDEFGHI) were found in HR_M at low frequencies but not in cliff (Wong et al. 2015).

Hydrocarbon degradation pathways expand the microbial metabolic versatility and the carbon source range for growth. In alkane and aliphatic hydrocarbon degradation, successive oxidations produce carboxylic acids that can be degraded by the β-oxidation pathway. In PAH degradation, metabolic intermediates are channeled into central aromatic routes such as catechol, gentisate, and protocatechuate pathways.

15.6 Antibiotics

Antibiotics are used globally instead of their conventional use in medicine. Antibiotics are commonly used in research experiments, genetic engineering, crop production, aqua culture, animal breeding, and fish farming (Dietze et al. 2005; Yanong 2006). Since the extensive usage of antibiotics, the microorganisms present in the waste have a good opportunity for developing resistance to the antibiotics. Due to the unsuccessful handling, or inappropriate disposal, antibiotics are released into the aquatic ecosystem via wastewater discharge, and as a result sulfamethoxazole, tetracycline, sulfamethazine, trimethoprim, ciprofloxacin, and erythromycin have been noticed in numerous wastewater treatment plants (WWTPs) which release their treated wastes to both ground and surface waters (Karthikeyan and Meyer 2006). Wastewater treatment plants (WWTPs) are one of the major hotspots for spreading antibiotic-resistant microorganisms (Baquero et al. 2008; Manaia et al. 2011). During the biological treatment of wastewater that effluent from variuos sources, the continuous contacting between bacteria and antibiotics (even at very low concentration) leads to increase and spread of antibiotic resistance (Da Silva et al. 2005; García-Galán et al. 2011; Lucas et al. 2016). Antibiotics resistance genes(ARGs) are considered the danger that threaten the public health within the twenty-first century as mentioned by the World Health Organization (WHO) (2014). Few articles have discussed the removal of antibiotics and antibiotics resistance genes (ARGs) from the environment (Gao et al. 2012; Lucas et al. 2016; Xu et al. 2015; Rodriguez-Mozaz et al. 2015).

Five ARGs, bla_{TEM} (resistance to β-lactams), *qnrS* (reduced susceptibility to fluoroquinolones), *ermB* (resistance to macrolides), *sulI* (resistance to sulfonamides), and *tetW* (resistance to tetracyclines), were detected and quantified using qPCR assays in wastewater samples (Lucas et al. 2016). The treatment of these samples with fungal strain *Trametes versicolor* ATCC 42530 resulted in decreasing the copy number and the expression of these. It had been showed that the *ermB* and *tetW* genes completely disappeared both after treatment with *Trametes versicolor* ATCC 42530 and in the non-inoculated control bioreactor (Gao et al. 2012; Rodriguez-Mozaz et al. 2015).

The copy number of bla_{TEM}gene exhibited a marked decrease during fungal treatment (Lucas et al. 2016). On the other hand, the copy numbers of bla_{SHV} and *sulI* genes, increased by thousandfold and tenfold, respectively in both fungal and control bioreactors; however, the increase was significantly lower ($p < 0.05$) in the fungal bioreactor than in the control bioreactor.

There is a correlation between the copy number of antibiotic-resistant genes and the occurrence of antibiotics in the environment (Lucas et al. 2016). The concentration of *ermB* gene decreased by three orders of magnitude in the presence of macrolides (even higher concentrations) (Lucas et al. 2016).

The tetW gene disappeared totally in both bioreactors, even though tetracycline antibiotics were hardly removed along the treatment (29% and 26% removal in the fungal and the control bioreactors, respectively). The concentration of this gene has

also been reported to decrease by three or four orders of magnitude in presence of small amounts of tetracycline antibiotics.

The concentration of b-lactam antibiotics in raw wastewater was quite high (c.a.10 mg L^1) although removal in both bioreactors was very efficient, reaching values close to zero. Nevertheless, levels of bla_{TEM} and bla_{SHV} along the treatment were quite different. In the fungal bioreactor, the bla_{TEM} gene also disappeared (100% removal), in agreement with previous studies (Rodriguez-Mozaz et al. 2015), whereas ARG concentration in control bioreactor did not undergo noteworthy change after treatment. In contrast, the bla_{SHV} gene increased in both bioreactors almost to the same extent, in agreement with the assumption that ARGs increase is favored by the presence of selective agents, such as antibiotics (Allen et al. 2010). The hypothesis here is that despite the decrease in the concentration of b-lactams in both bioreactors, the concentration was high enough to exert a selective pressure; however further studies are required to understand the relationship between the evolution of the bla_{TEM} and bla_{SHV} genes and the concentration of b-lactam antibiotics, including the exposure to sub-therapeutic concentrations.

The concentration of sulfonamides and the sulI gene increased in both bioreactors, whereas in another study in an urban WWTP (Lucas et al. 2016; Rodriguez-Mozaz et al. 2015), both antibiotics and the gene decreased their concentrations. These positive correlations between the gene and antibiotics are in line with the classical knowledge about the emergence of antibiotic resistance (Allen et al. 2010).

The relationship between the *qnrS* gene and quinolones showed a similar trend to that found between the bla_{SHV} gene and b-lactams. An increase of the *qnrS* genes was observed, whereas the antibiotic decreased. Quinolones are the most abundant group in wastewater, and therefore, despite their depletion, they may exert enough selective pressure to increase the gene concentration. Some studies have also suggested that qnr genes may have other functions (e.g., regulation of cellular DNA-binding proteins) in addition to the antibiotic resistance that contribute to its spread (Wang et al. 2004).

15.7 Trace Organic Contaminants (TrOCs)

Trace organic contaminants (TrOCs) include diverse groups of chemicals such as pharmaceuticals and personal care products (PPCPs), surfactants, pesticides, and industrial chemicals. Due to their ineffective removal by conventional wastewater treatment processes, these TrOCs commonly existed in the aquatic environment including groundwater and surface water and even seawater (Sui et al. 2015). The majority of these compounds is biologically active even at trace concentrations (in the range of few ng/L) and can impose detrimental impacts on aquatic environment as well as on human health (Gavrilescu et al., 2015; Pal et al., 2014) [4, 5]. Synthetic hormones can induce endocrine-disrupting effects on aquatic lives (Vandenberg et al., 2012; Chen and Ying, 2015) [6, 7]. Excessive exposure to a nonlethal dose of antibiotics may result in the development of antibiotic-resistant genes in bacteria

which became an emerging concern for human health according to the World Health Organization (de García et al., 2013; Camargo et al., 2014; Rizzo et al., 2013; Nazaret and Aminov, 2014) [8–11]. Bioremediation processes are environmentally friendly and cost-effective (Zhang et al., 2011; Benner et al., 2013; Hai et al., 2007; Hai et al., 2014b) [12–14]. The conventional activated sludge and membrane bioreactor processes can efficiently remove bulk organics, nutrients, and pathogens. However, certain groups of TrOCs such as pharmaceutical and personal care products (PPCPs) with strong electron-withdrawing functional groups are poorly removed by the conventional microbial treatment processes (Alturki, 2013; Hai et al., 2014a; Hai et al., 2014b) [2, 15–17]. Thus, effective treatment strategy to remove TrOCs from wastewater is urgently needed.

Pharmaceutical and personal care products (PPCPs) represent a wide group of TrOCs used for human and veterinary medicine and as fragrances in perfumes and other household products. These types of compounds are considered to be emerging pollutants due to their recalcitrant nature (Kosjek et al. 2007; Ternes et al. 2006). Previous studies detected PPCP concentrations in the environment in the range of nanograms per liter (ng/l) to micrograms per liter (lg/l) (Suárez et al. 2008).

Several treatments have been proposed for PPCP removal. Conventional physicochemical processes, advanced oxidation processes (AOPs), membrane filtration, and activated carbon demonstrated remarkable removal efficiencies for degradation of certain compounds, including tranquilizers, fragrances, and anti-inflammatory drugs, whereas other compounds, such as anti-epileptics, showed less efficient degradation (Ikehata et al. 2006). An emerging technology for the effective degradation of PPCPs involves the application of white-rot fungi. These microorganisms are capable of degrading lignin and several persistent pollutants. This ability is related to the secretion of oxidative enzymes, such as laccase, lignin peroxidase (LiP), manganese peroxidase (MnP), and versatile peroxidase (VP) (Marco-Urrea et al. 2009). Specifically for ligninolytic fungi, Marco-Urrea et al. (2009) studied the ability of four white-rot fungi, *Trametes versicolor*, *Irpex lacteus*, *Ganoderma lucidum*, and *Phanerochaete chrysosporium*, to degrade carbamazepine, ibuprofen, and clofibric acid. This study demonstrated that after 7 days of incubation, ibuprofen was degraded by all four strains, while carbamazepine and clofibric acid were much more recalcitrant; only T. versicolor attained significant degradation of both of these compounds. The use of oxidative enzymes to oxidize PPCPs in vitro has been demonstrated to remove the estrogenic activities from genistein, bisphenol A, nonylphenol, estrone (E1), 17b-estradiol (E2), estriol (E3), and ethinyl estradiol (EE2) (Cabana et al. 2007).

Extracellular enzymes of white-rot fungi (WRF) are characterized by their capacity to degrade the complex structure of lignin, WRF, and lignin-modifying enzymes which have been investigated recently for the degradation of a broad spectrum of PPCPs (Cruz-Morató et al., 2013; Yang et al., 2013a; Nguyen et al., 2013) [24–27]. The potential of WRF for the removal of PPCPs has been investigated mostly in batch mode. There are only a few studies on continuous flow reactor configurations (Rodriguez Porcel et al., 2007; Cruz-Morató et al., 2013; Yang et al., 2013a; Modin et al., 2014; Hai et al., 2013) [24, 26, 28–30].

Three different types of extracellular lignin-modifying enzymes, namely, lignin peroxidases (LiPs), laccase, and manganese-dependent peroxidases (MnPs), are secreted by WRF. The main difference between laccase and peroxidases is the electron acceptor where oxygen and hydrogen peroxide are the respective electron acceptors (Lundell et al., 2010; Guillén et al., 2000) [38, 39]. Not every WRF species produces all three enzymes, and combination of major lignin-modifying enzymes varies from one WRF species to another. Even the secretion pattern of enzymes varies within a WRF species. For instance, different strains of *Trametes versicolor* has been reported to secrete all three enzymes, but laccase is the main enzyme secreted by the strain ATCC 7731 (Bending et al., 2002; Yang et al., 2013b; Nguyen et al., 2014) [40–42]. In addition, composition of growth medium and culture conditions can influence the secretion of a specific enzyme. Degradation of some pollutants such as phenolic compounds, peptides, and organic acids by WRF may result in the formation of low-molecular-weight mediators which can enhance the spectrum of compounds degraded by WRF (Marco-Urrea et al., 2010; Pointing, 2001; Guillén et al., 2000) [37, 39, 43]. Based on the secretion patterns of enzymes, WRF can be categorized as (Hatakka, 1994) [44] (i) MnP-laccase group such as *T. versicolor*, *Pleurotus ostreatus*, *Dichomitus squalens*, and *Panus tigrinus*; (ii) LiP-laccase group such as *Phlebia ochraceofulva*; and (iii) LiP-MnP group such as *Phanerochaete chrysosporium*. In addition to the extracellular enzymes, intercellular enzymes may play an important role in the degradation of xenobiotics. Intracellular cytochrome P450 enzyme system has been observed to play a vital role in the degradation of some PPCPs such as chlorinated hydrocarbons and polycyclic aromatic hydrocarbons (Golan-Rozen et al., 2011; Marco-Urrea et al., 2006; Marco-Urrea et al., 2008) [45–47]. Cytochrome P450 is a group of monooxygenases which can degrade PPCPs by catalyzing a number of reactions such as heteroatom oxygenation, dehalogenation, and hydroxylation (Bernhardt, 2006) [48].

15.8 Bioremediation of Synthetic Dyes

Large volume of water and chemicals are used in textile industries during wet processing. Diverse chemical substances are used in different composition, extending from inorganic composites to polymers and organic products (Banat et al. 1996). The occurrence of dyes in very low concentrations in wastewater is extremely visible and objectionable (Nigam et al. 2000). Commercially more than 100,000 dyes are available globally with over 7×10^5 ton of dye stuff being produced yearly (Meyer 1981; Zollinger 1987). Moreover dyes are resistant to be degraded when they exposed to water, light, and numerous chemicals due to their chemical structure and composition (Puvaneswari et al., 2006). Different types of fungi belonging to *Ascomycota* and *Basidiomycota* were isolated from wastewater of textile dye plant. *Ascomycota* contain fungal strains, e.g., *Verticillium*, *Colletotrichum*, *Fusarium*, and *Paecilomyces variotii*, with potent dye-degrading enzymes such as manganese-dependent peroxidases, ligninases, and laccases (Shanmugapriya et al.,

2019). Recently, the laccases have attracted substantial interest for biotechnological solutions (Theerachat et al., 2019). *Fusarium oxysporum* utilize the β-ketoadipate pathway to degrade aromatic compounds (Porri et al., 2011). Also, we detected three members of *Tremellaceae* known to carry enzymes, e.g., laccases, particularly suitable for degradation of aromatic compounds (Puvaneswari et al., 2006; Theerachat et al., 2019). Furthermore, *Basidiomycota* include several white-rot fungi, such as *Bjerkandera*, *Trametes versicolor*, and *Pleurotus ostreatus*, which have displayed a capability to degrade different dyes (Anastasi et al., 2010). Some species also produce several types of lignolytic enzymes such as laccase, lignin peroxidase, and manganese peroxidase used in various applications in industry today to degrade pulp, dyes, and other xenobiotics (Theerachat et al., 2019). It can be noted that the white-rot fungi group carries several ligninolytic enzymes, lignin peroxidase (LiP), manganese peroxidase (MnP), versatile peroxidase, and laccase. We note that these enzymes degrade phenolic and aromatic substrates through radical reactions with H_2O_2 under aerobic conditions (Shanmugapriya et al., 2019). Moreover these enzymes are also used for the degradation of lignin (Shanmugapriya et al., 2019). Among the dyes the major class of commercially produced azo dyes are not freely degraded by microorganisms, but these can be degraded by *P. chrysosporium* (Paszczynski and Crawford 1995). Further fungi such as *Inonotus hispidus*, *Hirschioporus larincinus*, *Coriolus versicolor*, and *Phlebia tremellosa* have also the ability to decolorize dye-containing effluent (Banat et al. 1996).

Lignin-modifying enzymesLME (MnP, LiP, Lac) are produced in multiple isoforms and encoded by gene families with complex regulation. Nutrient levels, mediator compounds, and required metal ions (Mn^{2+} for MnP, Cu^{2+} for Lac) affect transcription of respective genes. Judicious manipulation of the chemical environment may allow the production of an adequate mixture of LME giving good decolorization without side products; however, this approach is not optimal. Gene amplification and expression in appropriate hosts could be promising for abundant production and affordable price of LME, as is already the case with laccases used commercially in the pulp and paper industry. Further potential benefits of genetically improved LME could be extended to substrate range, catalytic activity, and stability for industrial application of LME (Wesenberg et al. 2003).

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