Chapter 8
Mycoremediation of Organic Pollutants: Principles, Opportunities, and Pitfalls

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Abbreviations

LEs	Ligninolytic enzymes
LiP	Lignin peroxidase
MnP	Manganese-dependent peroxidase
VP	Versatile peroxidases
DyP	Dye-degrading peroxidases
Lac	Laccase
Tyr	Tyrosinase
UPO	Unspecific peroxigenases
CYP450	Cytochrome P450
CDH	Cellobiose dehydrogenase
SRFs	Soft rot fungi
BRFs	Brown rot fungi
WRFs	White rot fungi
LDFs	Litter-decomposing fungi
PCBs	Polychlorobiphenyls
PCDDs	Polychlorinated dioxins
DDT	1,1-bis(4-chlorophenyl)-2,2,2-trichloroethane
BaP	Benzo[*a*]pyrene
POPs	Persistent organopollutants
PAHs	Polycyclic aromatic hydrocarbons
LFs	Ligninolytic fungi
NLFs	Non-ligninolytic fungi

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ROS	Reactive oxygen species
CBAs	Chlorobenzoic acids
EDs	Endocrine disruptors
ECMs	Ectomycorrhizal fungal species
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
PCDFs	Polychlorinated dibenzofurans
diCDD	Dichlorodibenzo-dioxin
OCDD	Octachlorodibenzo-*p*-dioxin
MPs	Micropollutants
PPCP	Pharmaceuticals and personal care products
NPs	Nonylphenols
BPA	Bisphenol A
DEHP	Diethylhexyl phthalate
EE2	17α-ethinylestradiol
WWTP	Wastewater treatment plant
HBT	Hydroxybenzotriazole
NSAID	Non-steroidal anti-inflammatory drugs
IBU	Ibuprofen
CA	Clofibric acid
CBZ	Carbamazepine
DBQ	Dimethoxy-1,4-benzoquinone
CLEAs	Cross-linked enzyme aggregates
TNT	Trinitrotoluene
TPH	Total petroleum hydrocarbons
qPCR	Quantitative PCR
PCP	Pentachlorophenol
SMC	Spent Mushroom Compost

8.1 Introduction

Fungi are a diverse group of microorganisms ubiquitously present in natural ecosystems and they are regarded as the major decomposers of complex biomaterials. Fungi can thrive in a huge variety of habitats, ranging from surface waters to terrestrial environments—including extreme zones of the biosphere like deserts or polar regions—and they use air to disperse their propagules. Regardless of their taxonomic classification, all fungi are essentially heterotrophic microorganisms, i.e., they assimilate nutrients by absorption from the extracellular environment. In general terms, they can be subdivided into biotrophic and saprotrophic organisms based on their ecophysiology. The first group encompasses symbionts and pathogens of other species, while the second is the group of "decomposers" of biomass.

Although several studies have highlighted the ecological importance of some biotrophic fungi (for example mycorrhizas associated with plant roots in polluted soils), for the rationale of this chapter, which deals with the importance of fungi in the decontamination of polluted environments, saprotrophic fungi are more significant.

Saprophytic fungi are able to decompose and transform a wide array of organic (and inorganic) substrates. In contrast to bacteria, which need to transport nutrients into their intracellular compartment prior to their utilization, fungal mycelia can be seen as "externalized stomachs" which secrete their hydrolytic enzymes and organic acids into the extracellular environment and transport digested substances and chelates inside their cell wall. Although bacteria are, to some extent, faster and more efficient than fungi in utilizing readily assimilable substrates (proteins, lipids, starch and free sugars), fungi have evolved extremely efficient enzymatic machineries for the degradation of complex nonprotein polymers, such as lignocellulose and chitin, which are inaccessible and/or recalcitrant to the majority of prokaryotes. Due to these unique capabilities, fungi are considered to play key roles in a number of biogeochemical processes and, more generally, in the cycling of carbon and other elements.

8.1.1 Lignocellulolytic Fungi and Their Enzymes[1]

Lignocellulose is the most abundant renewable biomass on earth. All woody materials are composed mainly of complex carbohydrates (cellulose and hemicellulose) and lignin, the most complex and heterogeneous biopolymer known till date. Thanks to the production of lignocellulolytic enzymes, different saprotrophic fungi can degrade and utilize plant polymers. In general, extracellular lignocellulolytic enzymes from fungi can be subdivided into two major groups. The first one comprises a series of hydrolytic enzymes capable of breaking down polysaccharide constituents of the plant cell wall, namely cellulases (e.g., glucanases, glucosidases, cellobiohydrolases) and hemicellulases (xylanases, mannanases, xylosidase). The other group, referred to as ligninolytic system, promotes the radical-mediated nonspecific decomposition of lignin besides cleaving the aromatic moieties thereof. It includes heme peroxidases (lignin peroxidase, LiP, E.C. 1.11.1.14; manganese-dependent peroxidase, MnP, E.C.1.11.1.13; versatile peroxidases, VP, E.C. 1.11.1.16; dye-degrading peroxidases, DyP, E.C. 1.11.1.19), phenol oxidases (laccase, Lac, E.C. 1.10.3.2; tyrosinase, Tyr, E.C. 1.14. 18.1) and a series of accessory

[1]Additional information on decomposition of lignocellulase redsidues and waste by white and brown rot fungi is presented in Chap. 9—*White and brown rot fungi as decomposers of lignocellulosic materials and their role in waste and pollution control*.

enzymes for the production of H_2O_2 (e.g., glyoxal oxidase, glucose oxidase and aryl alcohol oxidase). During the last decade, a new type of H_2O_2-requiring enzyme has been described for the first time in the Basidiomycete *Agrocybe aegerita* (Ullrich et al. 2004) and, hence, in other fungi (Hofrichter and Ullrich 2014). These proteins are heme-thiolate haloperoxidases, nowadays classified as fungal unspecific peroxigenases (UPO, E.C. 1.11.2.1). Their catalytic cycle combines the typical pathways of extracellular heme peroxidases and intracellular monooxygenases (unspecific cytochrome P450 system, CYP450, E.C. 1.14.14.1), thus enabling them to catalyze an outstanding array of reactions (Hofrichter and Ullrich 2014).

By reason of the different strategy to decompose wood polymers—and owing to the appearance of the wood following the decay process—it is possible to distinguish soft rot (SRFs), brown rot (BRFs), and white rot fungi (WRFs). An additional group ecophysiologically close to the white rot is formed by litter-decomposing fungi (LDFs), which inhabit the organic horizon of forests and grassland soil. These basidiomycetes produce ligninolytic oxidases and peroxidases as WRFs, but their typical niche is soil, where they cause the decay of the leaf-litter and other plant-derived debris.

Ligninolysis per se does not support fungal growth, but fungi can cause a certain deterioration of the amorphous structure of lignin to access the carbohydrates intertwined in the lignin structure of the plant cell walls. To this end, SRFs almost exclusively produce cellulases that diffuse in the lignocellulose structure providing the fungus with nourishment, without significantly altering the structure of the cell wall. Ubiquitous SRFs include species of *Chaetomium*, *Fusarium*, *Paecilomyces* and other common soil genera—not typically wood-inhabiting fungi. Any timber which is in contact with the soil (for example, fence posts or telegraph poles) is ultimately attacked by these fungi, especially under condition of high moisture. SRFs such as *Trichoderma* spp. and *Chaetomium* spp. preferably attack plant biomass characterized by low lignin content. BRFs, instead, use a Fenton-type catalytic system to disrupt the lignin and holocellulose structure in wood (Rabinovich et al. 2004; Baldrian and Valášková 2008), thus facilitating the penetration of hydrolases in the plant cell wall. Strong and diffusible $OH^{\cdot}$ radicals are produced starting from reduced iron (Fe^{2+}) and H_2O_2, which are, in turn, produced either endogenously or *via* cellobiose dehydrogenase (CDH) catalyzed reactions, quinone-redox cycling or low molecular mass glycopeptides (Rabinovich et al. 2004; Baldrian and Valášková 2008). WRFs are also known to use Fenton-type reactions (Baldrian and Valášková 2008; Gómez-Toribio et al. 2009; Dashtban et al. 2010), although the distinctive feature of this fungal group is the production of extracellular high redox potential ligninolytic enzymes (LEs), which are responsible for the so called "enzymatic combustion" of lignin (Kirk and Farrell 1987). LDFs also produce ligninolytic enzymes and attack lignocellulose in a fashion very similar to that of wood-inhabiting white rots, although their niche is the superficial organic layer of soil.

8.1.2 Mycoremediation: Origin and Principles

The link between bioremediation potential of fungi and the decay of wood relies on the similarity between the components of the lignin macromolecules and the majority of aromatic pollutants. Indeed, the catabolic enzymes that fungi have evolved to access the cellulose and hemicellulose fibrils embodied in the wood structure were also found to be active towards a vast range of aromatic pollutants. Concerning this, an overview of the mechanism that ligninolytic and non-ligninolytic fungi use to attack both lignocellulose and organic contaminants is provided in Fig. 8.1.

The first evidence about fungal degradation of aromatic pollutants dates back more than 50 years (Anastasi et al. 2013), when Lyr (1963) reported the chlorophenol-removing potential of a wood-inhabiting fungus and awareness of fungal degradation of wood preservative agents had developed (Duncan and Deverall 1964). In the same period, the "kerosene fungus" *Cladosporium resinae* was also described for the first time upon its isolation from fuel tanks (Hendey 1964). However, the actual driving force which led to the birth of a new branch of bioremediation was the study of the model white rot fungus *Phanerochaete chrysosporium*. In fact, the ligninolytic machinery of *P. chrysosporium* became the focus of several researchers' attention in the early 1980s and a ligninase from the same fungus was characterized for the first time in 1984 (Tien and Kirk 1984). Driven by the capability of *P. chrysosporium* to degrade chlorinated lignin-derived by-products of the kraft pulping process, Bumpus and colleagues demonstrated one year later, in 1985, the ability of the same fungus to degrade (and partially mineralize) a set of persistent organopollutants, namely polychlorobiphenyls (PCBs), dioxins (PCDDs), lindane, 1,1-bis(4-chlorophenyl)-2,2,2-trichloroethane (DDT)

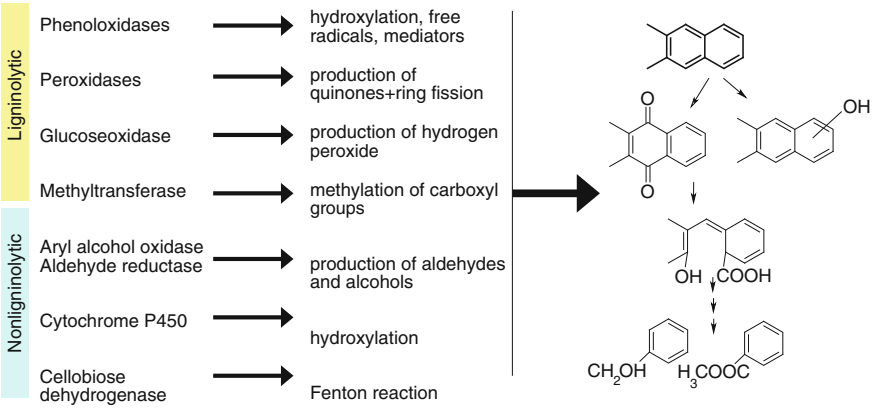

Fig. 8.1 Outline of the mechanisms used by ligninolytic and non-ligninolytic fungi to decompose lignocellulose and organic pollutants

and benzo[*a*]pyrene (BaP) (Bumpus et al. 1985). Since then, an intense period of research on the bioremediation potential of *P. chrysosporium* and other wood-degrading fungi has started (Hammel 1995; Rabinovich et al. 2004; Harms et al. 2011). Concerning this issue, the term "Mycoremediation" has been coined by the eminent mycologist Paul Stamets to specifically address the use of fungal mycelia and their enzymes in bioremediation. The majority of studies concerning fungal remediation have mainly considered the use of lignin degrading fungi (WRFs and LDFs) with typical target matrices being soils and sediments contaminated by persistent aromatic pollutants, such as wood preservatives, oil-derived products, explosives, dielectric fluids, pesticides and other man-made chemical products (Mougin 2002; Baldrian 2008; Cerniglia and Sutherland 2006; Cajthaml and Svobodová 2012).

Several physiological and biochemical features of ligninolytic fungi make them potential candidates for soil bioremediation strategies. As already mentioned, they produce a large number of oxidative enzymes, mainly Lac, LiP and MnP, which exhibit very low substrate specificity and, being active in the extracellular environment, are able to reach and attack scarcely bioavailable contaminants by non-specific radical-based reactions. In addition to the extracellular one, WRFs possess an intracellular enzymatic system involving CYP450 monooxygenases (Črešnar and Petrič 2011). This intracellular pathway occurs in all eukaryotic organisms and it mainly regulates the bioconversion of hormones and the detoxification of drugs and xenobiotics (Bernhardt 2006). In wood rotting fungi, cytochrome P450 is supposed to cooperate with the ligninolytic system in the general mechanism of xenobiotic degradation (van den Brink et al. 1998). Moreover, white rots develop a spatially extensive hyphal growth, enabling them to penetrate across air-filled soil pores, air–water interfaces and even rock matrices (Bornyasz et al. 2005) and to act as dispersion vectors for bacteria (Kohlmeier et al. 2005; Bonfante and Anca 2009). Fungi can also tolerate high concentrations of organic contaminants and heavy metals with limited or no deleterious effects on their own enzymatic activities (Baldrian et al. 2000; Baldrian 2003; Tuomela et al. 2005). In spite of their extraordinary degrading capabilities, the vast majority of fungi cannot assimilate contaminants as a source of carbon and energy like bacteria, except in some cases of non-ligninolytic fungi. For this reason, lignocellulosic residues are used as amendants to support the fungal growth and, thus to improve the mycoremediation performances (Singh 2006). Furthermore, it is well known that fungi are involved in soil humification process: in this respect, the use of these organisms in soil remediation could lead, not only to the decontamination, but also to the reuse of the soil for agricultural purposes once the remediation goals are met (Bollag 1992; Michels 1998).

In the following sections, we present the principles and mechanisms behind fungal degradation of persistent organic pollutants (POPs) and other emerging organic micropollutants (MPs).

8.2 Polycyclic Aromatic Hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are contaminants of great concern due to their worldwide distribution. Although they are natural compounds, PAHs also originate from incomplete combustion of fossil fuels and biomasses. For this reason, human activities are considered as the major source of PAH contamination of the environment. Chemically, PAHs consist of two or more fused benzene rings arranged either in a linear or a cluster mode and several members of this class—e.g., benzo[*a*]anthracene, chrysene and benzo[*a*]pyrene—have been included among priority pollutants owing to their toxic, mutagenic and carcinogenic properties (Haritash and Kaushik 2009). The persistence of PAHs in the environment is mainly due to their hydrophobicity, toxicity and recalcitrance to microbial degradation, which largely increase as the molecular weight of PAHs increases.

PAHs are, by far, the most extensively studied contaminants with respect to their susceptibility to fungal biodegradation. Owing to the chemical stability of PAHs, which is favored by the dense cloud of delocalized π electrons on the coplanar structure, the critical step for aerobic organisms is the initial incorporation of oxygen on the aromatic ring. In general, the way fungi use to attack PAHs is the same as that of mammalian and other eukaryotes, i.e., oxidation of PAHs to PAH *trans*-dihydrodiols by Phase I intracellular enzymes (CYP450 monooxygenases and epoxide hydrolase) followed by Phase II conjugation reactions mediated by transferases. This detoxification reaction was first described in *Cunninghamella elegans* by Cerniglia and Gibson (1977). Cerniglia (1997) also used this member of Mucoraceae as a model organism for subsequent PAH degradation studies, which prompted him to identify high molecular weight PAH-dihydrodiols, quinones and phenols metabolites. However, the above-cited study of Bumpus et al. (1985) about the xenobiotic degradation capabilities of *P. chrysosporium*, shifted the attention of many researchers towards ligninolytic fungi (LFs) and their powerful extracellular machineries. Since then, several other genera of LFs (mainly WRFs) such as *Trametes*, *Pleurotus*, *Bjerkandera*, *Irpex*, *Phlebia*, *Nematoloma*, and *Lentinus* have been demonstrated to metabolize PAHs efficiently under model liquid culture conditions or in the soil (Sack et al. 1997; Pointing 2001; Giubilei et al. 2009; Novotný et al. 2009; Baldrian 2008; Covino et al. 2010a, b, c). Additionally, it was proved that PAHs can be oxidized under in vitro conditions using Lac and peroxidases from LFs (Hammel et al. 1986; Majcherczyk et al. 1998; Eibes et al. 2006; Baborová et al. 2006; Covino et al. 2010c) and that the use of redox mediators can improve the rate of PAH degradation as well as expand the spectrum of substrates oxidized by such catalysts (Sack et al. 1997; Johannes and Majcherczyk 2000; Camarero et al. 2008; Cañas and Camarero 2010; Covino et al. 2010c). Besides, the involvement of CYP450 monooxygenase-epoxide hydrolase system was shown to be active in the initial degradation of PAHs also in LFs (Bezalel et al. 1997). Using ^{14}C-labeled compounds, it was proved that LFs are able to completely mineralize PAHs to carbon dioxide (Bumpus et al. 1985; Bezalel et al. 1996a; Wolter et al. 1997; Sack et al. 1997). However, evidence for PAH ring-cleavage by fungi are poorly described. Hammel

et al. (1991) showed that *P. chrysosporium* was able to decompose anthracene to phthalic acid while Bezalel et al. (1996b) presented the mechanism of 2,2′-diphenic acid production from phenanthrene. These authors suggested that CYP450 of *Pleurotus ostreatus* was responsible for the attack to phenanthrene, enabling further ring opening reactions. Moen and Hammel (1994) reported the formation of 2,2′-diphenic acid from phenanthrene as a result of lipid peroxidation by MnP, while other authors found several ring-cleavage products of acenaphthylene and acenaphthene after incubation with *Trametes versicolor* Lac as well as a laccase-mediator system (Johannes et al. 1998; Majcherczyk et al. 1998). A few years later, Cajthaml et al. (2002) identified several PAH metabolites in *Irpex lacteus* axenic cultures spiked with phenanthrene, anthracene, fluoranthene and pyrene: the presence of ring-cleavage products and PAH-dihydrodiols reinforced the hypothesis that ligninolytic fungi use both the CYP450 system and extracellular ligninases. Baborová et al. (2006) not only confirmed that purified MnP from *I. lacteus* can attack recalcitrant representative of PAHs, but also that it can cleave the benzene ring to form 2-(2′-hydroxybenzoyl)-benzoic acid. A pathway similar to that proposed for anthracene has been also hypothesized by Cajthaml and colleagues (2006) for benzo(*a*)anthracene, with emphasis on intermediates possessing two aromatic rings, e.g., 1,4-naphthalenedione, 1,4-naphthalenediol, and 1,2,3,4-tetrahydro-1-hydroxynaphthalene. The breakdown products in the metabolic pathway of benzo[*a*]anthracene by *I. lacteus* are shown in Fig. 8.2.

The WRF *P. ostreatus*, well known as oyster mushroom, is one of the most efficient degrader of PAHs (Bezalel et al. 1996b; Wolter et al. 1997). When it was grown in the presence of several PAHs (benzo[*a*]pyrene, pyrene, fluorene, phenanthrene, anthracene) metabolization and mineralization was shown to occur. The main PAH degradation products identified were: phenanthrene trans-9,10-dihydrodiol and 2,2′-diphenic acid, pyrene trans-4,5-dihydrodiol, anthracene trans-1,2-dihydrodiol, and 9,10-anthraquinone. For instance, the fungus was able to decompose phenanthrene, anthracene and pyrene by 50, 92 and 35 % in 5 days, respectively, in bran flakes media (Pickard et al. 1999). Schützendübel et al. (1999) found that *Bjerkandera adusta* removed 56 and 38 % of fluorene and anthracene 3 days after spiking these PAH in the fungal cultures, while *P. ostreatus* degraded 43 and 60 % of these compounds; other PAHs were degraded to a lower extent. All PAHs were removed uniformly during the period of incubation by *P. ostreatus* cultures, but fluorene and anthracene were degraded faster than other compounds in basidiomycete's rich media. The detected intermediates were mostly keto compounds (Schützendübel et al. 1999).

Another member of the Polyporales with remarkable PAH-degrading capabilities is the white rot fungus *Lentinus tigrinus* (Valentin et al. 2006; Covino et al. 2010a, c). Regardless of the N-content in the culture media, this fungus was able to remove up to 97 % of a mixture of 7 PAHs from 3 to 5 fused benzene rings. In addition, the major ligninolytic isoenzymes (Lac and MnP) from *L. tigrinus* were shown to efficiently degrade individual PAH congeners under in vitro conditions, either in the presence or in the absence of low molecular weight redox mediators, although MnP showed a wider PAH substrate range and faster oxidation rates than

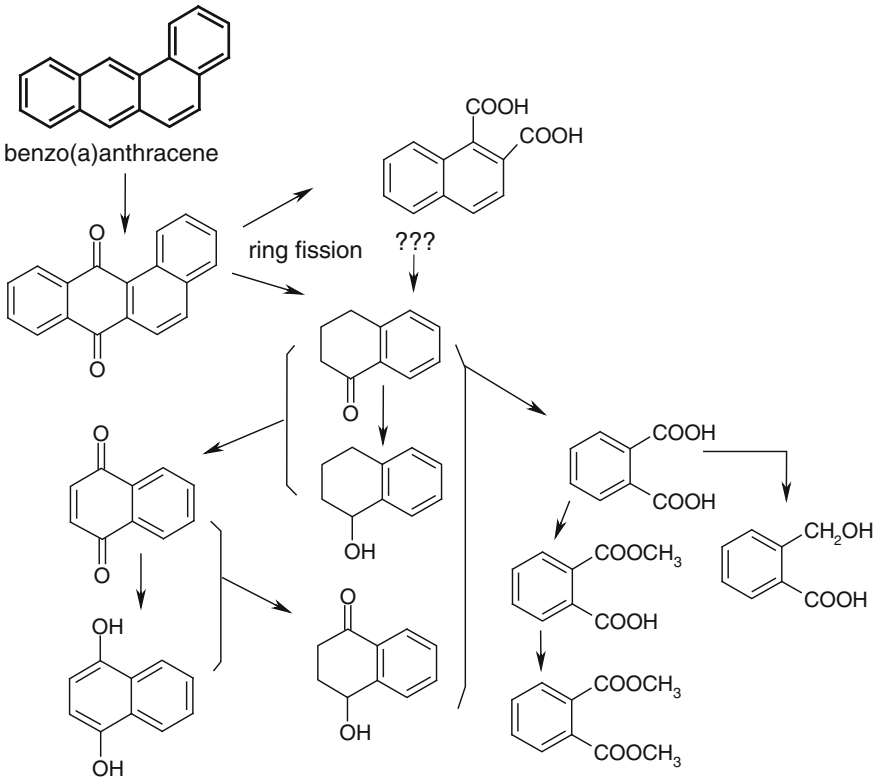

Fig. 8.2 Benzo[*a*]anthracene degradation by *I. lacteus*—adapted from Cajthaml et al. (2006)

the Lac produced by the same strain. When *L. tigrinus* was bioaugmented in a PAH-contaminated soil and in creosote wood, it removed PAHs to a higher extent than *I. lacteus* (Covino et al. 2010a). Moreover, Valentin et al. (2006) reported that *L. tigrinus*, *I. lacteus* and *B. adusta* were capable of growing and degrading PAHs under the typical halophilic conditions of a marsh soil.

Litter-decomposing fungi (LDFs) were claimed to have a greater potential than white rots in the mycoremediation of contaminated soil, mainly due to their innate capability to compete with natural soil microflora (Šašek 2003; Steffen et al. 2007). In addition to an early report on the BaP-degradation by *Marasmiellus troyanus* (Wunch et al. 1999), the first extensive screening of the PAH degrading potential of LDFs was performed by Gramss et al. (1999). Outstanding results in term of removal of a mixture of 5 PAHs were reported for two fungi, namely *Gymnophilus sapineus* and *Agrocybe praecox* (92 and 90 %, respectively, after 14 d). Steffen et al. (2002) reported the bioconversion of PAHs by nine litter decomposers in model liquid systems and concomitantly assessed the extent of ^{14}C-benzo[*a*]pyrene (BaP) mineralization. The results showed that all fungi were capable of oxidizing PAHs, with two strains belonging to the genus *Stropharia* (*S. coronilla* and

S. rugosoannulata) that were more efficient than others. The authors also highlighted the positive correlation between Mn^{2+} supplementation, expression of MnP activity and overall degradation/mineralization of PAHs (Steffen et al. 2002). As a consequence, *S. coronilla* MnP was further purified and incubated under in vitro condition with ^{14}C-BaP. The results confirmed the involvement of this enzyme in the mineralization of BaP and a 12-fold higher increase in $^{14}C\text{-}CO_2$ production upon Mn^{2+} supplementation. In addition, the two *Stropharia* spp. mentioned above were the most efficient PAHs degraders among other litter decomposers in an artificially contaminated soil (Steffen et al. 2007). Similarly, Lac from the oak-litter decomposer *Marasmius quercophilus* was shown to degrade PAHs with ionization potential lower than 7.55 eV, i.e., anthracene and BaP (Farnet et al. 2009). As for the PAH degradation in real contaminated matrices, the shaggy mane mushroom *Coprinus comatus* was used in a laboratory-scale mycoremediation test targeting historically creosote-contaminated soil and wood. Regardless of lignocellulose support selected as growth substrate, the PAH degradation performances of this fungus were similar to that of representative white rots (*Dichomitus squalens* and *P. ostreatus*), and some high molecular weight PAHs were degraded by *C. comatus* beyond their threshold of bioavailability (Covino et al. 2010b).

Comparatively, BRFs were less investigated than ligninolytic fungi with respect to PAH degradation. However, in spite of early studies claiming the inadequacy of brown rots for mycoremediation applications (Martens and Zadrazil 1998), recent reports have highlighted the PAH biotransformation potential of the button mushroom *Agaricus bisporus* and the possible use of *A. bisporus* spent compost in soil decontamination (Mayolo-Deloisa et al. 2011; García-Delgado et al. 2015).

Soft-rotting fungi include common soil saprotrophs that usually grow on substrates other than wood, but they can occasionally cause its decay. Indeed, Ascomycetes such as *Trichoderma*, *Chaetomium*, *Fusarium*, *Aspergillus*, *Penicillium* and *Paecilomyces* are still regarded by many authors as non-ligninolytic fungi (Rabinovich et al. 2004; Marco-Urrea et al. 2015) although several SRFs genera are known to produce Lac (Verdin et al. 2004; Baldrian 2006; Wu et al. 2010; Cázares-García et al. 2013; Viswanath et al. 2014) and ligninolytic peroxidases (Yang et al. 2003, 2013; Silva et al. 2009). The redox potential of these enzymes, however, is lower than those typically produced by wood-rotting fungi and their role in lignin and PAH biotransformation has not yet been fully understood (Baldrian 2006). In general, WRFs and LDFs have attracted more attention than soft-rotters for their possible applicability in bioremediation. In spite of this, soil saprotrophs are frequently isolated from crude oil- and PAH-contaminated soils, where they constitute a large proportion of the fungal community (Rafin et al. 2000, 2012; Covino et al. 2015; Bourceret et al. 2015: Cébron et al. 2015). Very recently, an interesting review on NLFs with potential application in bioremediation of organopollutants has been published (Marco-Urrea et al. 2015) and an exhaustive list of species with reported PAH degrading capability can be found in the literature (Gadd 2001; Cerniglia and Sutherland 2006; Fernández-Luqueño et al. 2011; Harms et al. 2011; Marco-Urrea et al. 2015). The mechanism by which these fungi attack PAHs is the same as that described for *C. elegans*, i.e., *via* CYP450

monooxygenase-epoxide hydrolase system, which yields hydroxylated, (*trans*-) di-hydroxylated and transient dihydro-epoxide derivatives. These activated structures, that are capable of binding to nucleophilic groups of DNA, can be also transformed into sulfate-, methyl-, glucose-, xylose-, or glucuronic acid conjugates by Phase II enzymes (e.g., aryl sulfotransferase, glutathione S-transferase, UDP-glucuronosyltransferase and UDP-glucosyltransferase, methyl transferases). This step, followed by the excretion of these compounds into the extracellular environment, results in a significant detoxification with respect to both parent molecules and mutagenic dihydroxy-epoxides, as it is known to occur in higher eukaryotes (Cerniglia and Sutherland 2006; Marco-Urrea et al. 2015). Interestingly, some members of this group, e.g., the yeasts *Pichia anomala* and *Rhodotorula glutinis* or the hyphomycetes *Fusarium solani*, *Trichoderma* and *Penicillium*, possess the capability to use PAHs with up to 5 fused benzene rings as sole carbon source (Romero et al. 1998, 2002; Rafin et al. 2000; Pan et al. 2004; Verdin et al. 2004; Cerniglia and Sutherland 2006), and some of them can do so even in the presence of microaerobic ($5 < O_2 < 15$ %) or very-low oxygen conditions ($1\ \% < O_2$) (Silva et al. 2009). Detailed studies on BaP-degradation by *F. solani* demonstrated that this fungus (i) produces a surfactant-like hydrogel (polysaccharides and glycoproteins) to increase the BaP solubility and uptake; (ii) transports BaP in the intracellular environment and accumulate it in lipid vesicles; (iii) degrades the substrates by means of either CYP450 or reactive oxygen species (ROS) and (iv) mineralizes BaP to an extent similar to that reported for the white rot fungus *P. ostreatus* (Verdin et al. 2004, 2005).

8.3 Chlorinated Aromatic Pollutants

8.3.1 Polychlorinated Biphenyls

Polychlorinated biphenyls (PCBs) are synthetic compounds the structure of which consists of a biphenyl molecule (two aromatic rings linked by a C–C bond) carrying from one to ten chlorine atoms. Theoretically, the entire set of PCB homologs comprises 209 congeners. In the past, PCB mixtures were widely used for a number of industrial applications due to their thermal and chemical stability, flame resistance and dielectric properties. As a consequence, their use led to extensive environmental contamination either through accidental releases and/or inappropriate methods of disposal. Owing to their aforementioned inertness, PCBs are therefore still present in a number of areas where their production had been carried out for decades, although their application was drastically restricted long time ago. Nowadays, PCBs are considered among the most hazardous contaminants in the world, placing them at the forefront of public health concern. Indeed, the teratogenic, carcinogenic and endocrine disrupting effects of these xenobiotics have been widely reported in the literature (Crinnion 2011; Helmfrid et al. 2012; Kramer et al.

2012; El Majidi et al. 2013) especially for coplanar PCBs (not substituted at the ring positions *ortho*-) which exhibit "dioxin-like" toxicity features (US Environmental Protection Agency 2000; Van den Berg et al. 2006).

In the light of all these considerations, the cleaning-up of PCB-contaminated sites has become a global priority. Among diverse remediation approaches, the use of biological systems represents an efficient, cost-effective, and environmentally friendly alternative to the more commonly used thermal and physico-chemical technologies (Passatore et al. 2014).

Bacteria play a key role in PCB biodegradation processes: anaerobes can use highly chlorinated biphenyls as electron acceptors and convert them into less chlorinated congeners, while aerobic bacteria can co-metabolize lower chlorinated biphenyls (Pieper 2005; Field and Sierra-Alvarez 2008). However, the co-metabolism of PCBs *via* the biphenyl pathway leads to the formation of chlorobenzoic acids (CBAs), which tend to be accumulated as dead-end products. The build-up of these metabolites exerts an inhibitory feedback effect on the upper biphenyl degradation pathway resulting in the impediment or slowing down of the PCB biotransformation process as a whole (Adebusoye et al. 2008; Furukawa and Fujihara 2008). Therefore, it is worthwhile to search for other microorganisms that are able to mineralize PCBs completely.

Due to their wide metabolic capabilities, a large number of ligninolytic fungi belonging to the *Basidiomycota* phylum were tested in laboratory-scale model liquid systems for their ability to degrade technical PCB mixtures or single PCB congeners, notably: *P. chrysosporium* (Eaton 1985; Thomas et al. 1992; Vyas et al. 1994; Yadav et al. 1995; Kamei et al. 2006a) *T. versicolor* (Zeddel et al. 1993; Vyas et al. 1994; Cloete and Celliers 1999), *Lentinus edodes* (Ruiz-Aguilar et al. 2002), *P. ostreatus* (Kubátová et al. 2001; Čvančarová et al. 2012), *Grifola frondosa* (Seto et al. 1999), *Coriolopsis polyzona* (Vyas et al. 1994; Novotný et al. 1997), *Phlebia brevispora* (Kamei et al. 2006b), *I. lacteus* (Čvančarová et al. 2012); *B. adusta* (Beaudette et al. 1998; Čvančarová et al. 2012), *Pycnoporus cinnabarinus* (Čvančarová et al. 2012), *Phanerochaete magnoliae* (Čvančarová et al. 2012).

Generally, all these studies confirmed that the extent of degradation significantly decreases with increasing chlorine content. For instance, a negligible mineralization of individual tetra- and hexa-chlorobiphenyls was reported for *P. chrysosporium* (Dietrich et al. 1995), whereas the level of degradation remarkably increased for three- and di-chlorinated congeners. Similar trends were also observed with technical PCB mixtures. For example, Yadav et al. (1995) showed that *P. chrysosporium* was able to remove 60.9, 30.5, and 17.6 % of Aroclor 1242, 1254, and 1260 PCB mixtures (42, 54 and 60 % chlorinated), respectively, in 30 days.

Moreover, some fungal strains (e.g., *P. ostreatus*) have shown to selectively remove PCB congeners with a preference for compounds with chlorine atom in *ortho* > *meta* > *para* position (Kubátová et al. 2001), while others (e.g., *P. chrysosporium*) did not exhibit any noticeable specificity for the position of chlorine substitutions (Yadav et al. 1995).

A number of studies have dealt with the interpretation of PCB degradation mechanisms in fungi. A few works indicated that purified fungal extracellular phenoloxidases and peroxidases were unable to oxidize PCB congeners (Beaudette et al. 1998; Krčmar et al. 1999; Takagi et al. 2007). For instance, Krčmar et al. (1999) performed a biodegradation experiment with technical PCB mixtures containing low and high chlorinated congeners (Delor 103 and Delor 106) using *P. chrysosporium* mycelium, crude extracellular liquid and crude extract enriched in MnP and LiP activities. A decrease in the PCB concentration after 44 h of treatment with mycelium (74 %) or crude extracellular liquid (60 %) was observed whereas MnP and LiP isolated from the extracellular liquid did not catalyze any degradation.

Afterwards, based on the identification of PCB transformation products in liquid fungal cultures, the involvement of both extracellular ligninolytic system and intracellular CYP450 monooxygenases system in the degradation process of PCBs was hypothesized (Kamei et al. 2006a, b; Čvančarová et al. 2012). In this respect, other studies proved that the ligninolytic enzymes were able to breakdown some of the PCB degradation intermediates such as their hydroxylated derivatives, which are produced by CYP450 system (Keum and Li 2004; Takagi et al. 2007; Kordon et al. 2010).

Kamei et al. (2006a) investigated the transformation products of 4,4′-dichlorobiphenyl in *P. chrysosporium* in the attempt to determine its degradation pathway. Methoxylated- and hydroxylated-PCB derivatives were detected in *P. chrysosporium* liquid cultures and, thus the involvement of cytochrome CYP450 in the degradation process was supposed. The addition of piperonyl butoxide, a well-known CYP450 inhibitor, to fungal cultures prevented the formation of hydroxylated metabolites, further supporting the initial hypothesis. Chlorobenzoic acids, chlorobenzaldehydes and chlorobenzylalcohols were also identified as PCB degradation products. In particular, Cvancarová et al. (2012) reported the formation of chlorobenzoates from hydroxylated PCBs and hypothesized the further transformation *via* reductive pathways. Once CYP450 oxidizes the aromatic structure of PCBs, ring fission reaction can be mediated by other enzymatic systems (i.e., LEs) (Cajthaml et al. 2006; Čvančarová et al. 2012); subsequently, a reductive mechanism can operate on the carboxyl group of CBAs leading to the formation of chlorinated aldehydes and alcohols (Muzikář et al. 2011; Stella et al. 2013). An overview of the PCB degradation pathway in ligninolytic fungi, including the formation and further biotransformation of CBAs, is provided in Fig. 8.3.

Despite the promising outcomes achieved in liquid cultures, few studies investigated the ability of white rot fungi to degrade chlorinated biphenyls either in artificially contaminated (Zeddel et al. 1993; Kubátová et al. 2001) or in actual PCB-contaminated soils (Borazjani et al. 2005; Federici et al. 2012). With regard to historically PCB-polluted matrices, a recent study demonstrated that a bioaugmentation approach based on the use of maize stalk-immobilized fungus *L. tigrinus* brought about 33.6 % of Aroclor 1260 depletion and 23.2 % of dechlorination in 60 days (Federici et al. 2012).

Compared to PCB biotransformation processes mediated by basidiomycetes, few studies investigated the PCB biodegradation potential of NLFs. One of the first

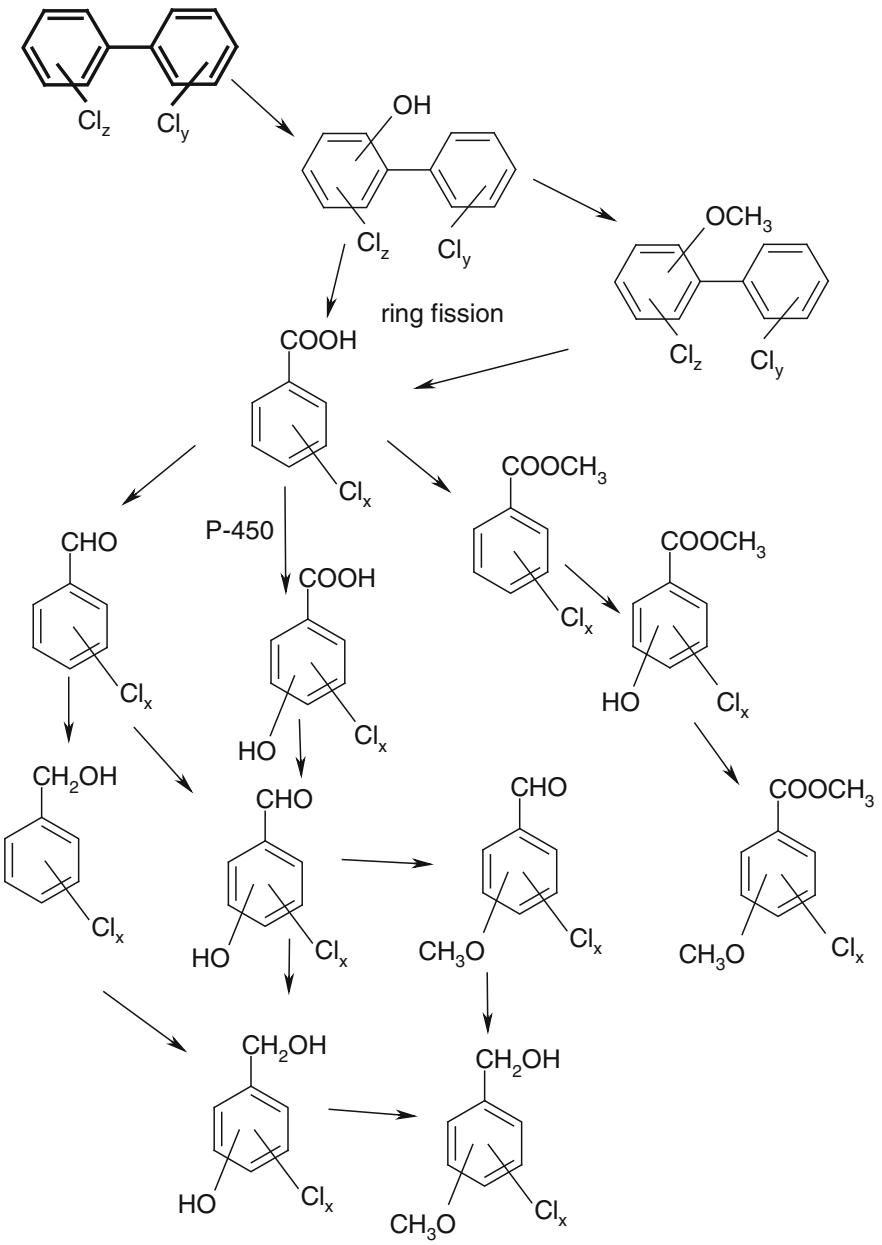

Fig. 8.3 Degradation pathway of PCBs and CBAs by *P. ostreatus*—adapted from Čvančarová et al. (2012) and Muzikář et al. (2011)

report (Dmochewitz and Ballschmiter 1988) revealed that the ascomycete *Aspergillus niger* was able to degrade a technical mixture of PCBs (Chlophen A) with a low chlorine content (42 %), while PCB mixtures with higher chlorination levels (54 and 60 %) remained untransformed. Recently, six fungal strains belonging to *Aspergillus*, *Penicillium*, *Fusarium* and *Scedosporium* genera were isolated from an aged contaminated soil (0.9 g kg^{-1} PCB mixture Aroclor 1260) and tested for their ability to degrade three target congeners (2-monoCB, 4,4′-diCB and 2,2′,5,5′-tetraCB) in liquid mineral medium in the presence of glucose (Tigini et al. 2009). The degradation experiments demonstrated that PCBs were cometabolically transformed by all the isolates at a similar rate, regardless of the number of halogenated substituents. A similar experimental approach was adopted by Mouhamadou et al. (2013). The PCB degradation ability of autochthonous fungal strains isolated from former industrial sites highly contaminated with PCBs was tested in liquid cultures. Among all the isolates, the ascomycetes *Doratomyces nanus*, *Doratomyces purpureofuscus*, *Doratomyces verrucisporus*, *Myceliophthora thermophila*, *Phoma eupyrena,* and *Thermoascus crustaceus* showed remarkable degradation ability (>70 %). As for the previous study, the number of chlorine substituents on the biphenyl nucleus did not affect the degradation rate. Thereafter, a consortium of the aforementioned fungal strains was used for the remediation of both PCB-contaminated soil and sediment (Sage et al. 2014). After 6 months of treatment, 18.7 and 33.3 % of 15 target PCBs were degraded in soil and sediment mesocosms, respectively. The fungal strains were re-inoculated and incubated for additional 2 months to further promote the biodegradation of PCBs. However, no additional PCB depletion was observed. This result suggested that the bioavailable fraction of PCB was completely removed after 6 months and the remaining fraction was inaccessible to fungi.

Research regarding the PCB degradation pathways in other non-white rot fungi, revealed that no dechlorination occurred during the transformation of 4-chlorobiphenyl by *Paecilomyces lilacinus*, but five chlorinated metabolites, including ring fission products, were identified (Sietmann et al. 2006). Another study by Tigini et al. 2009, where mitosporic fungi were incubated in the presence of PCBs, showed that no chloride ions were released. Moreover, peroxidase activities were not detected in the liquid cultures, either in the absence or in the presence of PCBs, providing further evidence that these extracellular enzymes were not involved in the biotransformation process. Only Lac activity was detected at significant levels, irrespective of the presence of PCBs, but the role played by this enzyme in the PCBs degradation process was not clarified.

In conclusion, despite the large number of studies regarding the mycoremediation of PCBs, further research is needed to explore the physiological and biochemical characteristics of fungi prior to any practical application. Moreover, as suggested in a recent study (Stella et al. 2015), a comprehensive evaluation of both physico-chemical factors (i.e., interaction of PCBs with soil organic matter) and biological factors (i.e., interaction with native microorganisms) is strongly required to develop an effective fungal-based remediation approach for the treatment of PCB contaminated matrices.

8.3.2 Chlorobenzoic Acids

Chlorobenzoic acids (CBAs) are a class of relevant environmental pollutants consisting of benzoic acid with different chlorination degrees of the aromatic ring. CBAs primarily result from bacterial biodegradation of polychlorinated biphenyls (PCBs) under aerobic conditions as reviewed by Field and Sierra-Alvarez (2008). During this process, CBAs tend to accumulate as dead-end products, acting as inhibitors for the upper biphenyl pathway and therefore restricts further PCB transformation (Pieper 2005; Adebusoye et al. 2008). Due to their relatively high solubility in water, CBAs are characterized by a mobility that is several orders of magnitude higher than that of PCBs. In addition, some of CBA isomers are highly toxic for aquatic organisms (Lee and Chen 2009), exhibit genotoxicity toward higher plants (Gichner et al. 2008) and act as endocrine disruptors (EDs) (Svobodová et al. 2009).

The number of publications dealing with the fungal degradation of CBAs is very limited. Four white rot fungi (*P. chrysosporium*, *P. ostreatus*, *T. versicolor*, *Heterobasidion annosum*) and two ectomycorrhizal (ECMs) fungal species (*Paxillus involutus*, *Suillus bovinus*) were tested for their capability to grow on different concentrations of 3-chlorobenzoic acid (0.1, 1, and 3 mM) and to degrade it in 4 weeks (Dittmann et al. 2002). Even low concentrations of 3-CBA led to a remarkable reduction in the growth of both ECMs and *P. chrysosporium*, whereas that of *P. ostreatus*, *T. versicolor* and *H. annosum* was not affected. This study showed, in particular, that the great ability of the latter group of white rot fungi to grow in the presence of 3-CBA correlated to the highest extent of degradation achieved at the end of incubation period.

Muzikár et al. (2011) performed a more comprehensive study dealing with fungal degradation of CBA. Eight ligninolytic fungal strains (*I. lacteus*, *B. adusta, P. chrysosporium*, *P. magnoliae*, *P. ostreatus*, *T. versicolor*, *P. cinnabarinus* and *D. squalens*) were tested for their ability to degrade a mixture of 12 CBAs (mono-, di- and tri-chlorobenzoates) in both model liquid systems and contaminated soils. In liquid media, *I. lacteus*, *P. cinnabarinus* and *D. squalens* were the most effective fungi in terms of CBA removal and toxicity reduction, whereas *I. lacteus* and *P. ostreatus* were the most efficient in an artificially ("ad hoc") contaminated soil. Analysis of the degradation products revealed that methoxy and hydroxy derivatives were produced along with reduced forms of the original acids (benzaldehydes and benzyl alcohols) and a general degradation pathway was proposed. However, the mechanism by which individual enzymes can degrade CBAs was not fully clarified. Subsequently, the white rot fungus *L. tigrinus* was selected for the evaluation of its efficiency in degrading a mixture of chlorinated benzoates (Stella et al. 2013). The in vivo degradation experiment showed that this organism was able to deplete most of the target CBA isomers within 20 days of incubation, with the exception of 2,6-diCBA, 2,3,6-triCBA and 2,4,6-triCBA. The authors suggested that the

recalcitrance of these isomers might be due to the steric hindrance of the two chlorine substituents adjacent to the carboxyl moiety and to the general electron-withdrawing effect of halides. Furthermore, to gain insights into the CBA degradation pathway of *L. tigrinus*, in vitro experiments were performed with both extracellular LEs (Lac and MnP) and intracellular enzymatic systems (microsomal fraction containing CYP450 monooxygenases) extracted from *L. tigrinus* cultures. Lac and MnP were shown not to be involved in the initial transformation steps of CBAs, even in the presence of redox mediators, whereas CYP450 were able to oxidize chlorobenzoates. The key role played by CYP450 in the degradation of CBAs under in vitro condition was inferred by the NADPH-dependency of the reaction and by its susceptibility to piperonyl butoxide and carbon monoxide, two well-known CYP450 inhibitors (Stella et al. 2013). Additionally, the detection of a hydroxylated chlorobenzoic acid further supported the pivotal role of CYP450 system in the initial CBA bioconversion steps by *L. tigrinus*.

8.3.3 Chlorinated Dioxins and Furans

Polychlorinated dibenzodioxins (PCDDs) are a group of polyhalogenated organic compounds which consist of two benzene rings joined by two oxygen bridges (dibenzo-1,4-dioxin) with one or several of the hydrogens replaced by chlorines and polychlorinated dibenzofurans (PCDFs) are a group of organopollutants with properties and chemical structures similar to polychlorinated dibenzodioxins. PCDDs and PCDFs are released in the environment as by-products of several industrial processes, including incineration of waste, chemical and manufacturing of pesticides. Because of their lipophilic properties, they get accumulated in humans and wildlife causing developmental disorders and cancer as already reported several decades ago (Huff et al. 1980). For this reason, they were listed among the most persistent hazardous organic pollutants in the Stockholm convention. A large number of white rot fungi, namely *P. chysosporium*, *Panellus stypticus*, *Phlebia* spp., and *Bjerkandera* spp., were assessed as potential dioxin degraders. The capability of *P. chysosporium* to mineralize PCDDs was demonstrated in 1985 (Bumpus et al. 1985) without the elucidation of the metabolic pathway. Later, Valli et al. (1992) demonstrated that *P. chrysosporium* degraded 50 % of 2,7-dichlorodibenzo-*p*-dioxin (2,7-diCDD) under ligninolytic conditions while only 10 % was degraded under non-ligninolytic conditions, implying the involvement of LiP and MnP. The proposed pathway for 2,7-diCDD transformation involved oxidative cleavage of 2,7-diCDD by LiP resulting in the production of 4-chloro-1,2-benzoquinone and 2-hydroxy-1,4-benzoquinone. Afterwards, hydroquinones or catechols were produced via LiP and/or MnP mediated oxidation followed by methylation leading to the formation of methoxybenzenes. Interestingly, though the white rot fungus *Panellus stypticus* does not produce either LiP or MnP, it

could transform 2,7-diCDD. The formation of 4-chlorocatechol as an intermediate and the inhibition of the degradation process upon addition of piperonyl butoxide, a well-known CYP450 inhibitor, suggested a degradation pathway involving CYP450 different from that described for *P. chrysosporium* (Sato et al. 2002). The same degradation mechanism was proposed for several *Phlebia* species that mineralized 6.5 % of [^{14}C]-2,7-diCDD producing hydroxylated and methoxylated intermediates (Mori and Kondo 2002a, b; Kamei and Kondo 2005). Moreover, Kamei et al. (2005) demonstrated that different *Phlebia* species were also able to degrade higher chlorinated dioxins, such as 2,3,7-triCDD, 1,2,8,9-tetraCDD and 1,2,6,7-tetraCDD. Several strains of the genus *Bjerkandera* were also screened for their ability to transform PCDDs and the production of key enzymes that catalyze the conversion of these pollutants was evaluated in either nitrogen-sufficient or -limited conditions (Manji and Ishihara 2004). Among the tested strains, *Bjerkandera* MS325, that could produce high levels of both MnP and LiP even under non-ligninolytic conditions, was proved to be the most efficient degrader of 1,3,6,8-tetraCDD (21 % of the original content removed in 10 days). Other WRFs were also studied for their ability to degrade dibenzo-*p*-dioxins as reviewed by Pinedo-Rivilla et al. (2009). In this respect, *Trametes* sp. CH2, *Irpex* sp. W3 and *Pleurotus pulmonarius* were reported for their capability to hydroxylate and methoxylate PCDDs (Yamaguchi et al. 2007; Nam et al. 2008).

In contrast to the above, evidence of PCDDs degradation by NLFs is limited to a few ascomycetes, but the enzymatic systems involved in such biotransformations remain unknown. A dioxin-degrading organism was isolated from the activated sludge of a landfill treatment plant and identified as *Acremonium* sp., (Nakamiya et al. 2002). This fungus (later identified as *Pseudallescheria boydii*) was able to remove 82 % of a mixture containing tetrachlorinated up to octachlorinated dioxins (2 ng/mL) under denitrifying conditions in 1 day. The incubation with octachlorodibenzo-*p*-dioxin (OCDD, 100 ng) under aerobic conditions led to the formation of 1- and 2-hydroxydibenzo-*p*-dioxin indicating that OCDD was transformed to non-chlorinated dibenzo-*p*-dioxins through a dechlorination process. However, the degradation mechanism of OCDD and other highly chlorinated dioxins was not described in detail. In another study, *P. boydii* was tested in a slurry bioreactor system for the treatment of dioxin-contaminated soils (Ishii and Furuichi 2007). Surprisingly, in spite of its great capability to remove dioxins, *P. boydii* failed to lower the dioxins level to the environmental standard in this particular experimental setup and, thus the biological treatment was coupled with a physico-chemical process (ethanol extraction) to reach the required quality standard. Two years after, the same authors clarified that *P. boydii* use glucose as carbon source to grow and degrade PCDDs during its logarithmic growth phase regardless of the concentration of dioxins (Ishii et al. 2009). Another study showed that the cyclic ether degrading fungus, *Cordyceps sinensis*, was able to degrade dibenzo-*p*-dioxin (DD), 2,3,7-trichlorodibenzo-*p*-dioxin (2,3,7-triCDD)

and octachlorodibenzo-*p*-dioxin (OCDD) using either glucose or 1,4-dioxane as a carbon source (Nakamiya et al. 2005). Less chlorinated catechols and catechols themselves along with *cis, cis*-muconates were identified as by-products, this leading to the postulation of a new dioxin degradation pathway.

Considering the fungal degradation studies on PCDFs, the number of publications dealing with furans is very limited. A mixture of 10 of tetra- to octachlorodibenzofurans with chlorine substituents on the aromatic rings at 2-, 3-, 7-, and 8- positions was partially degraded by *Phanerochaete sordida* YK-624 stationary cultures in low-nitrogen medium and 4,5-dichlorocatechol and tetrachlorocatechol were formed from the degradation of 2,3,7,8-tetra- and octaCDD, respectively (Takada et al. 1996). The white rot fungus *Phlebia lindtneri* was able to degrade 2,8-diCDF producing hydroxyl-diCDF as an intermediate differently from *P. sordida* (Mori and Kondo 2002b). A yeast-like fungal strain was isolated from a dioxin-contaminated soil under selective conditions: the soil sample was suspended in a mineral salt medium supplemented with 1 g L^{-1} of dibenzofuran, a model compound for highly chlorinated dibenzofurans (Hammer et al. 1998). The yeast strain, identified as *Trichosporon mucoides*, metabolized dibenzofuran at fast rate (more than 50 % of the compound added was degraded within 8 h) and water-soluble intermediates were detected during the incubation period. In detail, different isomers of mono- and di-hydroxylated dibenzofurans were observed, along with the ring-cleavage product (2-(1-carboxy methylidene)-2,3-dihydrobenzo [*b*]furanylidene glycolic acid). The ability of the imperfect soil fungus *Paecilomyces lilacinus* to transform dibenzofurans was also investigated and 14 degradation intermediates were identified by UV spectroscopy, mass spectrometry, and proton nuclear magnetic resonance spectroscopy (Gesell et al. 2004). According to this study, the biotransformation of dibenzofuran was initiated by two separate hydroxylation steps leading to the accumulation of monohydroxylated- and dihydroxylated-dibenzofurans. Further oxidation yielded to the ring-cleavage of dibenzofuran structure, which was not previously described for filamentous fungi. The ring fission products were identified as benzo[*b*]furo[3,2-d]-2-pyrone-6-carboxylic acid and [2-(1-carboxy-methylidene)-benzofuran-3-ylidene]-hydroxy-acetic acid and its derivatives hydroxylated at 7 and 8- positions on the non-cleaved ring. Riboside conjugates of 2-hydroxydibenzofuran and 3-hydroxydibenzofuran were also detected. These results suggested the involvement of CYP450 in the initial steps of the furans transformation process as already reported for other chlorinated environmental pollutants; the formation of the riboside conjugates that increased with the dibenzofuran concentration supported the common opinion that different types of conjugation (glycosylation, sulfate, or glutathion conjugation) are used by eukaryotic cells to encounter toxic hydroxylated compounds.

8.4 Emerging Organic Micropollutants: Endocrine Disruptors, Pharmaceuticals and Personal-Care Products[2]

In recent years, the presence of organic micropollutants (MPs) in the aquatic environment (i.e., surface waters, groundwaters and drinking water reservoirs) has become an issue of worldwide concern (Luo et al. 2014). The development of powerful and sensitive analytical techniques contributed to the detection and quantification of a large number of organic chemicals in the environment, thus compelling the scientific community to consider this type of contamination as a subject that merits attention (Santos et al. 2010). Such MPs, also termed as "emerging" organic contaminants (Gasser et al. 2014), encompass a vast and ever-expanding array of substances, mainly of anthropogenic origin, that are detected in surface waters at trace concentrations (ng to μg per liter). Among these substances, pharmaceuticals and personal care products (PPCPs) such as antibiotics, anti-inflammatories, cytostatic drugs, disinfectants, β-blockers, UV-filters, fragrances, and other compounds are common water MPs (Verlicchi et al. 2010; Luo et al. 2014). The other group is represented by endocrine disrupting compounds, i.e., chemical compounds that interfere with the endocrine system and therefore could be detrimental to human and wildlife health. The most studied and detected EDs are the natural hormones estrone and 17β-estradiol, the synthetic estrogen 17α-ethinylestradiol, industrial compounds and additives, nonylphenols (NPs), bisphenol A (BPA) and also the personal care product triclosan (Cajthaml et al. 2009a). Besides, endocrine disrupting effects have been reported for PAHs (Arcaro et al. 1999), PCBs, CBAs, PCDDs and other POPs (Cajthaml 2014).

The occurrence of emerging organic MPs in the environment has been associated with a number of negative effects, mainly regarding the aquatic ecosystems (see below). Till date, however, discharge guidelines and standards for most of the MPs do not exist. For example, environmental quality standards for a minority of MPs (e.g., NPs, BPA, diethylhexyl phthalate-DEHP and diuron) were stipulated in Directive 2008/105/EC (European Parliament and The Council 2008). Nonylphenol and nonylphenol ethoxylates were also recognized as toxic substances by the Canadian government (Canadian Environmental Protection Act 1999); but other potent MPs, such as PPCP and EDs have not been included yet in the list of regulated substances (Luo et al. 2014).

[2]Additional information on fungal treatment of emerging pollutant in municipal wastewater is presented in Chap. 5—*Fungal bioremediation of emerging micropollutants in municipal wastewater*.

8.4.1 Toxicity of Emerging Micropollutants

The adverse effects associated with the occurrence of MPs in the aquatic environment include short-term and long-term toxicity, endocrine disrupting effects and induced antibiotic resistance of microorganisms—although the latter phenomenon is still largely disputed (Luo et al. 2014). Some damaging health effects that occur in human and wildlife are due to the exposure to particular organic pollutants, many of which could be detected in all human beings, which cause damage or impairment of neural, immune and reproductive systems (Luo et al. 2014). Although the majority of EDs and PPCP are not considered as recalcitrant as other POPs (e.g., PAHs, PCBs, PCDDs, etc.), the widespread usage and continuous delivery of low concentration of contaminants to the receiving ecosystems might give rise to toxicity even without high rates of persistence (Santos et al. 2010). It has been demonstrated that environmental concentrations of EDs induce the "feminization" of male tissues and/or the presence of female reproductive organs in males, along with other dysfunctions of the reproductive systems in many aquatic animals (Liu et al. 2009; Santos et al. 2010; Eggen et al. 2014). Since the 1940s, decline in semen quality and increasing incidence of testicular cancer in humans has been observed, although a direct link between exposure to EDs and manifestation of negative effects could be proved only in model organisms (UNEP 2012), which is also applicable to a number of scientific studies that reported the thyroid-disrupting effects of EDs and other environmental MPs. (e.g., BPA, phthalates, and perfluorinated chemicals) (Boas et al. 2012).

The challenges facing environmental scientists are significant. However, it is essential to conduct research that increases our scientific knowledge on the wide range of water MPs, including a detailed understanding of their environmental cycling and toxicological impact. To understand the dimension and the importance of such problem, it is interesting to look at the strategies adopted by the leading EU countries. As an example, the Swiss Federal Office for the Environment (FOEN) conducted the project "Strategy Micropoll" in the period 2006–2010 in order to develop a strategy to reduce the input of organic MPs from domestic wastewater into Swiss surface waters. Results from this project led to the development of the VSA-Platform "Verfahrenstechnik Mikroverunreinigungen," for which the Swiss Federation is going to grant ca. 1 billion euro funds in the following years.

8.4.2 Fungal Biodegradation of EDs

The number of scientific contributions dealing with fungal degradation of EDs and other water MPs has been growing steadily through the last decades. The majority of such studies, although not all, focused on the groups of wood-rotting fungi, mainly because of their capability to produce unspecific, high redox potential LEs (Ashger et al. 2008; Cajthaml et al. 2009a). These potent biocatalysts can, bring

about the attack on the phenolic moieties present in the chemical structure of many of the MPs, resulting in the formation of phenoxyl radicals, which subsequently undergo oxidative coupling. Once dimers, tetramers or oligomers are formed, the overall biological and endocrine disrupting activity is significantly reduced or completely removed (Cabana et al. 2007b; Cajthaml 2014). Intracellular enzymatic complexes like CYP450 were also shown to be involved in the degradation of several compounds by the intact fungal cells, as well as Fenton-like reactions (Marco-Urrea et al. 2010d; Cajthaml 2014). Due to the number and diversity of compounds in the MPs family, we will only select some of the compounds which were extensively studied and provide a brief overview of the most significant results reported in the scientific literature.

Typical man-made EDs with estrogen-like action include NPs, BPA and 17α-ethinylestradiol (EE2). NPs isomers mainly occur in the environment as degradation products of nonylphenol-polyethoxylates which are widely used as nonionic surfactants in many industrial processes. BPA is a key building block for polycarbonate plastics and epoxy resins, but it is also used in a number of other materials and applications. Synthetic estrogens, such as EE2, are used worldwide as oral contraceptives. Non-metabolized EE2 and its conjugates are first excreted into the urban sewage systems and, after incomplete degradation in the wastewater treatment processes, they reach the surface water ecosystems. The capability of the above-cited compounds to bind human receptors and cause endocrine disruptions has been documented (Cabana et al. 2007b).

NPs: NPs were found to be very efficiently degraded by LF cultures and purified LEs (see Corvini et al. 2006; Cabana et al. 2007b; Cajthaml et al. 2009a; Cajthaml 2014). The intermediate steps of NPs biotransformation by LFs, including (C–O) coupling reactions and oxidation at the terminal carbon of the alkyl chain, are summarized in Fig. 8.4. Several studies reported the formation of dimeric and holigomeric NP transformation products using Lac of *Coriolopsis gallica* and *C. polyzona* (Cabana et al. 2007a; Torres-Duarte et al. 2012), a mechanism that is known to suppress the estrogenic activity of the parent compounds. Tsutsumi et al. (2001) suggested similar transformation products following oxidation with MnP of *P. chrysosporium*. This coupling metabolite is probably produced as a result of the action of LEs on phenolic hydroxyl groups that results in the formation of phenoxyl radicals which subsequently undergo different spontaneous reactions, including oxidative coupling (Hofrichter 2002; Dec et al. 2003). Dubroca et al. (2005) proposed the formation of a direct C–C bond between the phenolic rings and the analogous structure was finally identified by Wang et al. (2012) with respect to *p*-t-octylphenol. Production of NP oligomers was similarly observed by other authors who tested, e.g., the degradation abilities of Lac from the aquatic fungus *Clavariopsis aquatica* (Junghanns et al. 2005). Syed et al. (2011, 2013) investigated the involvement of CYP450 (CYP5136A3 and CYP63A2) from *P. chrysosporium* in the NP transformation mechanisms and discovered a very versatile function of these intracellular monooxygenases. They documented the oxidation of NP and other alkylphenols at the terminal alkyl chain carbon (omega oxidation) *via* alcohols resulting in aldehydes. Based on the literature and known pathway in

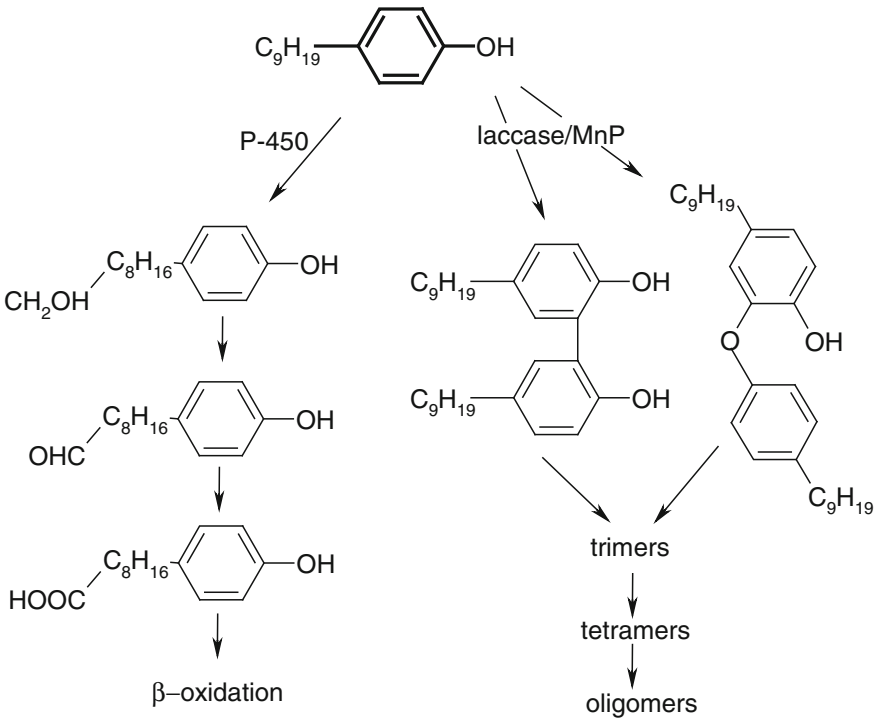

Fig. 8.4 Nonylphenols biotransformation by LFs—adapted from Cajthaml (2014)

yeasts/fungi for the degradation of an alkylphenol moiety, the authors concluded that the terminal oxidation of the alkyl side-chain is followed by the removal of the terminal carbons via the β-oxidation of NPs *via* carbon–carbon (C–C) and carbon–oxygen pathway as noted in other fungi, e.g., the yeast *Candida aquatextoris* (Vallini et al. 2001; Corvini et al. 2006; Rozalska et al. 2010). The possible entry into the basal metabolism is also supported by a study of Dubroca et al. (2005), who observed mineralization of NPs by a culture of *T. versicolor* using ^{14}C-labeled NP. Surprisingly, the potentially anticipated metabolites following direct hydroxylation of NPs by CYP450 of LFs have not been detected yet (Corvini et al. 2006). It was shown that the production of the coupled NPs leads to a reduction of estrogenic activity (Tsutsumi et al. 2001), an aspect which has not been investigated with respect to alkylphenol carboxylic acids so far.

BPA: BPA has also been shown to be susceptible to biodegradation by several fungi and fungal enzymes. Fukuda et al. (2001) demonstrated that purified Lac from *Trametes villosa* efficiently transformed BPA under in vitro conditions without any requirement for electron-shuttling mediator. Structural analysis of the BPA degradation products using nuclear magnetic resonance spectroscopy indicated that the oligomers of BPA were formed as a result of successive oxidation–condensation entailing C–C coupling (Fukuda et al. 2004). The presence of oligomeric fragments,

each including a phenol moiety, suggested the cleavage of the oligomeric products to release 4-isopropenylphenol or simultaneous fission of the original BPA molecule. Neither the soluble nor the insoluble fractions of the BPA reaction products had estrogenic activity, even at high concentrations (Fukuda et al. 2004). Similar metabolites were also detected by Michizoe et al. (2005) who identified 4-isopropylphenol following the transformation of BPA by Lac (*Trametes* sp.) in a reverse micelle system in organic media, and by Cabana et al. (2007a), who detected BPA polymeric structures. Huang et al. (2005) proposed that the formation of C–O coupled polymer occurred *via* radical-mediated mechanisms triggered by horseradish peroxidase and reported the formation of 4-isopropenylphenol. Wang et al. (2012, 2013a) tested LiP from *P. sordida* and identified dimerized BPA with C–C coupling and also with a C–O bridge. The same authors also detected hydroxylated BPA and suggested the involvement of CYP450 in the transformation (Wang et al. 2013b). Hirano et al. (2000) used a MnP isoenzyme from *P.ostreatus* and detected mostly breakdown products such as phenol, *p*-cresol, 4-isopropenylphenol and its dimer. Structures akin to these were reported by Torres-Duarte et al. (2012) following the treatment of BPA with Lac of *C. gallica*. It is noteworthy that in all the studies where the authors monitored the estrogenic potency of samples after treatment, the removal of BPA by either purified LEs or fungal cultures was always accompanied by a simultaneous depletion of the estrogenic activity (Tsutsumi et al. 2001; Cabana et al. 2007b; Cajthaml et al. 2009a; Torres-Duarte et al. 2012).

EE2: Synthetic and natural estrogens are considered to be major contributors to the estrogenic activity associated with wastewater treatment plant (WWTP) effluents due to their incomplete removal in conventional wastewater treatment process (Clouzot et al. 2008). EE2 was generally reported to be more resistant to biodegradation than other natural estrogens (Cajthaml et al. 2009b; Tran et al. 2013). Numerous authors reported the high efficiency of LFs and LEs in biodegradation or transformation of EE2 (see reviews Cabana et al. 2007a; Cajthaml et al. 2009b; Demarche et al. 2012b) and effective EE2 degradation by whole cultures of several LFs has been described (Blánquez and Guieysse 2008; Cajthaml et al. 2009a). Estrogenic activity generally decreased with the progression of degradation. However, during EE2 biodegradation tests with *I. lacteus*, *P. ostreatus*, *P. cinnabarinus* and *P. magnolia*, residual or increased estrogenic activity was observed, suggesting the production of estrogen-like degradation products (Cajthaml et al. 2009a). Suzuki et al. (2003) investigated the removal of the steroidal hormones E2 and EE2 in organic solvent under biphasic conditions using MnP and the Lac-mediator (hydroxybenzotriazole—HBT) systems from *P. chrysosporium* ME-446 and *T. versicolor* IFO-6482. They also monitored the evolution of estrogenic activity and documented the high potential of LEs for the biodegradation of EE2. Similar results were obtained by other authors (Tanaka et al. 2001) who worked with Lac from *Trametes* sp. and *Pycnoporus coccineus* in a rotating reactor. Auriol et al. (2007) studied the *T. versicolor* Lac-catalyzed conversion of natural oestrogens and EE2 and showed that the Lac-catalyzed system was not significantly affected by the presence of a real municipal wastewater matrix.

Moreover, when HBT was used as a mediator, the Lac-catalyzed system showed an improved efficiency. However, limited information is available about the fungal biotransformation products of EE2. Kresinová et al. (2012) published a study including a broad set of in vivo and in vitro experiments using sub-cellular and cellular fractions as well as purified LEs from *P. ostreatus* and suggested that the EE2 degradation mechanisms could involve intracellular microsomal enzymes, mycelium-associated laccase-like isoenzymes and extracellular ligninases (MnP and Lac). The synergic contributions of the individual transformation steps were documented and each phase of transformation was reported to individually reduce estrogenic activity. The authors also detected a set of intermediates clearly indicating that in spite of its recalcitrance, EE2 is efficiently transformed by the ligninolytic system and also by the CYP450 monooxygenase system. The intermediates included methoxylated estron (E1) and dioxo 17β-estradiol (E2), which were probably formed by the action of Lac, and dehydrogenated and hydroxylated EE2. Metabolites similar to the latter ones were also described by Choudhary et al. (2004) and Della et al. (2008) following the incubation of EE2 with the mucoromycete *Cunninghamella elegans* and microalgae, respectively. In a previous study, Tanaka et al. (2001) detected dimeric products of EE2 after treatment with Lac from *Trametes* sp.and observed a significant decrease in estrogenic activity but they did not perform any further analysis of the intermediates in detail. Other authors studied EE2 and natural estrogen biotransformation by Lac from the ascomycete *Myceliphthora thermophila* in a membrane bioreactor (Lloret et al. 2013) and detected two dimers and trimers of EE2 along with several transformation products of 17β-estradiol (E2). They identified E1 following Lac oxidation of E2 and characterized two C–C and two C–O dimers as well as four trimers with various coupling combinations and, concluding that the Lac transformation of E2 is analogous to that of EE2. The authors generally observed a decrease in the estrogenic activity during the course of the transformation. Similar results were achieved using Lac from *Myceliophthora* and *Trametes pubescens*, both adsorbed on glass beads. The enzymes were used for the transformation of natural estrogen E2 (Nicotra et al. 2004) and the reaction products were also tentatively identified as C–C dimeric compounds with coupling between either 4′-4 or 2′-2 and C–O (phenolic ring) at the 4′ or 2′ positions.

8.4.3 Fungal Degradation of Pharmaceuticals and Personal Care Products (PPCP)

The ability of fungi, especially belonging to the group of wood-inhabiting Basidiomycetes, to degrade PPCP detected in surface and process waters and sludge biosolids, has been demonstrated in recent years. In relation to this issue, non-steroidal anti-inflammatory drugs (NSAID) (e.g., diclofenac, naproxen, ibuprofen, ketoprofen), blood lipid regulators (clofibric acid), β-blockers (e.g.,

propanolol, atenolol), anti-epileptic drugs, (carbamazepine) and antibiotics (e.g., sulfamethoxazole, ciprofloxacin, erythromycin) are the most intensively studied pharmaceutical compounds (Marco-Urrea et al. 2009, 2010a, b, c, d; Accinelli et al. 2010; Rodarte-Morales et al. 2011, 2012; Domaradzka et al. 2015).

Marco-Urrea et al. (2009) screened four white rot fungi (*T. versicolor*, *I. lacteus*, *G. lucidum* and *P. chrysosporium*) for their ability to co-metabolize ibuprofen (IBU), clofibric acid (CA) and carbamazepine (CBZ) in model (glucose-supplemented) liquid systems. All strains efficiently removed IBU while the turkey-tail fungus *T. versicolor* was the most efficient in the biotransformation of the recalcitrant CA and CBZ. Failure of PPCP degradation by Lac and Lac-mediator systems under in vitro condition, reduced biotransformation rates in the presence of CYP450 inhibitors and the detection of hydroxylated and di-hydroxylated derivatives of IBU prompted the authors to conclude that CYP450 monooxygenase system is responsible for the initial oxidative attack to this NSAID. Further experiments in which *T. versicolor* was incubated in the presence of other NSAIDs (naproxen, ketoprofen and diclofenac) showed the involvement of intracellular (CYP450) and to a lesser extent, extracellular (Lac) enzymatic systems in pharmaceuticals degradation by this fungus (Marco-Urrea et al. 2010a, b, c). In all cases, the time course of the degradation of NSAIDs was accompanied by a significant drop in toxicity as assessed by the test with the luminescent bacterium *Vibrio fisheri*, and also by the presence of transient biotransformation products, the chemical structure of which had been elucidated by nuclear magnetic resonance techniques. The same authors took care to demonstrate whether *T. versicolor* could use mechanisms other than those reported above (CYP450-Lac) to oxidize representative PPCP (Marco-Urrea et al. 2010d). To rule this out, the fungus was incubated under conditions triggering the production of ROS (e.g., $OH^{\cdot}$) *via* quinone-redox cycling (see also Gómez-Toribio et al. 2009). The degradation of CBZ, CA, and the β-blockers atenolol and propranolol occured (80 % of 10 mg L^{-1} compounds initially added was removed after 6 h) only under 2,6-dimethoxy-1, 4-benzoquinone (DBQ) redox cycling conditions (i.e., fungus mediated Fenton-like mechanism) although the NSAID-bioconversion process was short-lived due to the depletion of DBQ by the same $OH^{\cdot}$. Several hydroxylated metabolites where reported for the first time, although the authors noted the complete disappearance of degradation products at the end of the incubation period (Marco-Urrea et al. 2010d).

Scale-up processes of *T. versicolor*-mediated degradation of PPCP from flasks to bubble column reactors were successful under both batch and continuous mode of operation (Blánquez and Guieysse 2008; Jelić et al. 2012), even in the presence of non-sterile real wastewaters from WWTP (Cruz-Morató et al. 2013), although sources of C and N (glucose and ammonium tartrate) were necessary for the growth of the fungus. In some cases, however, the production of hydroxylated metabolites with increased toxicity with respect to parent molecules has also been reported (Cruz-Morató et al. 2013).

8.4.3.1 White Rot Inocula for the Treatment of Sludge Biosolids[3]

Depending on their hydrophobicity, water MPs tend to partition in wastewater processes and adsorb onto sludges and therefore, considerable concentrations of PPCP and EDs are often detected in sludge biosolids (Cabana et al. 2007b; Clarke and Smith 2011). In consideration of this, Rodriguez-Rodriguez and colleagues (2012) tested a sludge-bioaugmentation approach using *T. versicolor* mycelia to remove the load of organic MPs therein. The fungus, pre-grown on lignocellulose-based pellets, was mixed (38 % w/w dry weight) with sewage sludge and control microcosms were set up with non-inoculated, sterile lignocellulose. The fungus was deemed responsible of a faster disappearance of some MPs (ranitidine and fenofibrate) and enhanced the removal of atorvastatin, diclofenac and hydrochlorothiazide in the initial phase of incubation. However, *T. versicolor* was rapidly overgrown by members of the sludge microflora and the overall degradation of several compounds at the end of the incubation period remained similar to that observed in the non-inoculated control. The authors therefore concluded that the bioaugmentation with white rot inoculant is feasible and advantageous with respect to the rates of contaminants removal but the biostimulation of the sludge microbiota, as assessed *via* denaturing gradient gel electrophoresis (DGGE), merits equal attention in the light of future applications (Rodríguez-Rodríguez et al. 2012).

8.4.3.2 Enzyme Aggregates and Immobilized Forms for the Treatment of Process Waters

The pollutant degradation potential of LEs was widely evidenced in the previous sections of this chapter. However, the application of fungal catalysts in free solution is unfeasible due to the denaturing and/or inactivating conditions (temperature, pH, metal ions etc.) often encountered in sewage or waste waters (Cabana et al. 2007b; Demarche et al. 2012a, b; Kües 2015). Therefore, for the optimal retention of catalytic properties and to promote an enduring reuse of such biocatalysts in continuous-flow processes, immobilization, encapsulation, and insolubilization are techniques of great interest nowadays.

Immobilization of Lac for its potential use in a variety of applications, including bioremediation, has been the subject of intensive research in the past decades (see the review Durán et al. 2002; D'Annibale et al. 1999, 2000). Among the carriers for the immobilization/encapsulation pre-activated for ionic or covalent binding with the enzyme, glass and mesoporous silica beads, ceramics and clay minerals, natural (chitin, chitosan, agarose, cellulose nanofibers) and synthetic (polystyrene, nylon 6, poly acrylamide) polymers were successfully used in a number of studies and

[3]Further information on the use of white rot and brown rot fungi in wastewater and sludge treatment can be found in Chap. 4—*Application of biosorption and biodegradation function of fungi in wastewater and sludge treatment*.

applications (Durán et al. 2002; Ba et al. 2013; Kües 2015). The only technical disadvantage of such immobilized or encapsulated forms is the low ratio of enzyme to support in the overall volume of biocatalyst (Cabana et al. 2007b; Ba et al. 2014). To overcome this, carrier-free insolubilization of laccase and other enzymes can be achieved by producing cross-linked enzyme aggregates (CLEAs). The technique relies on the precipitation of enzymes (e.g., Lac) and the subsequent addition of cross-linking agents, which bind together amino-acid residues of proteins to form stable and insoluble aggregates. The most widely used cross-linking agents are homobifunctional aldehydes (dialdehydes) such as glutaraldehyde, glyoxal and dextran polyaldehydes, although these chemical agents can have adverse effect on aquatic organisms (and humans) besides causing a certain loss of enzymatic activity during biocatalyst preparation (Ba et al. 2013). Chitosan represents a valuable alternative to dialdehydes as cross-linkers, provided that the carboxylic groups of the protein of interest are pre-activated by carbodiimides such as 1-ethyl-3-(3-dimethylaminoisopropyl) carbodiimide hydrochloride (EDC) (Zhang et al. 2009; Arsenault et al. 2011; Cabana et al. 2011; Ba et al. 2013).

Several studies reported that regardless of the cross-linker used, Lac- or peroxidases-based CLEAs are biocatalysts with improved properties (i.e., stability, catalytic efficiency and reiterate use) as compared to enzymes in their free-form and offer greater potential for applicability in the treatment of wastewater MPs (Cabana et al. 2007b; Taboada-Puig et al. 2011; Ba et al. 2013; Kües 2015). In an attempt to broaden the range of substrates oxidized by these biocatalysts, some authors have recently tested the co-aggregation of different type of enzymes, i.e., combi-CLEAs. For instance, Ba et al. (2014) characterized combi-CLEAs of *T. versicolor* Lac and mushroom-derived Tyr and then tested its efficiency for the removal of acetaminophen. Taboada-Puig et al. (2011) instead optimized the co-insolubilization of *B. adusta* VP and *A. niger* glucose oxidase (GOD), the latter serving as ancillary H_2O_2-producing enzyme. A good example of combi-CLEAs is represented by the concomitant aggregation of Lac, VP, and GOD from *T. versicolor, B. adusta* and *A. niger*, respectively (Touahar et al. 2014). This versatile combi-CLEA was able to oxidize a wider spectrum of MPs (pharmaceuticals) than Lac-based one, although the requirement of co-substrates (e.g., GOD substrates and Mn^{2+}) for its optimal activity might generate additional costs in case of practical applications.

Different types of reactors bearing immobilized or insolubilized enzymes have been tested for the removal of MPs from artificial or real wastewaters, although most of the research work has been carried out at bench-scale. Packed bed, fluidized bed, perfusion basket, and membrane reactors (Cabana et al. 2007a, 2009a, b; Taboada-Puig et al. 2011; Lloret et al. 2012a, b; Nair et al. 2013) were successfully used in batch or continuous mode of operation. Among these, membrane reactors appear to be the most promising for the treatment of micropollutant-containing waters under continuous-flow conditions since the porosity of the membrane allows the retention of biocatalyst while letting the permeate to flow through (Ba et al. 2013; Kües 2015).

The pilot-scale experiment of Gasser et al. (2014) demonstrated that a fluidized bed reactor (460 L total working volume) equipped with *Thielavia* (*Myceliophthora thermophila*) laccase immobilized on fumed silica nanoparticles (fsNP, 0.5 kg) and an ultra-filtration membrane unit (UF porosity 0.04 μm) could effectively and continuously remove BPA from wastewater treatment plant effluents. The experiment was conducted under field conditions, at a Swiss wastewater treatment plant, over a period of 45 days and the reactor was fed with WWTP effluents and operated continuously in a cyclic filtration mode which included membrane backwash in order to prevent clogging (net permeate production78 L h^{-1}). At the end of the test, the Lac-fsNP biocatalyst retained around 30–40 % of its initial activity and approximately 66 % of BPA in the reactor's inlet was removed (Gasser et al. 2014). The authors also estimated the overall economic feasibility of this novel enzymatic treatment at WWTP and concluded that the costs for the Lac-fsNP bioreactor process (0.130 € m^{-3}) are just slightly higher than other suitable treatments, namely ozonation (0.078 € m^{-3}) and powdered activated carbon adsorption (0.114 € m^{-3}). Further improvement of the treatment efficiency and additional reduction of costs might be achieved by optimizing some of the running parameters of the process (e.g., mixing conditions, nanobiocatalyst load, and hydraulic retention time). Moreover, as already stated above for combi-CLEAs, the co-immobilization of enzyme mixtures on the same nano-carrier could widen the range of wastewater MPs removed by such bioreactor treatment, thus enhancing the overall applicability potential of enzymatic treatment technologies at WWTP.

8.5 Pilot- and Field-Scale Mycoremediation

Pilot- and full-scale applications are essential to evaluate the effectiveness and economic viability of fungal technologies. However, till date, there are few reports concerning the use of fungi in the treatment of contaminated matrices at field-scale and thus many technical and engineering challenges remained in the area of its application. For instance, the model WRF *P. chrysosporium* was very efficiently degrading organic pollutants in laboratory-scale experiments but never showed similar effectiveness in any field-scale tests.

In the early 1990s, *P. chrysosporium* was used in a pilot-scale treatability study at a trinitrotoluene (TNT) contaminated-site—a former USA Naval submarine base (Bangor, Washington). The initial TNT concentration of 1844 ppm was reduced to 1087 ppm in 120 days (degradation of 41 % of the original TNT content). However, the final concentration was still significantly above the target level of 30 ppm and thus the test was considered a failure (US EPA Handbook 1993).

A decade later, a field-scale trial for the remediation of crude oil-contaminated soil was performed using composting biopiles technology where the efficiency of the traditional process (soil aerated and amended with bulking agents, such as straw, sawdust, wood chips, etc.) was compared with that of a bioaugmentation approach (Li et al. 2002). In particular, three fungal strains characterized by high

lipase activities (*Mucor* sp., *Cunninghamella* sp. and *Fusarium* sp.) were isolated and reintroduced into the contaminated matrices either with or without *P. chrysosporium.* The results showed that the inoculum composed of only indigenous fungi remarkably increased the degradation rate of total petroleum hydrocarbons (TPH) with respect to non-inoculated soil, while the introduction of the allochthonous fungus did not improve the efficiency of TPH removal process.

A similar outcome was also achieved a few years later in a pilot-scale experiment for the treatment of soil contaminated by TNT, PAHs, PCDDs and PCDFs (Tuomela et al. 2012). Pine bark was selected as a substrate to support the growth of the litter-decomposing fungus *Phanerochaete velutina*. Once the inoculum was prepared, the allochthonous fungus was introduced into soil and the extent of degradation of the target pollutants was evaluated. *P. velutina* growth was completely inhibited when introduced in soil with high concentration of PAHs (5000 mg kg^{-1}) and TNT (>10 g kg^{-1}) and no degradation occurred, moreover, the presence of molds such as *Trichoderma* clearly prevented its growth. On the other hand, dilution of PAH and TNT-contaminated soil with garden compost significantly enhanced the degradation process. However, since the growth of both *P. velutina* and indigenous microorganisms was clearly stimulated under such conditions, the role of the litter-decomposing soil fungus in the removal of PAHs and TNT could not be clarified. These results were also confirmed two years later when a field-scale experiment (2 t) for the treatment of a PAH-contaminated soil was carried out (Winquist et al. 2014). At first, the degradation of PAHs was assessed in a laboratory-scale experiment where soil was mixed with composted green waste (1:1) and incubated with or without *P. velutina*. The higher extent of degradation (96 % for 4-ring PAHs and 39 % for 5- and 6-ring PAHs) observed in microcosms inoculated with the fungus after three months of incubation as compared to the biostimulated ones prompted the researchers to scale-up the process. Unfortunately, even if the percentage of degraded PAHs in soil under field condition was extremely high, the results obtained in the case of *P. velutina*-inoculated treatment (bioaugmentation) or non-inoculated treatment (biostimulation) were comparable. This again suggested that the degradation of PAHs could not be ascribed univocally to the exogenous fungus.

P. velutina inoculated on pine bark was also used to remediate a TNT-contaminated soil diluted with composted green waste (1:20) at pilot-scale level (0.3 t) (Anasonye et al. 2015). TNT was efficiently degraded up to 80 % in 107 days. The quantification of the fungal ITS region DNA copy number by quantitative PCR (qPCR) using specific primers for *P. velutina* proved that the fungus grew in such contaminated soil withstanding high concentration of TNT and autochthonous microorganisms. However, due to economical and practical issues, no control with non-inoculated soil was prepared and thus the real efficiency of the fungus in the removal of TNT could not be compared with the degradation ability of indigenous microbiota present in the green waste.

The capability of the white rot fungus *T. versicolor* to remediate a pentachlorophenol (PCP)-contaminated soil at field-scale level was also evaluated (Walter et al. 2005). The contaminated soil was collected at a timber treatment plant

where PCP was widely used as a wood preservative. In a previous study performed at laboratory scale, PCP was removed from both biostimulated (soil mixed only with a lignocellulosic substrate) and bioaugmentated microcosms (*T. versicolor*-colonized lignocellulosic substrate) when the contaminated soil was diluted with uncontaminated soil. The role of the fungal inoculum versus indigenous microorganisms in the PCP degradation was not completely clarified, but complete mineralization of PCP was observed only in the presence of the allochthonous fungus. In view of this last result, soil "cells" (final volume approx. 500 L) were designed to carry out exclusively a *T. versicolor*-based treatment. The contaminated soil (1000 mg kg^{-1}) was diluted with non-contaminated soil and mixed with wood chips obtained from cuttings and shreds. Thereafter, such blended pile of material was mixed with a specific formulation composed of sawdust, cornmeal and starch previously inoculated with *T. versicolor* to obtain a final ratio of soil: woodchips: fungal inoculum of 40: 20: 40 or 60: 20: 20, respectively. After a rapid decline in PCP concentration within the first 25 weeks of incubation, the degradation rate remarkably decreased. Nonetheless, after 2.5 years, more than 90 % of the original PCP content was removed in both cells (20 or 40 % of fungal inoculum used) indicating that PCP levels at the end of treatment were not statistically different with respect to the differences in the amount of fungal inoculum.

P. ostreatus is one of the best candidates for potential applicability in mycoremediation of soils, therefore several field-scale tests based on the use of such were designed. This WRF was used for the filed-scale remediation of explosive-contaminated soil from the Yorktown Naval Weapons Station (Virginia, USA) where munitions manufacturing process was carried out for decades (Axtell et al. 2000). Two plots (6 cubic yards) of contaminated soil were blended with 3 cubic yards of a substrate mixture previously colonized by *P. ostreatus*. Concentrations of the target explosives, namely TNT, HMX (octogen) and RDX (cyclonite), were noticeably reduced during a 62-day incubation period. Nonetheless, in soil amended only with the substrate mixture, the concentrations of TNT, HMX, and RDX were also reduced substantially during the same period suggesting that the addition of amendments enhanced the growth and activity of indigenous microorganisms and thus sufficient to promote the degradation of these compounds in soil. However, the role of the *P.ostreatus* in this field-scale test was not elucidated.

Further application of fungal bioaugmentation was evaluated for the remediation of creosote-contaminated soil at a wood-preserving site in West Virginia (Lamar et al. 2002). After a preliminary bench-scale evaluation of the degradation capabilities of two WRFs, namely *P. ostreatus* and *I. lacteus*, *P. ostreatus* from a commercial spawn provider was selected to scale up the process. Two pilot-scale biocells comprising the contaminated soil, a sterile substrate (wood splinters or wood splinters with sawdust) and *P. ostreatus* spawn were monitored for 276 days. Concentration of all the 16 PAHs was reduced in both biocells below the industrial risk-based level with the exception of benzo[*a*]pyrene (Lamar et al. 2002). However, the involvement of indigenous microorganisms in the degradation process was not assessed.

The influence of *P. ostreatus* mycelia on the degradation of selected PAHs in soil was investigated under field conditions also in the Northern temperate zone (Hestbjerg et al. 2003). Homogenized *P. ostreatus* refuse from commercial mushroom production was added to PAH-contaminated soils collected from two sources: a former shipyard and coal tar storage from an asphalt factory. Treatments (soil control, soil mixed with autoclaved sawdust substrate and soil mixed with *P. ostreatus* refuse) were set up in concrete cylinders for both soils. In the case of the soil from the coal tar storage, the addition of the oyster mushroom significantly enhanced the degradation rate of 4-ring PAHs compared to the rate in either soil control or biostimulated cylinder, while 5- and 6-ring PAHs were only slightly degraded and no significant difference was observed between the two treatments. On the contrary, the growth and the activity of *P. ostreatus* were completely prevented when inoculated in the other soil, although the factors that resulted in its inefficacy were not clarified.

8.6 Mycoremediation of Soils: Opportunities and Pitfalls[4]

As it was evidenced in the previous sections, there is no doubt that both LFs and NLFs are potent biological tools for the degradation of persistent soil organopollutants and other emerging water MPs. Considering the soil compartment, fungal inoculants were successfully used since the 1980s for remediation studies at laboratory scale but only a limited number of field-scale trials, which were attempted in the past two decades, showed remarkable limitations with regard to the practical application of technologies based on the use of LFs. As a consequence, all the attempts to promote soil mycoremediation on the market and propose it as a valuable on-site bioremediation option have failed (Šašek 2003; Baldrian 2008).

The reasons that lie behind this failure are diverse, as it was already observed by several authors. The first and most important is, without any doubt, the competition between introduced fungi and the resident soil microflora. The idea that a single species, in most cases allochthonous to the soil, should bring about the complete restoration of a contaminated matrix is absolutely unrealistic and unsustainable. Fungi, like bacteria and plant roots introduced into the soil, are influenced by the type and activity of other resident soil organisms and this has a great influence on their colonization capacity. Moreover, the inoculation support onto which the fungi are immobilized prior to their introduction in non-sterile soil (e.g., straw, hay, corn cobs, seeds and seeds husks, wood chips or shavings, bark, etc.) represents a source of nutrients for the autochthonous soil microbiota, this generating divergent effects: an ensuing positive impact on bioremediation efficiency as has been reported in some cases (Lang et al. 2000; Steffen et al. 2007; Federici et al. 2012) and

[4]Additional information on using fungi and mycoaugmented compost to treat polyaromatic hydrocarbon can be found in Chap. 1—*Fungi in composting*.

growth-inhibition and mycophagy in other circumstances (de Boer and van der Wal 2008; Baldrian 2008).

The strict growth conditions required by most white rot fungi represent another significant constraint for the application of fungal technologies at field-scale. For example, *P. chrysosporium* requires high temperature to grow (37 °C) and to produce LEs (30 °C) making its application inadequate under actual environmental conditions (i.e., due to daily and seasonal fluctuations of temperatures) (Hestbjerg et al. 2003). On the other hand, several studies demonstrated that *P. ostreatus* was less affected by temperature and remained active in the degradation of organic pollutants even at very low temperatures (up to 8 °C) (Eggen and Sveum 1999; Lang et al. 2000; Hestbjerg et al. 2001). Moreover, in contrast to *P. chrysosporium* and other white rot fungi such as *D. squalens*, *Pleurotus* sp.was less affected by the presence of soil organisms and being highly competitive with soil microbiota, was more successful at colonizing the contaminated matrix (in der Wiesche et al. 1996; Lang et al. 1997, 1998).

One of the parameters which deeply influence the success of bioremediation application is the bioavailability of contaminants. In turn, this parameter depends on soil organic matter content, texture (e.g., percentage of clay minerals), structure (e.g., peds size, microporosity, etc.) and hydrophobicity of the contaminants. It has been claimed several times that biodegradation hardly ever reaches the complete removal of POPs, for example PAHs and PCBs, this being particularly valid in the case of historically contaminated matrices with high proportion of non-bioavailable compounds. It should be borne in mind that thorough extractions of lipophilic contaminants under harsh conditions that allow the quantification of total contaminant load in a defined matrix (soil) would also include the portion of "aged" pollutants tightly bound to soil components which are inaccessible to microbes in reality. Therefore, the concept of contaminant bioavailability is more readily accepted by decision makers and regulatory organizations (Naidu et al. 2013). Several authors in fact assert that bioavailability should be the underlying basis for risk assessment and in setting the remediation goals at contaminated sites (Latawiec et al. 2011; Naidu et al. 2013). In view of this, establishing contaminant bioavailability and careful testing of the treatability of contaminated matrices prior to field-scale application might help to clarify the feasibility of certain bioremediation interventions. Moreover, accurate compound-specific and ecotoxicological analyses (during and after mycoremediation trials) can help to assess whether the oxidized and more water-soluble derivatives are likely to exert a higher toxicity than parent molecules themselves, or if the residual portion of contaminants that has been immobilized in the soil humus, is inaccessible. In case polar metabolites are formed, as in the case of oxidized high molecular weight (HMW)-PAHs, it is of utmost importance to identify the members of the bacterial community which are able to cooperate with fungi and to consider their contribution.

Another critical factor that requires consideration while dealing with mycoremediation approaches is the formulation and the amount of inoculum which can favor an efficient and long-lasting colonization of the soil matrix. Ligninolytic fungi, both white rots and litter decomposers, are not endowed with intrinsic

capabilities to grow in soil unless an external substrate is also provided. Therefore, lignocellulose pre-colonized by the fungus constitutes the inoculum/amendment for soil. However, using large amount of lignocellulose-immobilized fungi is not economically feasible and would significantly increase the volume of the soil after treatment. A valid alternative to bypass the high costs associated with the production of fungal inoculum is the reuse of Spent Mushroom Compost (SMC) which is a cheap and readily available source of both fungal mycelia and enzymes (Phan and Sabaratnam 2012). In fact, in recent years, the worldwide market of commercially grown edible fungi (i.e., *P. ostreatus*) has been growing exponentially and spent residues from mushroom industries are still considered as a waste to be disposed. In this respect, *P. ostreatus* refuse from commercial mushroom production was exploited as a source of viable mycelia to remediate contaminated soils under field conditions as reported in the previous section.

8.7 Conclusions

The potential of fungi in the remediation of contaminated matrices (soil, sediments, surface water) were highlighted and the shortcomings that make this technology not suitable yet for large-scale application were also analysed in this chapter.

A deeper understanding of the ecology of fungi in their natural environment and a detailed knowledge of the physiology of mycoremediation process, with specific regard to the condition when fungi come in contact with the recalcitrant organic pollutants, could enable us to take a step forward in the application of fungal-based technology for the treatment of polluted environmental matrices.

In this respect, advances in genomics have triggered a revolution in understanding even the most complex biological systems. Indeed, the composition of microbial community and its dynamics during the fungal colonization of contaminated solid matrices as well as the interactions between exogenously introduced mycelia and resident soil microorganisms can be described in depth by means of innovative molecular biology techniques.

Transcriptomics and proteomics are steadily progressing field of science and their application to study mycoremediation processes could clarify how the fungal gene expression and the production of enzymes involved in the degradation process are regulated in response to the variations of environmental condition (i.e., chemicals/pollutants concentration, physiological and nutritional stress condition, etc.). However, due to some methodological biases and the high costs associated to their applications, the analysis of metatranscriptome and/or metaproteome in contaminated/treated matrices is still limited to a few studies at bench-scale level. In the near future, as soon as these limitations could be surmounted, these approaches might be used as a tool for understanding the real potential of fungal inocula in the clean up of polluted environmental compartments.

Concerning this, mycoremediation cannot be considered as a method of choice nowadays, but it still deserves attention because the combination of fungal

treatment with bacteria-based approaches or with phytoremediation could overcome the limitations associated with their stand-alone application. Moreover, despite the failure in the bioaugmentation of allochthonous species in soil, the importance of fungi in some widely accepted bioremediation approaches (e.g., co-composting of polluted matrices, biopiling, etc.) should not be overlooked. In some specific cases, such as low permeability clay soils where conventional (e.g., in situ) technologies are not applicable or ineffective, the mere addition of lignocellulose might represent a valuable alternative to stimulate the growth of resident mycoflora, and, in turn, bacterial populations that could attack the target pollutants.

On the other hand, the application of high redox potential fungal enzymes (laccases, peroxidases and peroxigenases) for the treatment of micropollutant-contaminated wastewaters appears more realistic and feasible than other approaches. In this respect, the development of bioreactors with immobilized/insolubilized biocatalysts appears to be at a technologically mature stage. In the authors' opinion, this is the most promising and likely fungal-derived technology that have a chance to be practically applied in the near future. The work of Gasser et al. (2014), along with a plethora of studies targeting the implementation of tertiary treatments at wastewater treatment plants, reinforces this assertion. Nonetheless, such biocatalyst-based bioreactors should not be viewed exclusively as an end-of-pipe (tertiary) treatment unit at urban WWTP because similar technologies might also be applied to reduce the chemical burden arising from critical point source of MPs contamination, such as chemical and pharmaceutical production plants effluents.

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References

Accinelli C, Saccà ML, Batisson I, Fick J, Mencarelli M, Grabic R (2010) Removal of oseltamivir (Tamiflu) and other selected pharmaceuticals from wastewater using a granular bioplastic formulation entrapping propagules of *Phanerochaete chrysosporium*. Chemosphere 81: 436–443

Adebusoye SA, Picardal FW, Ilori MO, Amund OO (2008) Influence of chlorobenzoic acids on the growth and degradation potentials of PCB-degrading microorganisms. World J Microb Biot 24:1203–1208

Anasonye F, Winquist E, Räsänen M, Kontro J, Björklöf K, Vasilyeva G, Jørgensen K, Steffen K, Tuomela M (2015) Bioremediation of TNT contaminated soil with fungi under laboratory and pilot-scale conditions. Int Biodeter Biodegr 105:7–12

Anastasi A, Tigini V, Varese GC (2013) The bioremediation potential of different ecophysiological groups of fungi. In: Goltapeh EM et al (eds) Fungi as Bioremediators, Soil Biology 32, Springer, Berlin Heidelberg. pp 29–49

Arcaro KF, O'Keefe PW, Yang Y, Clayton W, Gierthy JF (1999) Antiestrogenicity of environmental polycyclic aromatic hydrocarbons in human breast cancer cells. Toxicology 133:115–127

Arsenault A, Cabana H, Jones JP (2011) Laccase-Based CLEAs: Chitosan as a Novel Cross-Linking Agent. Enzyme Res Artcle ID: 376015

Ashger M, Bhatti HN, Ashraf M, Legge RL (2008) Recent developments in bio-degradation of industrial pollutants by white rot fungi and their enzyme system. Biodegradation 19:771–783
Auriol M, Filali-Meknassia Y, Tyagi RD, Adams CD (2007) Laccase-catalyzed conversion of natural and synthetic hormones from a municipal wastewater. Water Res 41:3281–3288
Axtell C, Johnston CG, Bumpus JA (2000) Bioremediation of soil contaminated with explosives at the naval weapons station yorktown. J Soil Contam 9:537–548
Ba S, Arsenault A, Hassani T, Jones JP, Cabana H (2013) Laccase immobilization and insolubilization: from fundamentals to applications for the elimination of emerging contaminants in wastewater treatment. Crit Rev Biotechnol 33:404–418
Ba S, Haroune L, Cruz-Morato C, Jacquet C, Touahar IE, Bellenger J-P, Legault CY, Jones JP, Cabana H (2014) Synthesis and characterization of combined cross-linked laccase and tyrosinase aggregates transforming acetaminophen as a model phenolic compound in wastewaters. Sci Total Environ 487:748–755
Baborová P, Moder M, Baldrian P, Cajthamlová K, Cajthaml T (2006) Purification of new manganese peroxidase of the white-rot fungus *Irpex lacteus*, and degradation of polycyclic aromatic hydrocarbons by the enzyme. Res Microbiol 157:248–253
Baldrian P (2003) Interactions of heavy metals with white-rot fungi. Enzyme Microb Technol 32:78–91
Baldrian P (2006) Fungal laccases- occurrence and properties. FEMS Microbiol Rev 30:215–242
Baldrian P (2008) Wood-inhabiting ligninolytic basidiomycetes in soils: ecology and constraints for applicability in bioremediation. Fungal Ecol 1:4–12
Baldrian P, in der Wiesche C, Gabriel J, Nerud F, Zadrazil F (2000) Influence of cadmium and mercury on activities of ligninolytic enzymes and degradation of polycyclic aromatic hydrocarbons by *Pleurotus ostreatus* in soil. Appl Environ Microbiol 66:2471–2478
Baldrian P, Valášková V (2008) Degradation of cellulose by basidiomycetous fungi. FEMS Microbiol Rev 32:501–521
Beaudette LA, Davies S, Fedorak PM, Ward OP, Pickard MA (1998) Comparison of gas chromatography and mineralization experiment for measuring loss of selected polychlorinated biphenyl congeners in cultures of white rot fungi. Appl Environ Microbiol 64:2020–2025
Bernhardt R (2006) Cytochromes as versatile biocatalists. J Biotechnol 124:128–145
Bezalel L, Hadar Y, Cerniglia CE (1996a) Mineralization of polycyclic aromatic hydrocarbons by the white rot fungus *Pleurotus ostreatus*. Appl Environ Microbiol 62:292–295
Bezalel L, Hadar Y, Fu PP, Freeman JP, Cerniglia CE (1996b) Metabolism of phenanthrene by the white rot fungus *Pleurotus ostreatus*. Appl Environ Microbiol 62:2547–2553
Bezalel L, Hadar Y, Cerniglia CE (1997) Enzymatic mechanisms involved in phenanthrene degradation by the white rot fungus *Pleurotus ostreatus*. Appl Environ Microbiol 63: 2495–2501
Blánquez P, Guieysse B (2008) Continuous biodegradation of 17β-estradiol and 17α-ethynylestradiol by Trametes versicolor. J Hazard Mater 50:459–462
Boas M, Feldt-Rasmussen U, Main KM (2012) Thyroid effects of endocrine disrupting chemicals. Mol Cell Endocrinol 355:240–248
Bollag JM (1992) Decontaminating soil with enzymes. Environ Sci Technol 26:1876–1881
Bonfante P, Anca IA (2009) Plants, mycorrhizal fungi, and bacteria: a network of interactions. Annual Rev Microbiol 63:363–383
Borazjani H, Wiltcher D, Diehl S (2005) Bioremediation of polychlorinated biphenyl and petroleum contaminated soil. In: Lyon WG, Hong JJ, Reddy RK (eds) Proceedings of the international conference on environmental science and technology. American Science Press, New Orleans, USA, pp 502–507
Bornyasz MA, Graham RC, Allen MF (2005) Ectomycorrhizae in a soil-weathered granitic bedrock regolith: linking matrix resources to plants. Geoderma 126:141–160
Bourceret A, Cébron A, Tisserant E, Poupin P, Bauda P, Bequiristain T, Leyval C (2015) The bacterial and fungal diversity of an aged PAH- and heavy metal-contaminated soil is affected by plant cover and edaphic parameters. Soil Microbiol Microb Ecol Springer, US, pp 1–14

Bumpus JA, Tien M, Wright D, Aust SD (1985) Oxidation of persistent environmental pollutants by a white rot fungus. Science 228:1434–1436

Cabana H, Jiwan JLH, Rozenberg R, Elisashvili V, Penninckx M, Agathos SN, Jones JP (2007a) Elimination of endocrine disrupting chemicals nonylphenol and bisphenol A and personal care product ingredient triclosan using enzyme preparation from the white rot fungus *Coriolopsis polyzona*. Chemosphere 67:770–778

Cabana H, Jones JP, Agathos SN (2007b) Elimination of endocrine disrupting chemicals using white rot fungi and their lignin modifying enzymes: a review. Eng Life Sci 7:429–456

Cabana H, Alexandre C, Agathos SN, Jones JP (2009a) Immobilization of laccase from the white rot fungus *Coriolopsis polyzona* and use of the immobilized biocatalyst for the continuous elimination of endocrine disrupting chemicals. Bioresour Technol 100:3447–3458

Cabana H, Jones JP, Agathos SN (2009b) Utilization of cross-linked laccase aggregates in a perfusion basket reactor for the continuous elimination of endocrine-disrupting chemicals. Biotechnol Bioeng 102:1582–1592

Cabana H, Ahamed A, Leduc R (2011) Conjugation of laccase from the white rot fungus *Trametes versicolor* to chitosan and its utilization for the elimination of triclosan. Bioresour Technol 102:1656–1662

Cajthaml T (2014) Biodegradation of endocrine-disrupting compounds by ligninolytic fungi: mechanisms involved in the degradation. Environ Microbiol. doi:10.1111/1462-2920.12460

Cajthaml T, Möder M, Kačer P, Šašek V, Popp P (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

Cajthaml T, Erbanová P, Šašek V, Moeder M (2006) Breakdown products on metabolic pathway of degradation of benzo(*a*)anthracene by a ligninolytic fungus. Chemosphere 64:560–564

Cajthaml T, Křesinová Z, Svobodová K, Möder M (2009a) Biodegradation of endocrine disrupting compounds and suppression of estrogenic activity by ligninolytic fungi. Chemosphere 6: 745–750

Cajthaml T, Křesinová Z, Svobodová K, Sigler K, Řezanka T (2009b) Microbial transformation of synthetic estrogen 17α-ethinylestradiol. Environ Pollut 157:3325–3335

Cajthaml T, Svobodová K (2012) Biodegradation of aromatic pollutants by ligninolytic fungal strains. In: Singh SN (ed) Microbial degradation of xenobiotics. Springer-Verlag, pp 291–316

Camarero S, Cañas AI, Nousiainen P, Record E, Lomascolo A, Martínez MJ, Martínez ÁT (2008) *p*-hydroxycinnamic acids as natural mediators for laccase oxidation of recalcitrant compounds. Environ Sci Technol 42:6703–6709

Canadian Environmental Protection Act (1999). Priority substances list assessment report, "nonylphenol and its ethoxylates"

Cañas AI, Camarero S (2010) Laccases and their natural mediators: biotechnological tools for sustainable eco-friendly processes. Biotechnol Adv 28:694–705

Cázares-García SV, Vázquez-Garcidueñas MS, Vázquez-Marrufo G (2013) Structural and phylogenetic analysis of laccases from *Trichoderma*: a bioinformatic approach. PLoS ONE 8:e55295

Cébron A, Beguiristain T, Bongoua-Devisme J, Denonfoux J, Faure P, Lorgeoux C, Ouvrard S, Parisot N, Peyret P, Leyval C (2015) Impact of clay mineral, wood sawdust or root organic matter on the bacterial and fungal community structures in two aged PAH-contaminated soils. Environ Sci Pollut Res 22:13724–13738

Cerniglia CE (1997) Fungal metabolism of polycyclic aromatic hydrocarbons: past, present and future applications in bioremediation. J Ind Microbiol Biotechnol 19:324–333

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

Cerniglia CE, Sutherland JB (2006) Relative roles of bacteria and fungi in polycyclic aromatic hydrocarbon biodegradation and bioremediation of contaminated soils. In: Geoffrey Michael Gadd (ed) Fungi in biogeochemical cycles. Cambridge University Press, pp 182–211

Choudhary MI, Musharraf SG, Ali RA, Atif M, Atta-ur-Rahman (2004) Microbial transformation of antifertility agents, norethisterone and 17α-ethinylestradiol. Z. Naturforschung 59b:319–323

Clarke BO, Smith SR (2011) Review of 'emerging' organic contaminants in biosolids and assessment of international research priorities for the agricultural use of biosolids. Environ Int 37:226–247

Cloete TE, Celliers L (1999) Removal of Aroclor 1254 by the white rot fungus *Coriolus versicolor* in the presence of different concentration of Mn (IV) oxide. Int Biodeterior Biodegrad 44: 243–253

Clouzot L, Marrot B, Doumenq P, Roche N (2008) 17α-Ethinylestradiol: an endocrine disrupter of great concern. Analytical methods and removal processes applied to water purification. A review Environ Prog 27:383–396

Corvini PFX, Schaffer A, Schlosser D (2006) Microbial degradation of nonylphenol and other alkylphenols—our evolving view. Appl Microbiol Biotechnol 72:223–243

Covino S, Čvančarová M, Muzikar M, Svobodová K, D'Annibale A, Petruccioli M, Federici F, Křesinová Z, Cajthaml T (2010a) An efficient PAH-degrading *Lentinus* (*Panus*) *tirinus* strain: effect of inoculum formulation and pollutant bioavailability in solid matrices. J Hazard Mater 183:669–676

Covino S, Svobodová K, Čvančarová M, D'Annibale A, Petruccioli M, Federici F, Křesinová Z, Galli E, Cajthaml T (2010b) Inoculum carrier and contaminant bioavailability affect fungal degradation performances of PAH-contaminated solid matrices from a wood preservation plant. Chemosphere 79:855–864

Covino S, Svobodová K, Křesinová Z, Petruccioli M, Federici F, D'Annibale A, Čvančarová M, Cajthaml T (2010c) In vivo and in vitro polycyclic aromatic hydrocarbon degradation by *Lentinus (Panus) tigrinus* CBS 577.79. Bioresour Technol 101:3004–3012

Covino S, D'Annibale A, Stazi SR, Cajthaml T, Čvančarová M, Stella T, Petruccioli M (2015) Assessment of degradation potential of aliphatic hydrocarbons by autochthonous filamentous fungi from a historically polluted clay soil. Sci Total Environ 505:545–554

Črešnar B, Petrič Š (2011) Cytochrome P450 enzymes in the fungal kingdom. Review Biochim Biophys Acta 1814:29–35

Crinnion WJ (2011) Polychlorinated biphenyls: persistent pollutants with immunological, neurological and endocrinological consequences. Altern Med Rev 16(1)

Cruz-Morató C, Ferrando-Climent L, Rodriguez-Mozaz S, Barceló D, Marco-Urrea E, Vicent T, Sarrà M (2013) Duran Degradation of pharmaceuticals in non-sterile urban wastewater by *Trametes versicolor* in a fluidized bed bioreactor. Water Res 47:5200–5210

Čvančarová M, Křesinová Z, Filipová A, Covino S, Cajthaml T (2012) Biodegradation of PCBs by ligninolytic fungi and characterization of the degradation products. Chemosphere 88: 1317–1323

D'Annibale A, Stazi SR, Vinciguerra V, Di Mattia E, Sermanni GG (1999) Characterization of immobilized laccase from *Lentinula edodes* and its use in olive mill wastewater treatment. Process Biochem 34:697–706

D'Annibale A, Stazi SR, Vinciguerra V, Di Mattia E, Sermanni GG (2000) Oxirane-immobilized *Lentinula edodes* laccase: stability and phenolics removal efficiency in olive mill wastewater. J Biotechnol 77:265–273

Dashtban M, Schraft H, Syed TA, Qin W (2010) Fungal biodegradation and enzymatic modification of lignin. Int J Biochem Mol Biol 1:36–50

de Boer W, van der Wal A (2008) Chapter 8 interactions between saprotrophic basidiomycetes and bacteria. British Mycological Society Symposia Series. Ecol Saprotrophic Basidiomycetes 28:143–153

Dec J, Haider K, Bollag J (2003) Release of substituents from phenolic compounds during oxidative coupling reactions. Chemosphere 52:549–556

Della Greca M, Pinto G, Pistillo P, Pollio A, Previtera L, Temussi F (2008) Biotransformation of ethinylestradiol by microalgae. Chemosphere 70:2047–2053

Demarche P, Junghanns C, Mazy N, Agathos SN (2012a) Design-of-experiment strategy for the formulation of laccase biocatalysts and their application to degrade bisphenol A. New Biotechnol 30:96–103

Demarche P, Junghanns CH, Nair RR, Agathos SN (2012b) Harnessing the power of enzymes for environmental stewardship. Biotechnol Adv 30:933–953

Dietrich D, Hickey WJ, Lamar R (1995) Degradation of 4,4′-dichlorobiphenyl, 3,3′,4,4′-tetrachlorobiphenyl, and 2,2′,4,4′,5,5′-hexachlorobiphenyl by the white rot fungus *Phanerochaete chrysosporium*. Appl Environ Microbiol 61:3904–3909

Dittmann J, Heyser W, Bücking H (2002) Biodegradation of aromatic compounds by white rot and ectomycorrhizal fungal species and the accumulation of chlorinated benzoic acid in ectomycorrhizal pine seedlings. Chemosphere 49:297–306

Dmochewitz S, Ballschmiter K (1988) Microbial transformation of technical mixtures of polychlorinated biphenyls (PCB) by the fungus *Aspergillus niger*. Chemosphere 17:111–121

Domaradzka D, Guzik U, Wojcieszyńska D (2015) Biodegradation and biotransformation of polycyclic non-steroidal anti-inflammatory drugs. Rev Environ Sci Biotechnol 14:229–239

Dubroca J, Brault A, Kollmann A, Touton I, Jolivalt G, Kerhoas L, Mougin C (2005) Biotransformation of nonylphenol surfactants in soils amended with contaminated sewage sludges. In: Lichtfouse E, Dudd S, Robert D (eds) Environmental chemistry: green chemistry and pollutants in ecosystems. Springer, Berlin, Germany, pp 305–315

Duncan CG, Deverall FJ (1964) Degradation of wood preservatives by fungi. Appl Microbiol 12:57–62

Durán N, Rosa MA, D'Annibale A, Gianfreda L (2002) Applications of laccases and tyrosinases (phenoloxidases) immobilized on different supports: a review. Enzyme Microb Tech 31: 907–931

Eaton DC (1985) Mineralization of polychlorinated biphenyls by *Phanerochaete chrysosporium*: a ligninolytic fungus. Enzyme Microb Tech 7:194–196

Eggen RIL, Hollender J, Joss A, Schärer M, Stamm C (2014) Reducing the discharge of micropollutants in the aquatic environment: the benefits of upgrading wastewater treatment plants. Environ Sci Technol 8:7683–7689

Eggen T, Sveum P (1999) Decontamination of aged creosote polluted soil: The influence of temperature, white rot fungus *Pleurotus ostreatus*, and pretreatment. Int Biodeterior Biodegrad 43:125–133

Eibes G, Cajthaml T, Moreira MT, Feijoo G, Lema JM (2006) Enzymatic degradation of anthracene, dibenzothiophene and pyrene by manganese peroxidase in media containing acetone. Chemosphere 64:408–414

El Majidi N, Bouchard M, Carrier G (2013) Systematic analysis of the relationship between standardized prenatal exposure to polychlorinated biphenyls and mental and motor development during follow-up of nine children cohorts. Regul Toxicol Pharmacol 66:130–146

European Parliament and The Council (2008) Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC of the European Parliament and of the Council. Off J Eur Union L348:84–97

Farnet AM, Gil G, Ruaudel F, Chevremont AC, Ferre E (2009) Polycyclic aromatic hydrocarbon transformation with laccases of a white-rot fungus isolated from a mediterranean schlerophyllous litter. Geoderma 149:267–271

Federici E, Giubilei M, Santi G, Zanaroli G, Negroni A, Fava F, Petruccioli M, D'Annibale A (2012) Bioaugmentation of a historically contaminated soil by polychlorinated biphenyls with *Lentinus tigrinus*. Microb Cell Fact 11:35

Fernández-Luqueño F, Valenzuela-Encinas C, Marsch R, Martínez-Suárez C, Vázquez-Núñez E, Dendooven L (2011) Microbial communities to mitigate contamination of PAHs in soil-possibilities and challenges: a review. Environ Sci Pollut Res 18:12–30

Field JA, Sierra-Alvarez Reyes (2008) Microbial transformation and degradation of polychlorinated biphenyls. Rev Environ Pollut 155:1–12

Fukuda T, Uchida H, Suzuki M, Miyamoto H, Morinaga H, Nawata H, Uwajima T (2004) Transformation products of bisphenol A by a recombinant *Trametes villosa* laccase and their estrogenic activity. J Chem Technol Biotechnol 79:1212–1218

Fukuda T, Uchida H, Takashima Y, Uwajima T, Kawabata T, Suzuki M (2001) Degradation of bisphenol A by purified laccase from *Trametes villosa*. Biochem Biophys Res Commun 284:704–706

Furukawa K, Fujihara H (2008) Microbial degradation of polychlorinated biphenyls: biochemical and molecular features. J Biosc Bioeng 105:433–449

Gadd GM (2001) Fungi in bioremediation (No 23). Published for the British Mycological Society. Cambridge University Press. ISBN 0521781191

García-Delgado C., D'Annibale A., Pesciaroli L., Yunta F., Crognale S., Petruccioli M., Eymar E. (2015). Implications of polluted soil biostimulation and bioaugmentation with spent mushroom substrate (*Agaricus bisporus*) on the microbial community and polycyclic aromatic hydrocarbons biodegradation. *Sci. Total Environ.* 508: 20–28

Gasser CA, Yu L, Svojitka J, Wintgens T, Ammann EM, Shahgaldian P, Corvini PF-X, Hommes G (2014) Advanced enzymatic elimination of phenolic contaminants in wastewater: a nano approach at field scale

Gesell M, Hammer E, Mikolasch A, Schauer F (2004) Oxidation and ring cleavage of dibenzofuran by the filamentous fungus *Paecilomyces lilacinus*. Arch Microbiol 182:51–9

Gichner T, Lovecká P, Vrchotová B (2008) Genomic damage induced in tobacco plants by chlorobenzoic acids metabolic products of polychlorinated biphenyls. Mutat Res 657:140–145

Giubilei MA, Leonardi V, Federici E, Covino S, Šašek V, Novotný C, Federici F, D'Annibale A, Petruccioli M (2009) Effect of mobilizing agents on mycoremediation and impact on the indigenous microbiota. J Chem Technol Biotechnol 84:836–844

Gómez-Toribio V, García-Martín AB, Martínez MJ, Martínez AT, Guillén F (2009) Induction of extracellular hydroxyl radical production by white-rot fungi through quinone redox cycling. Appl Environ Microbiol 75:3944–3953

Gramss G, Kirsche B, Voigt KD, Gunther T, Fritsche W (1999) Conversion rates of five polycyclic aromatic hydrocarbons in liquid cultures of fifty-eight fungi and the concomitant production of oxidative enzymes. Mycol Res 103:1009–1018

Hammel KE (1995) Mechanisms for polycyclic aromatic hydrocarbon degradation by ligninolytic fungi. Environ Health Perspect 103:41–43

Hammel KE, Kalyanaraman B, Kirk TK (1986) Oxidation of polycyclic aromatic hydrocarbons and dibenzo[*p*]-dioxins by *Phanerochaete chrysosporium* ligninase. J Biol Chem 261: 16948–16952

Hammel KE, Green B, Gai WZ (1991) Ring fission of anthracene by a eukaryote. Proc Natl Acad Sci USA 88:10605–10608

Hammer E, Krowas D, Schafer A, Specht M, Francke W, Schauer F (1998) Isolation and characterization of a dibenzofuran-degrading yeast: identification of oxidation and ring cleavage products. Appl Environ Microbiol 64:2215–2219

Haritash AK, Kaushik CP (2009) Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): a review. J Hazard Mater 169:1–15

Harms H, Schlosser D, Wick LY (2011) Untapped potential: exploiting fungi in bioremediation of hazardous chemicals. Nat Rev Microbiol 9:177–192

Helmfrid I, Berglund M, Löfman O, Wingren G (2012) Health effects and exposure to polychlorinated biphenyls (PCBs) and metals in a contaminated community. Environ Intern 44:53–58

Hendey NI (1964) Some observations on *Cladosporium resinae* as a fuel contaminant and its possible role in the corrosion of aluminium alloy fuel tanks. T Brit Mycol Soc 47:467–475

Hestbjerg H, Brinch UC, Nielsen T (2001) Potential of *Pleurotus ostreatus* for bioremediation: Temperature requirements and competitiveness. Abstracts, SETAC Organic Soil Contaminants, Copenhagen, Denmark, September 2–5, p 24

Hestbjerg H, Willumsen PA, Christensen M, Andersen O, Jacobsen CS (2003) Bioaugmentation of tar-contaminated soils under field conditions using *Pleurotus ostreatus* refuse from commercial mushroom production. Environ Toxicol Chem 22:692–698

Hirano T, Honda Y, Watanabe T, Kuwahara M (2000) Degradation of bisphenol A by the lignin-degrading enzyme, manganese peroxidase, produced by the white rot basidiomycete, *Pleurotus ostreatus*. Biosci Biotechnol Biochem 64:1958–1962

Hofrichter M (2002) Review: lignin conversion by manganese peroxidase (MnP). Enzyme Microb Technol 30:454–466

Hofrichter M, Ullrich R (2014) Oxidations catalyzed by fungal peroxygenases. Curr Opin Chem Biol 19:116–125

Huang Q, Walter J, Weber JR (2005) Transformation and removal of bisphenol A from aqueous phase via peroxidase-mediated oxidative coupling reactions: efficacy, products, and pathways. Environ Sci Technol 39:6029–6036

Huff JE, Moore JA, Saracci R, Tomatis L (1980) Long-term hazards of polychlorinated dibenzodioxins and polychlorinated dibenzofurans. Environ Health Persp 36:221–240

in der Wiesche C, Martens R, Zadrazil F (1996) Two-step degradation of pyrene by white-rot fungi and soil microorganisms. Appl Microbiol Biotechnol 46:653–659

Ishii K, Furuichi T (2007) Development of bioreactor system for treatment of dioxin-contaminated soil using *Pseudallescheria boydii*. J Hazard Mater 148:693–700

Ishii K, Furuichi T, Tanikawa N, Kuboshima M (2009) Estimation of the biodegradation rate of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin by using dioxin-degrading fungus, *Pseudallescheria boydii*. J Hazard Mater 162:328–332

Jelić A, Gros M, Petrović M, Ginebreda A, Barceló D (2012) Occurrence and elimination of pharmaceuticals during conventional wastewater treatment. Emerging and Priority Pollutants in Rivers. The Handbook of Environmental Chemistry, Springer US, vol 19, pp 1–23

Johannes C, Majcherczyk A (2000) Natural mediators in the oxidation of polycyclic aromatic hydrocarbons by laccase mediator systems. Appl Environ Microbiol 66:524–528

Johannes C, Majcherczyk A, Hüttermann A (1998) Oxidation of acenaphthene and acenaphthylene by laccase of *Trametes versicolor* in a laccase-mediator system. J Biotechnol 61:151–156

Junghanns C, Moeder M, Krauss G, Martin C, Schlosser D (2005) Degradation of the xenoestrogen nonylphenol by aquatic fungi and their laccases. Microbiology 151:45–57

Kamei I, Kondo R (2005) Biotransformation of dichloro-, trichloro-, and tetrachlorodibenzo-*p*-dioxin by the white rot fungus *Phlebia lindtneri*. Appl Microbiol Biotechnol 68:560–566

Kamei I, Suhara H, Kondo R (2005) Phylogenetical approach to isolation of white rot fungi capable of degrading polychlorinated dibenzo-*p*-dioxin. Appl Microbiol Biotechnol 69: 358–366

Kamei I, Kogura R, Kondo R (2006a) Metabolism of 4,4′-dichlorobiphenyl by white-rot fungi *Phanerochaete chrysosporium* and *Phanerochaete* sp. MZ142 *Appl*. Microbiol Biotechnol 72:566–575

Kamei I, Sonoki S, Haraguchi K, Kondo R (2006b) Fungal bioconversion of toxic polychlorinated biphenyls by white-rot fungus, *Phlebia brevispora*. Appl Microbiol Biotechnol 73:932–940

Keum YS, Li QX (2004) Fungal laccase-catalyzed degradation of hydroxy polychlorinated biphenyls. Chemosphere 56:23–30

Kirk TK, Farrell RL (1987) Enzymatic "combustion": the microbial degradation of lignin. Annu Rev Microbiol 41:465–505

Kohlmeier S, Smits TMH, Ford RM, Keel C, Harms H, Lukas YW (2005) Taking the fungal highway: mobilization of pollutant-degrading bacteria by fungi. Environ Sci Technol 39: 4640–4646

Kordon K, Mikolasch A, Schauer F (2010) Oxidative dehalogenation of chlorinated hydroxybiphenyls by laccases of white-rot fungi. Intern Biodet Biodegrad 64:203–209

Kramer S, Moller Hikel S, Adams K, Hinds D, Moon K (2012) Current status of the epidemiologic evidence linking polychlorinated biphenyls and non-Hodgkin lymphoma and the role of immune dysregulation. Environ Health Perspect 120(8)

Krčmar P, Kubátová A, Votruba J, Erbanová P, Novotný C, Sašek V (1999) Degradation of polychlorinated biphenyls by extracellular enzymes of *Phanerochaete chrysosporium* produced in a perforated plate bioreactor. World J Microbiol Biotechnol 15:269–276

Křesinová Z, Moeder M, Ezechiáš M, Svobodová K, Cajthaml T (2012) Mechanistic study of 17α-ethinylestradiol biodegradation by *Pleurotus ostreatus*: tracking of extracelullar and intracelullar degradation mechanisms. Environ Sci Technol 46:13377–13385

Kubátová A, Erbanová P, Eichlerová I, Homolka L, Nerud F, Šašek V (2001) PCB congener selective biodegradation by the white-rot fungus *Pleurotus ostreatus* in contaminated soil. Chemosphere 43:207–215

Kües U (2015) Fungal enzymes for environmental management. Curr Opin Biotechnol 33: 268–278

Lamar RT, White RB, Ashley KC (2002) Evaluation of white-rot fungi for the remediation of creosote-contaminated soil. Remediation J 12:97–106

Lang E, Eller G, Zadrail F (1997) Lignocellulose decomposition and production of ligninolytic enzymes during interaction of white rot fungi with soil microorganisms. Microb Ecol 34:1–10

Lang E, Nerud F, Zadrazil F (1998) Production of ligninolytic enzymes by *Pleurotus* sp. and *Dichomitus squalens* in soil and lignocellulose substrate as influenced by soil microorganisms. FEMS Microb Lett 167:239–244

Lang E, Gonser A, Zadrazil F (2000) Influence of incubation temperature on activity of ligninolytic enzymes in sterile soil by *Pleurotus* sp. and *Dichomitus squalens*. Basic Microbiol 40:33–39

Latawiec AE, Swindell AL, Simmons P, Reid BJ (2011) Bringing bioavailability into contaminated land decision making: the way forward? Crit Rev Env Sci Tec 41:52–77

Lee PY, Chen CY (2009) Toxicity and quantitative structure–activity relationships of benzoic acids *to Pseudokirchneriella subcapitata*. J Hazard Mater 165:156–161

Li P, Sun T, Stagnitti F, Zhang C, Zhang H, Xiong X, Allinson G, Ma X, Allinson M (2002) Field-scale bioremediation of soil contaminated with crude oil. Environ Eng Sci 19:277–289

Liu ZH, Kanjo Y, Mizutani S (2009) Removal mechanisms for endocrine disrupting compounds (EDCs) in wastewater treatment—physical means, biodegradation, and chemical advanced oxidation: a review. Sci Total Environ 407:731–748

Lloret L, Eibes G, Feijo G, Moreira MT, Lema JM (2012a) Continuous operation of a fluidized bed reactor for the removal of estrogens by immobilized laccase on Eupergit supports. J Biotechnol 182:404–406

Lloret L, Hollmann F, Eibes G, Feijoo G, Moreira MT, Lema JM (2012b) Immobilisation of laccase on Eupergit supports and itsapplication for the removal of endocrine disrupting chemicals in a packed-bed reactor. Biodegradation 23:373–386

Lloret L, Eibes G, Moreira MT, Feijoo G, Lema JM (2013) Removal of estrogenic compounds from filtered secondary wastewater effluent in a continuous enzymatic membrane reactor. Identification of biotransformation products. Environ Sci Technol 47:4536–4543

Luo Y, Guo W, Ngo HH, Nghiem LD, Hai FI, Zhang J, Liang S (2014) A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. Sci Total Environ 473–474:619–641

Lyr H (1963) Enzymatische detoxification chlorieter phenole. Phytopathol Z 47:73–83

Majcherczyk A, Johannes C, Huttermann A (1998) Oxidation of polycyclic aromatic hydrocarbons (PAH) by laccase of *Trametes versicolor*. Enzyme Microb Technol 22:335–341

Manji S, Ishihara A (2004) Screening of tetrachlorodibenzo-*p*-dioxin-degrading fungi capable of producing extracellular peroxidases under various conditions. Appl Microbiol Biotechnol 63:438–444

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

Marco-Urrea E, Pérez-Trujillo M, Blánquez P, Vicent T, Caminal G (2010a) Biodegradation of the analgesic naproxen by *Trametes versicolor* and identification of intermediates using HPLC-DAD-MS and NMR. Bioresour Technol 101:2159–2166

Marco-Urrea E, Pérez-Trujillo M, Cruz-Morató C, Caminal G, Vicent T (2010b) Degradation of the drug sodium diclofenac by Trametes versicolor pellets and identification of some intermediates by NMR. J Hazard Mater 176:836–842

Marco-Urrea E, Pérez-Trujillo M, Cruz-Morató C, Caminal G, Vicent T (2010c) White-rot fungus-mediated degradation of the analgesic ketoprofen and identification of intermediates by HPLC-DAD-MS and NMR. Chemosphere 78:474–481

Marco-Urrea E, Radjenović J, Caminal G, Petrović M, Vicent T, Barceló D (2010d) Oxidation of atenolol, propranolol, carbamazepine and clofibric acid by a biological Fenton-like system mediated by the white-rot fungus *Trametes versicolor*. Water Res 44:521–532

Marco-Urrea E, García-Romera I, Aranda E (2015) Potential of non-ligninolytic fungi in bioremediation of chlorinated and polycyclic aromatic hydrocarbons. New Biotechnol 32: 620–628

Martens R, Zadrazil F (1998) Screening of white-rot fungi for their ability to mineralize polycyclic aromatic hydrocarbons in soil. Folia Microbiol 43:97–103

Mayolo-Deloisa K, Machín-Ramírez C, Rito-Palomares M, Trejo-Hernández MR (2011) Oxidation of polycyclic aromatic hydrocarbons using partially purified laccase from residual compost of *Agaricus bisporus*. Chem Eng Technol 34:1368–1372

Michels J (1998) Processes for the bioremediation of soil. Editor: Federal Environmental Agency BMBF Project Management Agency for Waste Management and Remediation of Hazardous Abandoned Sites (PT AWAS). Section III 3.6

Michizoe J, Ichinose H, Kamila N, Maruyama T, Goto M (2005) Biodegradation of phenolic environmental pollutants by a surfactant-laccase complex in organicmedia. J Biosci Bioeng 99:642–647

Moen MA, Hammel KE (1994) Lipid peroxidation by the manganese peroxidase of *Phanerochaete chrysosporium* is the basis for phenanthrene oxidation by the intact fungus. Appl Environ Microbiol 60:1956–1961

Mori T, Kondo R (2002a) Degradation of 2, 7-dichlorodibenzo-*p*-dioxin by wood-rotting fungi, screened by dioxin degrading ability. FEMS Microbiol Lett 213:127–131

Mori T, Kondo R (2002b) Oxidation of dibenzo-*p*-dioxin, dibenzofuran, biphenyl, and diphenyl ether by the white rot fungus *Phlebia lindtneri*. Appl Microbiol Biotechnol 60:200–205

Mougin C (2002) Bioremediation and phytoremediation of industrial PAH-polluted soils. Polycyclic Aromat Compd 22:1–33

Mouhamadou B, Faure M, Sage L, Marçais J, Souard F, Geremia RA (2013) Potential of autochthonous fungal strains isolated from contaminated soils for degradation of polychlorinated biphenyls. Fungal Biol 117:268–274

Muzikář M, Křesinová Z, Svobodová K, Filipová A, Čvančarová M, Cajthamlová K, Cajthaml T (2011) Biodegradation of chlorobenzoic acids by ligninolytic fungi. J Hazard Mater 196: 386–394

Naidu R, Channey R, McConnell S, Johnston N, Semple KT, McGrath S, Dries V, Nathanail P, Harmsen J, Pruszinski A, MacMillan J, Palanisami T (2013) Towards bioavailability-based soil criteria: past, present and future perspectives. Environ Sci Pollut Res Int 22:8779–8785

Nair RR, Demarche P, Agathos SN (2013) Formulation and characterization of an immobilized laccase biocatalyst and its application to eliminate organic micropollutants in wastewater. New Biotech 30:814–823

Nakamiya K, Furuichi T, Ishii K (2002) Isolation of a fungus from denitrifying activated sludge that degrades highly chlorinated dioxins. J Mater Cycles Waste Manage 4:127–134

Nakamiya K, Hashimoto S, Ito H, Edmonds JS, Yasuhara A, Morita M (2005) Degradation of dioxins by cyclic ether degrading fungus. Cordyceps sinensis FEMS Microbiol Lett 248:17–22

Nam I, Kim Y, Murugesan K, Jeon J, Chang Y (2008) Bioremediation of PCDD/Fs-contaminated municipal solid waste incinerator fly ash by a potent microbial biocatalyst. J Hazard

Nicotra S, Intra A, Ottolina G, Riva S, Danieli B (2004) Laccase-mediated oxidation of the steroid hormone 17b-estradiol in organic solvents. Tetrahedron Asymmetry 15:2927–2931

Novotný C, Cajthaml T, Svobodová K, Susla M, Šašek V (2009) *Irpex lacteus*, a white rot fungus with biotechnological potential—review. Folia Microbiol 54:375–390

Novotný C, Vyas BRM, Erbanová P, Kubátová A, Šašek V (1997) Removal of PCBs by various white-rot fungi in liquid cultures. Folia Microbiol 42:136–140

Pan F, Yang Q, Zhang Y, Zhang S, Yang M (2004) Biodegradation of polycyclic aromatic hydrocarbons by *Pichia anomala*. Biotechnol Lett 26:803–806

Passatore L, Rossetti S, Juwarkar AA, Massacci A (2014) Phytoremediation and bioremediation of polychlorinated biphenyls (PCBs): state of knowledge and research perspectives. J Hazard Mater 278:189–202

Phan CW, Sabaratnam V (2012) Potential uses of spent mushroom substrate and its associated lignocellulosic enzymes. Appl Environ Microbiol 96:863–873

Pickard MA, Roman R, Tinoco R, Vazquez-Duhalt R (1999) Polycyclic aromatic hydrocarbon metabolism by white rot fungi and oxidation by *Coriolopsis gallica* UAMH 8260 laccase. Appl Environ Microbiol 65:3805–3809

Pieper DH (2005) Aerobic degradation of polychlorinated biphenyls. Appl Microbiol Biotechnol 67:170–191

Pinedo-Rivilla C, Aleu J, Collado IG (2009) Pollutants biodegradation by fungi. Curr Org Chem 13:1194–1214

Pointing S (2001) Faesibility of bioremediation by white-rot fungi. Appl Microbiol Biotechnol 57:20–33

Rabinovich ML, Bolobova AV, Vasilchenko LG (2004) Decomposition of natural aromatic structures and xenobiotics by fungi. Appl Biochem Microbiol 40:5–23

Rafin C, Potin O, Veignie E (2000) Degradation of benzo[a]pyrene as sole carbon source by a non white-rot fungus, *Fusarium solani*. Polycycl Aromat Compd 21:311–329

Rafin C, de Foucault B, Veignie E (2012) Exploring micromycetes biodiversity for screening benzo[a]pyrene degrading potential. Environ Sci Pollut Res Int 20:3280–3289

Rodarte-Morales AI, Feijoo G, Moreira MT, Lema MJ (2011) Degradation of selected pharmaceutical and personal care products (PPCPs) by white-rot fungi. World J Microbiol Biotechnol 27:1839–1846

Rodarte-Morales AI, Feijoo G, Moreira MT, Lema MJ (2012) Biotransformation of three pharmaceutical active compounds by the fungus *Phanerochaete chrysosporium* in a fed batch stirred reactor under air and oxygen supply. Biodegradation 23:145–156

Rodríguez-Rodríguez CE, Barón E, Gago-Ferrero P, Jelić A, Llorca M, Farré M, Díaz-Cruz MS, Eljarrat E, Petrović M, Caminal G, Barceló D, Vicent T (2012) Removal of pharmaceuticals, polybrominated flame retardants and UV-filters from sludge by the fungus *Trametes versicolor* in bioslurry reactor. J Hazard Mater 233–234:235–243

Romero MC, Cazau MC, Giorgieri S, Arambarri AM (1998) Phenanthrene degradation by microorganisms isolated from a contaminated stream. Environ Pollut 101:355–359

Romero MC, Salvioli ML, Cazau MC, Arambarri AM (2002) Pyrene degradation by yeasts and filamentous fungi. Environ Pollut 117:159–163

Rozalska S, Szewczyk R, Dlugonski J (2010) Biodegradation of 4-n-nonylphenol by the non-ligninolytic filamentous fungus *Gliocephalotrichum* simplex: a proposal of a metabolic pathway. J Hazard Mater 180:323–331

Ruiz-Aguilar GML, Fernandez-Sanchez JM, Rodrıguez-Vazquez R, Poggi-Varaldo H (2002) Degradation by white-rot fungi of high concentrations of PCB extracted from a contaminated soil. Adv Environ Res 6:559–568

Sack U, Heinze TM, Deck J, Cerniglia CE, Martens R, Zadrazil F, Fritche W (1997) Comparison of phenanthrene and pyrene degradation by different wood-decaying fungi. Appl Environ Microbiol 63:3919–3925

Sage L, Périgon S, Faure M, Gaignaire C, Abdelghafour M, Mehu J, Geremia RA, Mouhamadou B (2014) Autochthonous ascomycetes in depollution of polychlorinated biphenyls contaminated soil and sediment. Chemosphere 110:62–69

Santos LH, Araújo AN, Fachini A, Pena A, Delerue-Matos C, Montenegro MC (2010) Ecotoxicological aspects related to the presence of pharmaceuticals in the aquatic environment. J Hazard Mater 175:45–95

Šašek V (2003) Why mycoremediations have not yet come into practice. The utilization of bioremediation to reduce soil contamination: problems and solutions. NATO Science Series. 19:247–266

Sato A, Watanabe T, Watanabe Y, Harazono K, Fukatsu T (2002) Screening for basidiomycetous fungi capable of degrading 2, 7-dichlorodibenzo-*p*-dioxin. FEMS Microbiol Lett 213:213–217

Schützendübel A, Majcherczyk A, Johannes C, Hüttermann A (1999) Degradation of fluorene, anthracene, phenanthrene, fluoranthene, and pyrene lacks connection to the production of extracellular enzymes by *Pleurotus ostreatus* and *Bjerkandera adusta*. Int Biodeterior Biodegrad 43:93–100

Seto M, Nishibori K, Masai E, Fukuda M, Ohdaira Y (1999) Degradation of polychlorinated biphenyls by a 'Maitake' mushroom, *Grifola frondosa*. Biotechnol Lett 21:27–31

Sietmann R, Gesell M, Hammer E, Schauer F (2006) Oxidative ring cleavage of low chlorinated biphenyl derivatives by fungi leads to the formation of chlorinated lactone derivatives. Chemosphere 64:672–685

Silva IS, Grossman M, Durrant LR (2009) Degradation of polycyclic aromatic hydrocarbons (2-7 rings) under microaerobic and very-low-oxygen conditions by soil fungi. Int. Biodeterior. Biodegrad. 63:224–229

Singh H (2006) Mycoremediation: fungal bioremediation. Wiley-Interscience

Steffen KT, Hatakka A, Hofrichter M (2002) Removal and mineralization of polycyclic aromatic hydrocarbons by litter-decomposing basidiomycetous fungi. Appl Microbiol Biotechnol 60:212–217

Steffen KT, Schubert S, Tuomela M, Hatakka A, Hofrichter M (2007) Enhancement of bioconversion of high-molecular mass polycyclic aromatic hydrocarbons in contaminated non-sterile soil by litter-decomposing fungi. Biodegradation 18:359–369

Stella T, Covino S, Křesinová Z, D'Annibale A, Petruccioli M, Cvančarová M, Cajthaml T (2013) Chlorobenzoic acid degradation by *Lentinus* (*Panus*) *tigrinus*: In vivo and in vitro mechanistic study-evidence for P-450 involvement in the transformation. J Hazard Mater 260:975–983

Stella T, Covino S, Burianová E, Filipová A, Křesinová Z, Voříšková J, Větrovský T, Baldrian P, Cajthaml T (2015) Chemical and microbiological characterization of an aged PCB-contaminated soil. Sci Total Environ 533:177–186

Suzuki K, Hirai H, Murata H, Nishida T (2003) Removal of estrogenic activities of 17α-estradiol and ethinylestradiol by ligninolytic enzymes from white rot fungi. Water Res 37:19721975

Svobodová K, Placková M, Novotná V, Cajthaml T (2009) Estrogenic, androgenic activity of PCBs, their chlorinated metabolites and other endocrine disruptors estimated with two in vitro yeast assays. Sci Total Environ 407:5921–5925

Syed K, Porollo A, Ying WL, Yadav JS (2011) A fungal P450 (CYP5136A3) capable of oxidizing polycyclic aromatic hydrocarbons and endocrine disrupting alkylphenols: role of Trp129 and Leu324. PLoS ONE 6:1–14

Syed K, Porollo A, Lam YW, Grimmett PE, Yadav JS (2013) CYP63A2, a catalytically versatile fungal P450 monooxygenase capable of oxidizing higher-molecular-weight polycyclic aromatic hydrocarbons, alkylphenols, and alkanes. Appl Environ Microbiol 79:2692–702

Taboada-Puig R, Junghanns C, Demarche P, Moreira MT, Feijoo G, Lema JM, Agathos SN (2011) Combined cross-linked enzyme aggregates from versatile peroxidase and glucose oxidase: production, partial characterization and application for the elimination of endocrine disruptors. Bioresour Technol 102:6593–6599

Takada S, Nakamura M, Matsueda T, Kondo R, Sakai K (1996) Degradation of polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans by the white rot fungus *Phanerochaete sordida* YK-624. Appl Environ Microbiol 62:4323–4328

Takagi S, Shirota C, Sakaguchi K, Suzuki J, Sue T, Nagasaka H, Hisamatsu S, Sonoki S (2007) Exoenzymes of *Trametes versicolor* can metabolize coplanar PCB congeners and hydroxy PCB. Chemosphere 67:54–57

Tanaka T, Tonosaki T, Nose M, Tomidokoro N, Kadomura N, Fujii T, Taniguchi M (2001) Treatment of model soils contaminated with phenolic endocrine disrupting chemicals with laccase from *Trametes* sp. in a rotating reactor. J Biosci Bioeng 92:312–316

Thomas DR, Carswell KS, Georgiou G (1992) Mineralization of biphenyl and PCBs by the white rot fungus *Phanerochaete chrysosporium*. Biotechnol Bioeng 40:1395–1402

Tien M, Kirk TK (1984) Lignin-degrading enzyme from *Phanerochaete chrysosporium*: purification, characterization, and catalytic properties of a unique H_20_2-requiring oxygenase. Proc Natl Acad Sci 81:2280–2284

Tigini V, Prigione V, Di Toro S, Fava F, Varese GC (2009) Isolation and characterisation of polychlorinated biphenyl (PCB) degrading fungi from a historically contaminated soil. Microb Cell Fact 8:5

Torres-Duarte C, Teresa VM, Vazquez-Duhalt R (2012) Laccase-mediated transformations of endocrine disrupting chemicals abolish binding affinities to estrogen receptors and their estrogenic activity in zebrafish. Appl Biochem Biotechnol 168:864–876

Touahar IE, Haroune L, Ba S, Bellenger JP, Cabana H (2014) Characterization of combined cross-linked enzyme aggregates from laccase, versatile peroxidase and glucose oxidase, and their utilization for the elimination of pharmaceuticals. Sci Total Environ 481:90–99

Tran NH, Urase T, Ngo HH, Hu JY, Ong SL (2013) Insight into metabolic and cometabolic activities of autotrophic and heterotrophic microorganisms in the biodegradation of emerging trace organic contaminants. Bioresour Technol 146:721–731

Tsutsumi Y, Haneda T, Nishida T (2001) Removal of estrogenic activities of bisphenol A and nonylphenol by oxidative enzymes from lignin-degrading Basidiomycetes. Chemosphere 42:271–276

Tuomela M, Steffen KT, Kerko E, Hartikainen H, Hofrichter M, Hatakka A (2005) Influence of Pb contamination in boreal forest soil on the growth and ligninolytic activity of litter-decomposing fungi. FEMS Microbiol Ecol 53:179–186

Tuomela M, Jørgensen K, Winquist E, Björklöf K, Schultz E, Anasonye F, Häkkinen L, Räsänen M, Sorvari J, Hartikainen ES, Steffen K, Valentin L (2012) Mycoremediation of contaminated soil in field scale. Conference abstract. Environ Eng Manag J 11:S38

Ullrich R, Nüske J, Scheibner K, Spantzel J, Hofrichter M (2004) Novel haloperoxidase from the agaric basidiomycete *Agrocybe aegerita* oxidizes aryl alcohols and aldehydes. Appl Environ Microbiol 70:4575–4581

UNEP, United Nations Environment Programme (2012) Annual report

US Environmental Protection Agency (2000) Exposure and human health.reassessment of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and related compounds. Part I: Estimating exposure to dioxin-like compounds. Vol. 3EPA/600/P-00/001

US EPA Handbook (1993) Approaches for the remediation of federal facility sites contaminated with explosive and radioactive wastes. EPA/625/R-93/013

Valentin L, Feijoo G, Moreira MT, Lema JM (2006) Biodegradation of polycyclic aromatic hydrocarbons in forest and salt marsh soils by white-rot fungi. Int Biodeterior Biodegrad 58:15–21

Valli K, Wariishi H, Gold MH (1992) Degradation of 2,7-dichlorodibenzo-*p*-dioxin by the lignin degrading basidiomycete *Phanerochaete chrysosporium*. J Bacteriol 174:2131–2137

Vallini G, Frassinetti S, D'Andrea F, Catelani G, Agnolucci M (2001) Biodegradation of 4-(1-nonyl)phenol by axenic cultures of the yeast *Candida aquaetextoris*: identification of microbial breakdown products and proposal of a possible metabolic pathway. Int Biodeterior Biodegrad 47:133–140

Van den Berg M, Birnbaum LS, Denison M, De Vito M, Farland W, Feeley M (2006) The 2005 World Health Organization reevaluation of human and mammalian toxic equivalency factors for dioxins and dioxin-like compounds. Toxicol Sci 93:223–241

van den Brink HM, van Gorcom RF, van den Hondel CA, Punt PJ (1998) Cytochrome P450 enzyme systems in fungi. Fungal Genet Biol 23:1–17

Verdin A, Sahraoui ALH DR (2004) Degradation of benzo[a]pyrene by mitosporic fungi and extracellular oxidative enzymes. Int Biodeterior Biodegrad 53:65–70

Verdin A, Sahraoui ALH, Newsam R, Robinson G, Durand R (2005) Polycyclic aromatic hydrocarbons storage by *Fusarium solani* in intracellular lipid vesicles. Environ Pollut 133:283–291

Verlicchi P, Galletti A, Petrovic M, Barceló D (2010) Hospital effluents as a source of emerging pollutants: an overview of micropollutants and sustainable treatment options. J Hydrol 389:416–428

Viswanath B, Rajesh B, Janardhan A, Kumar AP, Narasimha G (2014) Fungal laccases and their applications in bioremediation. Enzyme Res. doi:10.1155/2014/163242

Vyas BRM, Šašek V, Matucha M, Bubner M (1994) Degradation of 3,3',4,4'- tetrachlorobiphenyl by selected white rot fungi. Chemosphere 28:1127–1134

Walter M, Boyd-Wilson K, Boul L, Ford C, McFadden D, Chong B, Pinfold J (2005) Field-scale bioremediation of pentachlorophenol by *Trametes versicolor Int*. Biodeter Biodegr 56:51–57

Wang J, Majima N, Hirai H, Kawagishi H (2012) Effective removal of endocrine-disrupting compounds by lignin peroxidase from the white-rot fungus *Phanerochaete sordi*da YK-624. Curr Microbiol 64:300–303

Wang J, Yamamoto Y, Hirai H, Kawagishi H (2013a) Dimerization of bisphenol by hyper lignin-degrading fungus *Phanerochaete sordida* YK-624 under ligninolytic condition. Curr Microbiol 66:544–547

Wang J, Yamamoto R, Yamamoto Y, Tokumoto T, Dong J, Thomas P, Hirai H, Kawagishi H (2013b) Hydroxylation of bisphenol A by hyper lignin-degrading fungus *Phanerochaete sordida* YK-624 under non-ligninolytic condition. Chemosphere 93:1419–1423

Winquist E, Björklöf K, Schultz E, Räsänen M, Salonen K, Anasonye F, Cajthaml T, Steffen K, Jørgensen K, Tuomela M (2014) Bioremediation of PAH-contaminated soil with fungi- From laboratory to field scale. Int Biodeter Biodegr 86:238–247

Wolter M, Zadrazil F, Martens R, Bahadir M (1997) Degradation of eight highly condensed polycyclic aromatic hydrocarbons by *Pleurotus* sp. *florida* in solid wheat straw substrate. Appl Microbiol Biotechnol 48:398–404

Wu YR, Luo ZH, Chow KKR, Vrijmoed LLP (2010) Purification and characterization of an extracellular laccase from the anthracene-degrading fungus *Fusarium solani* MAS2. Bioresource Technol 101:9772–9777

Wunch KG, Alworth WL, Bennett JW (1999) Mineralization of benzo[a]pyrene by *Marasmiellus troyanus*, a mushroom isolated from a toxic waste site. Microbiol Res 154:75–79

Yadav JS, Quensen JF III, Tiedje JM, Reddy CA (1995) Degradation of polychlorinated biphenyl mixtures (aroclors 1242, 1254, and 1260) by the white rot fungus *Phanerochaete chrysosporium* as evidenced by congener-specific analysis. Appl Environ Microbiol 61:2560–2565

Yamaguchi M, Kamei I, Nakamura M, Takano M, Sekiya A (2007) Selection of *Pleurotus pulmonarius* from domestic basidiomycetous fungi for biodegradation of chlorinated dioxins as environmentally persistent organopollutants. Shinrin Sogo Kenkyusho Kenkyu Hokoku 6: 231–237

Yang Q, Yang M, Pritsch K, Yediler A, Hagn A, Schloter M, Kettrup A (2003) Decolorization of synthetic dyes and production of manganese-dependent peroxidase by new fungal isolates. Biotechnol Lett 25:709–713

Yang Q, Zhang H, Li X, Wang Z, Xu Y, Ren S, Chen X, Xu Y, Hao H, Wang H (2013) Extracellular enzyme production and phylogenetic distribution of yeasts in wastewater treatment systems. Bioresour Technol 129:264–273

Zeddel A, Majcherczyk A, Hüttermann A (1993) Degradation of polychlorinated biphenyls by white-rot fungi *Pleurotus ostreatus* and *Trametes versicolor* in a solid state systém. Toxicol Environ Chem 40:255–266

Zhang J, Xu Z, Chen H, Zong Y (2009) Removal of 2,4-dichlorophenol by chitosan-immobilized laccase from *Coriolus versicolor*. Biochem Eng J 45:54–59