Chapter 7

White-Rot Fungi and their Enzymes as a Biotechnological Tool for Xenobiotic Bioremediation

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Abstract

A huge amount of hazardous organopollutants, often persistent and toxic, is produced annually over the world and may contaminate soil, water, ground water, and air. Being from various sources such as wastewater, landfill leachates, and solid residues, xenobiotics include phenols, plastics, hydrocarbons, paints, dyes, pesticides and insecticides, paper and pulp mills, and pharmaceuticals. Among biological processes for degradation of xenobiotics, fungal ones, being eco-friendly and cost cheap, have been investigated extensively because most of basidiomycetes are more tolerant to high concentrations of pollutants. Fungal bioremediation is a promising technology using their metabolic potential to remove or reduce xenobiotics. Basidiomycetes are the unique microorganisms that show high capacities of degrading a wide range of toxic xenobiotics. They act via the extracellular ligninolytic enzymes, including laccase, manganese peroxidase, and lignin peroxidase. Their capacities to remove xenobiotic substances and produce polymeric products make them a useful tool for bioremediation purposes. During fungal remediation, they utilize hazardous compounds, even the insoluble ones, as the nutrient source and convert them to simple fragmented forms. The aim of this chapter is to elucidate the ability of basidiomycetes to degrade xenobiotics. This is an overview to present the importance of extracellular enzymes for efficient bioremediation of a large variety of xenobiotics.

Keywords: xenobiotics, white-rot fungi, enzymes, bioremediation, biodegradation

1. Introduction

The contamination of soil, water, ground water, and air with toxic chemicals is one of the major environmental problems, faced by the world today. With the intensive industrialization and

the extensive use of pesticides in agriculture and chemicals in various fields, the environmental pollution with organic compounds has become a serious threat. Indeed, pollution of aquatic system and soil is a worldwide problem that can result in accumulation of toxic chemicals in food chains and also harm the flora and fauna. Hence, environmental pollution with hazardous wastes containing recalcitrant chemicals, being often xenobiotic compounds, has become one of the major ecological problems, with an increasing awareness around the world [1, 2]. The quick rise of industrial activities has extremely increased the release of toxic effluents into water bodies along with ground water [3]. The pollution resulting in the release of these compounds causes disturbance to the natural bodies and their ecosystems, leading to climatic changes, water level reduction, and other negative impacts [4]. On the other hand, there are increasing concerns about potential adverse health and ecological effects resulting from the production, the use, and the disposal of numerous chemicals that otherwise offer improvements in human life and economic activities. Thus, a huge amount of hazardous organopollutants is produced annually over the world and only 10% of these are disposed safely. The most hazardous compounds are persistent in the environment and are carcinogenic and/or mutanogenic. Xenobiotics are chemicals that are "foreign to the biosphere" and may become available to microorganisms in different environmental compartments, depending on their fate in air, water, soil, and sediments [5]. Household chemicals, pharmaceuticals, and other consumables as well as biogenic hormones are released into the environment after passing through wastewater treatment processes, which are not designed for their removal [6]. The main sources of xenobiotics are wastewater, landfill leachates, and solid wastes released from the industries directly, such as phenols, plastics, hydrocarbons, paints, dyes from textile mills, pesticides and insecticides from agricultural industries and paper and pulp mills, or indirectly, including pharmaceuticals, especially the group of endocrine disrupters, and pesticide residues. These chemicals include biopolymers (cellulose, kraft lignin, and lignin), synthetic polymers (polyarylate, polyacrylamide, and nylon), polycyclic aromatic hydrocarbons (anthracene, benzo[a]pyrene, chrysene, naphthalene, pyrene, etc.), pentachlorophenols (PCP), polychlorinated biphenyls (PCB), pesticides and insecticides (1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane [DDT], benzene, toluene, ethylbenzene, and xylene [BTEX]), as well as trinitrotoluene (TNT), dyes (azo dyes, anthraquinone, etc.), and others (including azide, cyanides, aminotriazole, and carbon tetrachloride). Unlike the naturally occurring organic compounds that are readily degraded upon introduction into the environment, some of these synthetic chemicals are extremely resistant to degradation by native microorganisms [7].

Degradation of such compounds by physical and/or chemical processes is costly and often produces undesirable products which are toxic. Biological methods, being eco-friendly and cost cheap techniques, were proposed for xenobiotic degradation purposes in order to overcome these problems. Compared to bacteria, most of the fungi are robust organisms and generally more tolerant to high concentrations of pollutants. It explains why they have been extensively investigated since the mid-1980s for their bioremediation capacities. White-rot fungi (WRF) constitute an eco-physiological group comprising mostly of basidiomycetes and litter-decomposing fungi. Recently, there has been a great interest in white-rot fungi and their ligninolytic enzymes, including laccase, manganese peroxidase (MnP) and lignin peroxidase (LiP), for the degradation of a wide range of xenobiotics.

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These xenobiotics include phenols [2, 8–10], hydrocarbons [11–14], dyes and textile effluents [15–21], pharmaceuticals including the endocrine-disrupter compounds [6, 22], pulp and paper mills [23–25], pesticides, and insecticides [26–29]. Some studies reported the effect of ligninolytic enzymes on degradation of other organopollutants like PCB [1], chlorinated phenols including trichlorophenols [30, 31], and xenobiotics in landfill leachates, solid wastes [32–35], and munitions [26].

Because of the non-specific nature of fungal lignin depolymerization, white-rot fungi can degrade a wide range of persistent environment pollutants even the insoluble chemicals [26]. These microorganisms generally act via the extracellular ligninolytic system showing good potential applications in chemical, agro-food, paper, textile, and cosmetic industries. This group may be a useful and a powerful tool for bioremediation purposes thanks to fungal capacities to degrade many xenobiotic substances.

The expression of these enzymes depends on the strain itself: some white-rot fungi produce LiP and MnP, but not laccase, while others produce MnP and laccase, but not LiP, acting simultaneously or separately on xenobiotics released from the environment. The potential of white-rot fungi can be harnessed thanks to emerging knowledge of the physiology and the morphology of these organisms. This knowledge could be transformed into reliable and robust waste treatment processes. The importance of high extracellular levels of these enzymes to enable the efficient degradation of recalcitrant compounds under in vivo conditions relates to the sorption and complexation of enzymes in soil and the probable loss of their activity once externalized.

The lignin degradation system consists on peroxidases, H_2O_2-producing enzymes, veratryl alcohol (3,4-dimethoxybenzyl alcohol), oxalate, and manganese. LiP and MnP are glycosylated heme proteins that couple the reduction of H_2O_2 to water with the oxidation of a variety of substrates [36]. It was reported that *Phanerochaete chrysosporium*, producing simultaneously both MnP and LiP, was able to degrade many xenobiotics and recalcitrant compounds [26].

Laccases, which are also extracellular enzymes and being blue multicopper oxidases, catalyze the monoelectronic oxidation of a large spectrum of substrates, including phenolic and nonphenolic compounds as well as recalcitrant environmental pollutants [6, 11, 12, 22]. This explains their potential use for xenobiotic degradation, and bioremediation purposes.

2. Origin, threat, and biodegradation of xenobiotics; enzymatic system involved in xenobiotic biodegradation

While a huge amount of hazardous organopollutants is produced annually over the world, only 10% of these are disposed of safely. The most hazardous compounds are persistent in the environment and are known to have carcinogenic and/or mutanogenic effects. The prime source of xenobiotics is wastewater, landfill leachates [33], and solid residual releases from the industries [37]. Solid wastes may contain volatile organic compounds as residues or incorpo-

rated into the structure of materials such as plastic foams, packaging, floor and wall coverings, solvents, paints, and adhesives [38].

Wastewaters, including domestic and industrial wastewaters, contain a variety of compounds. Some of the common compounds present in wastewaters and in other effluents are phenolic compounds, hydrocarbons, dyes, endocrine disrupting compounds, and pesticides [37].

Landfills generate large amounts of leachates containing high levels of organics and ammonia nitrogen [32, 39]. These substances with others like phenols and hydrocarbons can be a major source of contamination of the groundwater. Indeed, the variety of contaminants in landfill leachates, their synergistic and antagonistic effects as well as their physicochemical properties make them serious toxicants, which may survive different treatments [32]. Landfill leachates exhibit consequently high toxicity levels [32, 33, 40, 41]. The efficiency of fungal remediation of landfill leachates has been proved on *Trametes trogii*, *Lentinus tigrinus*, and *P. chrysosporium* [32]. The strains were able, via their extracellular enzymes, to reduce organics (chemical oxygen demand (COD), phenols, and hydrocarbons) as well as toxicity, for twofolds diluted LFL. However, raw LFL caused growth inhibition and enzyme secretion reduction, indicating the sensitivity of these strains to high levels of toxic compounds such as phthalates and phenol derivatives [42]. Tigini et al. reported that autochthonous and allochthonous fungal strains were efficient in LFL treatment, showing a complete spectrum of action and being able to significantly reduce the wastewater toxicity for all the tested strains. Thus, *Porostereum spadiceum* showed the best activity with 40 % of decolorization within 1 week [33].

Solid waste residues can be domestic wastes, including food, paper, and garden wastes; waste from council activities associated with servicing residential areas: street sweepings, tree lopping, parks and gardens, and litter bins; and waste from institutional, commercial, industrial activities, generally containing higher proportions of metals and plastics than domestic wastes. They also can be derived from demolition and building activities, which contain high proportions of inert material (concrete, bricks) and low proportions of other materials. Many xenobiotic compounds are released from municipal solid waste and may be found in the leachates and the gaseous phase of landfills [43]. They include 1,1-dichloroethylene, 2,4,6-trichlorophenol, dimethyl phthalate, phenol, benzoate, and phthalic acid [44].

Phenols and phenolic compounds are widely distributed compounds in the nature, especially in the plants, but also in marine systems, produced by marine plants and animals where they can be degraded by indigenous microbial population [45]. Several types of industries, such as coal refineries, phenol manufacturing, pharmaceuticals, dying, petrochemical, pulp mill as well as agricultural wastes, contain phenols which are considered among the most prevalent pollutants due to their high toxicity even at low concentrations [37, 46, 47]. Phenol is also employed in the production of resins and also used in the manufacture of plastic, biocides, disinfectants, textiles, medicines, explosives, pinks, perfumes, and photographic materials [48]. Consequently, phenols have negative effects on the ozone layer and on the earth heat [47]. Phenol, being a carcinogenic compound, must be removed from industrial wastewaters prior to their discharge, via biodegradation processes resulting in minimum secondary metabolites and harmless end products [49]. Several studies have shown that phenol can be degraded by

a wide variety of fungi including *P. chrysosporium*, *Trametes versicolor*, *Trametes villosa*, and *Lentinus eodes* [25, 26, 50].

Furthermore, chlorinated phenols are one of the most serious environmental pollutants. Lignin-degrading fungi and their enzymes have been used to detoxify these compounds through their transformation into non-toxic or less toxic substances [51, 52]. Ehlers and Rose found immobilized WRF cultures to be effective in removing phenolic and chlorinated phenolic pollutants [52]. Leontievsky et al. reported that *Panus tigrinus* and *Coriolus versicolor* and their ligninolytic enzyme systems efficiently transform 2,4,6-trichlorophenol (TCP) to 2,6-dichloro-1,4-hydroquinol and 2,6-dichloro-1,4-benzoquinone [51]. However, MnP and laccase differed in their specificity: in *P. tigrinus* culture, primarily the MnP attacked 2,4,6-TCP, whereas in *C. versicolor* culture, predominantly laccase catalyzed the transformation. Besides, *P. chrysosporium* has been the most extensively studied among the ligninolytic fungi, as a model system, and the pathways for degradation of 2,4-di, 2,4,5-, and 2,4,6-trichlorophenols were investigated [26, 53].

Plastics are known to be hazardous materials due to the nature of components that are made of and including polystyrene, polyvinyl chloride, polyethylene, and its derivatives. They are very slowly degraded due to the molecular bonds and interactions. Biodegradation of plastics gained importance in the last few years, but the fragmented compounds released by this biodegradation also lead to other with environmental issues [37]. Cameron et al. reported that *P. chrysosporium* was able to degrade plastics like nylon [26].

Polycyclic aromatic hydrocarbons and saturated hydrocarbons are usually found in petroleum effluents at high concentrations and cause an environmental pollution. Because physical-chemical degradation of such compounds is cost-effective and may lead to further disturbances in the environment, biological treatments offer the alternative to reduce the impact of these pollutants [37]. Hence, bioremediation had a great potential as an alternative method for the rehabilitation of contaminated sites. The use of natural microorganisms, isolated for their ability to degrade a large variety of hydrocarbons [11–14], allows the elimination of such compounds from contaminated sites [54]. Microorganisms that can degrade hydrocarbons are particularly isolated from petroleum-contaminated sites [55]. Indeed, the microbial action depends on aromatics structure since the aromatic fraction is more difficult to degrade [56]. Olusola and Anslem reported that *Pleurotus pulmonarius* was able to degrade crude oil [14]. Other studies reported the effective fungal bioremediation of hydrocarbons [11–13]. Bioremediation of anthracene and pyrene in soil, using mycelia of *P. chrysosporium*, *T. versicolor*, and *Pleurotus ostreatus* was reported as effective, since MnP and LAC were secreted at high levels in the soil. However, these high enzyme levels allowed a more efficient degradation of recalcitrant compounds in liquid media [1].

Volatile organic compounds and additives, such as emulsifiers and texturizers in paint, can be degraded by different tools such as chemicals (water as solvent), hygroscopic stresses, and microbial sources [37]. Some fungi were reported as effective decomposers of paints. The development of microfungi on the surface of painting induces aesthetical, mechanical, and biochemical decay [57].

Textile effluents are one of the principal sources of pollution over the world. In particular, the release of colored effluents into the environment is undesirable, not only due to their color but also because many synthetic dyes with their complex aromatic molecular structure [58, 59] and their breakdown products are toxic and/or mutagenic [59, 60]. Due to the unspecific nature of their lignin-degrading enzymatic system, fungi can also degrade textile dyes [61]. However, the well understanding of the fungal degradation mechanism involved is essential to identify the degradation products and to verify the toxicity removal, consequently. Until now, much research has been done on dye degradation by fungi and or laccases [62, 63] but few studies have focused on the intermediate products toxicity [64]. Many fungal isolates and their enzymes were reported as efficient for the degradation or the decolorization of many polymeric dyes, including blue dextran and Poly R478 as well as the triphenylmethane dyes: cresol red, crystal violet, and bromophenol blue [19, 20]. Ben Younes et al. reported that laccase from the thermophilic fungal strain *Scytalidium thermophilum* catalyzed the decolorization and the detoxification of the azo dye Congo red and the triarylmethane dyes, commonly found in textile industry effluents [20]. The team also reported, in previous studies, that the crude enzyme as well as the purified laccase from *Perenniporia tephropora* was able to decolorize dyes of the textile industries, including neolane pink, neolane blue, and remazol brilliant blue R (RBBR) [18]. The latter was also efficiently decolorized by laccase from *T. trogii* [17]. The ability of *T. trogii* laccase to decolorize azo and triarylmethane dyes was approved in the absence of redox mediators, since MG and BCG were completely degraded with crude laccase within 6 h of treatment. Toxicity evaluation showed a final product detoxification [21]. On the other hand, the fungal decolorization of RBBR has been reported for other strains such as *Dichomitus squalens*, *Ischnoderma resinosum*, *Pleurotus calyptratus* [65], and *P. ostreatus* [66]. Tekere et al. reported the ability of *Trametes cingulata*, *T. versicolor*, *Datronia concentrica*, and *Pycnoporus sanguineus* to decolorize the Poly R478 [67]. Mohorcic et al. found that *Bjerkandera adusta* was able to decolorize the black-blue dye through violet and red to pale yellow via its extracellular enzyme; the MnP which was also reported for its ability to decolorize amaranth and remazol black B [68]. Previously, Swamy and Ramsay reported since 1999 the ability of *Bjerkandera* sp., *P. chrysosporium*, and *T. versicolor* to decolorize remazol orange, remazol brilliant blue, reactive blue, and tropaeolin O in agar plates [69]. Consequently, some strains including *T. trogii* and *S. thermophilum* were reported to be able to decolorize and detoxify textile effluents [19, 20]. Robinson et al. reported that *B. adusta* and *Phlebia tremellosa* provided a good efficiency to decolorize textile effluent in N-limited conditions [62].

Paper and pulp mills are effluents released from paper mill industries and cause serious environmental pollution because they contain chlorinated organic compounds, which are absorbable organic halides, including pentachlorophenols, tetrachlorocatechols, and tetrachloroguaiacols [70]. They are often released to anaerobic conditions, exhibiting high acute and chronic toxicity levels and mutagenicity and/or carcinogenicity. Fungal enzymes were used for bleaching these effluents to obtain high-quality paper pulps [23, 71]. Indeed, it was reported that the laccase from *Coriolopsis gallica* has been implicated in the decolorization of effluents from the pulp and paper industry [25, 72]. Laccases have also been shown to be applicable for the bioremediation of pulp and paper industry wastes by effecting direct dechlorination [73] for the removal of chorophenols and chlorolignins from bleach effluents

[74, 75]. Other uses of laccases for the pulp and paper industry include reduction of the kappa number of pulp [15] and an improvement in the paper-making properties of pulp [76].

A large variety of pesticides and insecticides, including organophosphorous compounds, and benzimidazoles, are intensively used and may contaminate the land due to their slow degradation [37]. Despite the slow process, microbial degradation is considered as a tool to minimize the negative effects of these compounds on the ecosystem. Many studies reported the effective degradation of pesticides by fungal strains, including *P. chrysosporium* and *T. versicolor*, and involving two different enzyme systems: laccase and peroxidases [26–29].

Pharmaceuticals are discharged directly by pharmaceutical manufacturers or in wastewaters from hospitals. These compounds have performed their biologically intended effect, but their degradation into toxic substances in the body is often a cause for concern [77] since they unfortunately get passed into the environment in either their complete or fragmented forms. These pharmaceuticals, used in personal care products (PCPs) or being endocrine-disrupting chemicals (EDCs), mainly include hormones, anesthetics, and antibiotics, and can be accumulated in an organism and passed on to the other through the common food chain [78]. Even though they are the indirect sources, they cause adverse effect on the ecological cycle [37]. Nonsteroidal anti-inflammatory drugs are also a large and diverse chemical group of drugs used on humans and animals for the treatment of inflammation, pain, and fever [6]. The use of diclofenac in animals has been reported to have led to a sharp decline in the vulture population reaching 99% [6]. These compounds, including nonylphenol (4-nonylphenol), bisphenol A (2,2-bis(4-hydroxyphenol) propane), triclosan (5-chloro-2(2,4-dichlorophenoxy) phenol) and others, are frequently detected in receiving waters downstream of intense urbanization [79, 80]. The latter can mimic or interfere with the action of animal endogenous hormones by acting as estrogen agonists, binding to the estrogen receptor or eliminating a normal biological response [6, 81, 82]. The promise of laccase for the transformation or the elimination of PCPs and EDCs from both aqueous solutions and polluted soils has been recently established [6, 83]. Cabana et al. demonstrated that the resulting chemicals do not have any estrogenic activity [84].

It is known that white-rot fungi can degrade lignin in the way that the mycelia of the organisms penetrate the cell cavity and release ligninolytic enzymes to decompose materials to a white sponge-like mass [85]. The ability of fungi to transform a wide variety of hazardous chemicals has aroused interest in using them in bioremediation [86]. Enzymatic treatment, involving mainly peroxidases and/or laccases, is currently considered as an alternative method for the removal of toxic xenobiotics from the environment [87].

2.1. Peroxidase system

The lignin degradation system consists on peroxidases, H_2O_2-producing enzymes, veratryl alcohol, oxalate, and manganese. All of these enzymes are glycosylated heme proteins that couple the reduction of hydrogen peroxide to water with the oxidation of a variety of substrates. The redox potentials of LiP and MnP are higher than for others peroxidases; that is why they have been shown to oxidize chemicals that are not easy to be oxidized by other microorganisms. These chemicals include Polycyclic aromatic hydrocarbons (PAH), phenol

and its derivatives, cyanide, TNT, and others [26]. This finding was reported for the fungus *P. chrysosporium*, which has been shown to degrade many xenobiotics and recalcitrant compounds, both in soil and in liquid cultures, suggesting the attractive use of such fungus in bioremediation.

Lignin peroxidases (LiPs) belong to the family of oxidoreductases [36, 88] and were firstly described in the basidiomycete *P. chrysosporium* in 1983 [89]. This enzyme has been recorded for several species of white-rot basidiomycetes [90]. LiP is dependent of H_2O_2, with an unusually high redox potential and low optimum pH [91, 92]. This enzyme is able to oxidize a variety of substrates including polymeric ones [93] and has consequently a great potential for application in various industrial treatment processes [92].

Manganese peroxidases (MnPs) belong to the family of oxidoreductases [36]. Following the discovery of LiP in *P. chrysosporium*, MnP secreted from the same fungus was found as another lignin-degrading enzyme [94] and was secreted by almost all white-rot fungi. MnP catalyzes the oxidation of phenolic structures to phenoxyl radicals [9]. The product Mn^{3+}, being highly reactive, complex with chelating organic acids, such as oxalate, lactate, or malonate. On the other hand, it was reported that MnP may oxidize Mn(II) without H_2O_2 and with decomposition of acids, and concomitant production of peroxyl radicals [95].

2.2. Laccase system

Laccases which are blue multicopper oxidases, catalyze the monoelectronic oxidation of a large spectrum of substrates, for example, ortho- and para-diphenols, polyphenols, aminophenols, and aromatic or aliphatic amines, coupled with a full, four electron reduction of O_2 to H_2O. Laccases act on both phenolic and nonphenolic lignin-related compounds as well as highly recalcitrant environmental pollutants, and they can be effectively used in paper and pulp industries, textile industries, xenobiotic degradation, and bioremediation and can act as biosensors. Some studies reported the identification of genes that are differentially regulated during fungal growth in the presence of different environmental pollutants. However, abiotic stress caused by many factors including water potential, temperature, and pH can influence the metabolism of the degradation process. Hence, considering bioremediation in soil, the conditions that favor fungal activity in soil, such as temperature, moisture, nutrient status, pH, and aeration, need to be optimized to promote metabolic degradation of xenobiotics. Magan et al. studied the effect of abiotic factors on the fungal degradation of pesticides by *T. versicolor* and *P. chrysosporium* for soil bioremediation purposes [96]. In fact, the potential property of laccase is its highly non-specific nature of substrates [97]. Furthermore, the common presence of one or more substructures in the lignin molecule and in xenobiotics explains the ability of white-rot fungi to degrade such a wide range of environmental organic pollutants, even at high levels [98, 99]. Otherwise, it has been shown that laccase metabolizes these compounds without any net energy gain [100]. Indeed, the oxidation of lignin is performed to access to wood polysaccharides, being their main energy source [101]. This implies that the presence of lignin-cellulosic substrates is required to ensure the degradation of xenobiotic compounds [102].

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3. Conclusion

One of the major environmental problems, causing a serious threaten over the world, is the contamination of atmosphere components with toxic chemicals. Unfortunately, the most xenobiotic compounds, produced annually at huge amounts, are persistent in the environment and have carcinogenic and/or mutanogenic effects. The main sources of xenobiotics are wastewater, landfill leachates, and solid wastes. Xenobiotics include phenols, plastics, hydrocarbons, paints, dyes, pesticides, insecticides, paper and pulp mills, pharmaceuticals, and others.

Biological processes, being eco-friendly and cost cheap techniques, were proposed for xenobiotic degradation to overcome these problems. White-rot fungi, especially the basidiomycetes, are the most tolerant microorganisms to high concentrations of pollutants, giving their exceptional abilities for biodegradation in aqueous environments and soil and have been investigated extensively for their bioremediation capacities. Fungal bioremediation is a promising tool since the metabolic potential of such microorganisms converts most of the environmental pollutants to less hazardous or non-hazardous compounds with less input of energy and time. White-rot fungi are the unique organisms that show the capacities of degrading highly toxic organics and recalcitrant compounds. The key enzymes of their metabolism are extracellular ligninolytic enzymes that enable fungi to tolerate high concentrations of toxic substrates. These enzymes have potential applications in a large number of fields, including the chemical, fuel, food, agricultural, paper, textile, and cosmetic industrial sectors. Their capacities to remove xenobiotic substances and to produce others, which are less or non-toxic, make them a useful tool for bioremediation purposes.

The potential of white-rot fungi can be harnessed thanks to emerging knowledge of the physiology and morphology of these microorganisms. This knowledge could be transformed into reliable and robust waste treatment processes. The importance of high extracellular levels of these enzymes to enable the efficient degradation of xenobiotic compounds under in vivo conditions relates to the sorption and complexation of enzymes in soil and the probable loss of much of their activity once externalized.

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