Degradation of xenobiotic compounds by lignin-degrading white-rot fungi: Enzymology and mechanisms involved

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White-rot fungi (WRF) are ubiquitous in nature with their natural ability to compete and survive. WRF are the only organisms known to have the ability to degrade and mineralize recalcitrant plant polymer lignin. Their potential to degrade second most abundant carbon reserve material lignin on the earth make them important link in global carbon cycle. WRF degrade lignin by its unique ligninolytic enzymatic machinery including lignin peroxidase, manganese peroxidase, laccase, cellobiose dehydrogenase, \( \text{H}_2\text{O}_2 \)-generating enzymes, etc. The ligninolytic enzymes system is non-specific, extracellular and free radical based that allows them to degrade structurally diverse range of xenobiotic compounds. Lignin peroxidase and manganese peroxidase carry out direct and indirect oxidation as well as reduction of xenobiotic compounds. Indirect reactions involved redox mediators such as veratryl alcohol and \( \text{Mn}^{2+} \). Reduction reactions are carried out by carboxyl, superoxide and semiquinone radicals, etc. Methylation is used as detoxification mechanism by WRF. Highly oxidized chemicals are reduced by transmembrane redox potential. Degradation of a number of environmental pollutants by ligninolytic system of white rot fungi is described in the present review.

Keywords: Lignin degradation, Ligninolytic enzymes, White-rot fungi, Xenobiotic compounds

The industrial development over the last five decades has resulted in an exponential increase in the production and consumption of chemicals. Production, use and disposal of numerous chemicals cause widespread contamination of soils as well as ground waters and surface waters. Indiscriminate applications, high persistence, unknown environmental pathway and pollutant’s potential to bioaccumulate have resulted in severe repercussions, including the loss of food sources, mutagenic and carcinogenic effects to the mankind. The recognition that environmental pollution is a worldwide threat to public health has given rise to a new industry for environmental restoration. Physical and chemical treatment processes (i) typically remove organic pollutants at low level, (ii) are highly selective in terms of the range of pollutants removed and (iii) prohibitively expensive for the treatment of wastes. Clean-up of environmental pollution also presents a serious economic burden and therefore cost effective yet efficient and environment-friendly methods of decontamination are vital in solving the hazardous waste problems. The use of indigenous or introduced microorganisms to decontaminate waste sites – bioremediation, provides a very attractive, eco-friendly and economic solution to many of our hazardous pollution problems. For both economic and ecological reasons, biological degradation (bioremediation) has become an increasingly popular alternative for the treatment of hazardous wastes. One such method of bioremediation involves the white rot fungi, a group of basidiomycetes characterized by their ability to degrade lignin in wood. This degrading ability is unique among these fungi and has made them an important link in the global carbon cycle.

Lignin-degrading white rot fungi

Most known white rot fungi (WRF) are basidiomycetes and are capable of white rot decay. White rot decay derives from the appearance of wood attacked by these fungi, in which lignin removal results in a bleached appearance of the substrate. The ability to catabolize lignocellulose and hemicellulose is fairly common as a primary metabolic process among WRF. As a result, it is not regarded as a rate-limiting step in the carbon flux. Lignin is extremely recalcitrant and is mineralized in an obligatorily oxidative process, carried out appreciably only by white rot fungi¹. The oxidation of lignin yields no net energy gain, and so lignin is degraded during secondary metabolism in order to access wood polysaccharides locked in lignin-carbohydrate complex.
Ligninolytic activity appears during nutrient C, N, or S limitation. Under such conditions, white rot fungi produce certain extracellular enzymes viz. lignin peroxidase, manganese peroxidase, laccases, \( \text{H}_2\text{O}_2 \)-generating enzymes, versatile peroxidase, cellobiose dehydrogenase, etc in various combinations. These enzymes are encoded by gene families that allow complex regulation and generation of isoforms, and catalyze one-electron oxidation resulting in the formation of reactive free radical species inside the lignin polymer. Subsequently, the radicals undergo spontaneous reactions leading to the incorporation of oxygen, bond cleavage and finally the breakdown of lignin molecule\(^2\). The physiology and biochemistry of lignin degradation has recently been reviewed\(^3\). The unique ligninolytic enzyme system of basidiomycetous fungi would be ideal for the biodegradation of organopollutants in the environment\(^4\).

Understanding the basic mechanism of lignin degradation is required to know that how highly oxidized environmental pollutants can be degraded by WRF.

**Advantages of white-rot fungi**

The white rot fungal technology is very different from other better-established methods of bioremediation. The mechanisms used by the fungi provide them with several advantages for pollutant degradation. The lignin-degrading system, being extracellular, has evolved to degrade insoluble chemicals such as lignin and many of hazardous environmental pollutants at considerably higher concentrations. The intracellular machinery of bacteria is poorly accessible to the pollutants, and their uptake may inhibit the growth.

The lignin-degrading system is non-specific, non-stereoselective and free radicals-based in nature, allows WRF to degrade a wide variety as well as complex mixtures of pollutants. Free radicals are highly reactive and reactions occur as chain reaction, carrying out complete degradation of pollutants. Whereas, bacterial enzymes are highly specific and a consortium may be required to degrade successfully and completely such chemicals.

The lignin-degrading system is expressed in response to nutrient (C, N or S) limitations and therefore organism does not require preconditioning with the pollutant to be degraded. Enzymes are not repressed even when the pollutant concentration is reduced to ineffective levels for enzyme induction. Bacteria fail to degrade the pollutants when the concentration is reduced below threshold value and ineffective for enzyme induction. WRF can effectively degrade very low concentrations of pollutants to non-detectable levels. WRF can be cultivated on inexpensive growth substrates like wheat straw, corn cobs, wood chips or other crop residues and also on liquid media as well as in soil that promote the use of WRF for bioremediation. In addition to being able to grow under nutrient limitation, the fungi also produce oxygen radicals such as \( \text{OH}^\bullet \), which is capable of oxidizing biomolecules, such as proteins and DNA that could result in the death of other microbes. Using the plasma membrane dependent redox system, the fungus is able to adjust the pH of its surrounding environment. Thus, microbes with pH optimum that differ from that of the fungus might not grow well after the fungus has been introduced.

The extreme non-specificity of the mechanisms described here makes the WRF an attractive solution to many of our ever-growing hazardous waste problems. Only through our understanding and continued research efforts, with regard to these mechanisms, we will be able to successfully design bioremediation strategies employing the WRF.

**Mechanisms for xenobiotic degradation**

**Lignin peroxidase**

Direct oxidation—Lignin peroxidase (LiP) has a classical peroxidase catalytic mechanism. Native enzyme is oxidized by \( \text{H}_2\text{O}_2 \) and generates two-electron deficient compound I. Compound I oxidizes a chemical and reduces to one electron deficient compound II. A subsequent oxidation of another molecule by compound II returns the peroxidase to its native resting stage (Fig. 1). LiP is having relatively high redox potential\(^5\), so the chemicals with high redox potentials that are not oxidized by other enzymes are oxidized by LiP. LiP can oxidize both phenolic and non-

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**Fig. 1—Mechanism of direct oxidation by lignin peroxidase**
phenolic compounds resulting in carbon-carbon bond cleavage, aromatic ring fission, phenolic oxidation, demethoxylation, methylation, hydroxylation and dimerization reactions same as with lignin.

Many pollutants including benzo[a]fluorene, cyanides, dyes, etc. are directly oxidized by LiP in vitro. Cyanide is oxidized directly to cyanyl radical by LiP. The ability of LiP to oxidize cyanide allows the fungus to efficiently degrade the pollutant to CO\textsubscript{2}. LiP also catalyzes direct oxidation of pyrene to pyrene-1,6-dione and pyrene-1,8-diones. Dibenzo[p]dioxin is also oxidized by LiP to its cation radical\textsuperscript{8}. Various dyes are directly oxidized by LiP\textsuperscript{9}. Oxidation of methylene blue by LiP results into formation of Azure C, which is a tri-demethylated methylene blue derivative\textsuperscript{10}.

**Indirect oxidation**—In many cases, chemicals are not directly accessible to heme of LiP and thus direct oxidation does not occur. In such cases involvement of redox mediator plays an important role. Veratryl alcohol (VA) produced by WRF is an excellent substrate for LiP. VA serves as an electron mediator to facilitate oxidation of pollutants. VA is oxidized by LiP to VA cation radical (VA\textsuperscript{+}) which is a strong oxidant responsible for indirect oxidation of lignin and pollutants (Fig. 2).

EDTA was found to be indirectly decarboxylated by LiP H2. The apparent inhibition of veratryl alcohol oxidase activity of LiP H2 by EDTA is suggestive of the reduction of VA\textsuperscript{+} back to VA during oxidation of EDTA\textsuperscript{11}. Chemicals that have been found to be indirectly oxidized by LiP include herbicide aminoti-triazol\textsuperscript{12}, pentachlorophenol\textsuperscript{13}, phenol\textsuperscript{14}, etc. Recently, we have reported direct as well as indirect oxidative decolourization of Remazol brilliant blue R by LiP produced by *Trametes versicolor*\textsuperscript{15}.

**Reduction**—LiP catalyzes reduction of various chemicals in the presence of VA (Fig. 3). VA\textsuperscript{+} generated in LiP reaction oxidizes carboxylic acids to respective acid derived anion radicals, which in turn serve as reductant. Such radicals effectively reduce cytochrome c, nitro blue tetrazolium, ferric ion and molecular oxygen and are also involved in the reduction of carbon tetrachloride to the trichloromethyl radical which is neither a substrate for enzyme nor a good reductant\textsuperscript{11}.

VA\textsuperscript{+} oxidizes EDTA as well as oxalate to their corresponding anion radicals. These carboxylate anion radicals, in the absence of another electron acceptor, reduce molecular oxygen to O\textsubscript{2}\textsuperscript{−}, which will reduce ferric iron to ferrous iron (and has been shown to reduce some chemicals). H\textsubscript{2}O\textsubscript{2} then readily reacts with chelated ferrous iron to produce OH\textsuperscript{−}\textsuperscript{16} (Fig. 3).
'OH is having incredible oxidizing ability and make-up a potential non-enzymatic biological system known as Fenton reagent. Fenton's reaction has been widely used for degradation of xenobiotic compounds including PCBs, herbicides and dyes.\textsuperscript{17,18}

**Manganese peroxidase (MnP)**

**Oxidation**—MnP oxidizes Mn\textsuperscript{2+} to Mn\textsuperscript{3+} which is stabilized by organic acid chelators and acts in turn as a low molecular mass, diffusible, redox mediator that attacks organic molecules and oxidizes various chemicals non-specifically via hydrogen and one electron abstraction\textsuperscript{19} (Fig. 4). Compound I can be reduced by Mn\textsuperscript{2+} and other electron donors such as ferrocyanide and phenolics. Compound II is slowly reduced by other substrates and requires Mn\textsuperscript{3+} to complete the catalytic cycle\textsuperscript{20}.

Chelates of Mn\textsuperscript{3+} with carboxylic acids (oxalate, malonate, malate, tartrate, lactate) cause one-electron oxidation of various substrates. Phenolic and amino-aromatic compounds are oxidized by hydrogen abstraction to form phenoxy and amino radicals, respectively\textsuperscript{19}. Certain non-phenolic aromatic substances with low redox potential such as tetramethoxybenzene or anthracene are subject to one-electron abstraction from the aromatic ring, giving rise to aryl cation radicals\textsuperscript{21}. Chelates of Mn\textsuperscript{3+} and carboxylic acids can react with each other and are converted to alkyl radicals, which undergo subsequent spontaneous reactions with dioxygen resulting in the formation of other radicals (e.g. superoxide)\textsuperscript{22}. Versatile peroxidases produced by some WRF possess the ability to oxidize, in addition to Mn\textsuperscript{2+} also phenolic (phenol red) and non-phenolic (veratryl alcohol) aromatic compounds\textsuperscript{23}.

**Reduction**—MnP catalyzes reduction reactions in the presence of hydroquinones and Mn\textsuperscript{2+} (Ref. 24). Mn\textsuperscript{3+} oxidizes hydroquinones to corresponding semiquinone radicals, which has been shown to reduce more oxidized chemical. The quinone formed by this process is reduced back to hydroquinone by quinone reductases. Thus, highly oxidized pollutants, are indirectly reduced by MnP and LiP facilitating further metabolism\textsuperscript{24} (Fig. 5).

**Methylation**

White-rot fungi methylate a wide variety of phenolic compounds by a trans-membrane methyl transferase, which is reported to be a detoxification mechanism\textsuperscript{25}. The primary methyl donors S-adenosylmethionine and methyl chloride, are synthesized by these fungi\textsuperscript{26}. Upon methylation of hydroquinones resulting methylated products are oxidized such that the aromatic ring is opened; quinones being degraded instead to redox cycle\textsuperscript{27}. Methylation of phenolic compound yields corresponding anisole\textsuperscript{28}. Phenols are not efficient substrates of LiP but \(\alpha\)-methylated aromatic compounds such as veratryl alcohol are efficiently oxidized by LiP. Chlorinated phenols such as 2,4,5-trichlorophenol and PCP are mineralized by WRF where methylation is the first step of such metabolism\textsuperscript{25}. PCP, potent inhibitor of oxidative phosphorylation is quite toxic to multitude of organisms, upon methylation is converted to pentachloroanisole that is less toxic\textsuperscript{29}. Therefore, WRF use methylation as a mechanism to detoxify pollutants and following detoxification LiP and MnP effectively metabolize the pollutants further.

**Transmembrane redox potential**

A number of highly oxidized chemicals including 2,4,6-trinitrotoluene (TNT) are reduced by cultures of WRF under non-ligninolytic conditions and the mechanism is independent of ligninolytic enzymes. A method of reduction used by many microbes, includ-
ing filamentous fungi, involves maintenance of a proton gradient across the plasma membrane. TNT is reduced by cultures of *P. chrysosporium* to mono- and di-amino congeners by transmembrane redox potential (Fig. 6), which are further oxidized by MnP and subsequent evolution of CO₂ is associated with the production of LiP³⁰.

**Xenobiotic degradation**

**Polycyclic aromatic hydrocarbons (PAHs)**

PAHs comprise an important group of environment pollutants having mutagenic, genotoxic and carcinogenic properties. PAHs are non-polar, hydrophobic organic compounds with two or more fused benzene rings. Low-molecular-weight PAHs, such as naphthalene, acenaphthene, acenaphthylene, fluorene, anthracene, and phenanthrene are transformed rapidly by many bacteria and fungi. High-molecular-weight PAHs, however, are more recalcitrant in the environment and resist both chemical and microbial degradation.

Ligninolytic enzymes of WRF oxidize PAHs to corresponding PAH quinones and subsequently degraded further to CO₂³¹. The two main enzyme groups involved in the initial attack on PAHs by fungi are the cytochrome P-450 monooxygenase and LiP. Both enzymes are relatively non-specific for PAHs that they metabolize. Cytochrome P-450 incorporates one atom of molecular oxygen into PAH molecule to form an arene oxide, which then undergoes either spontaneous isomerization to form a phenol, with subsequent conjugation with sulfate, glucuronic acid, glucose or xylose, or enzymatic hydration to form trans-hydrodiol³². LiP ionizes aromatic compounds to form aryl cation radicals, which undergo further oxidation to form quinones. Lignin peroxidase H₈ from *Phanerochaete chrysosporium* is able to catalyze oxidation of 9-phenanthrol forming phenanthrene-9,10-quinone. Purified forms of LiP and MnP have been shown to oxidize anthracene, pyrene, fluorene and benzo[α]pyrene to their corresponding quinones³³,³⁴. Some PAHs, up to six aromatic rings, are oxidized by manganese-dependent lipid peroxidation reactions, both *in vitro* and *in vivo*³³.

Laccase of *T. versicolor* oxidizes most of the 16 PAHs listed by the US EPA as priority pollutant chemicals. Benzo[α]pyrene and perylene are partially converted to polymeric products. Small amounts of quinones and ketones are the main oxidation products from anthracene, benzo[α]pyrene and fluorene³⁵,³⁶. Laccase in combination with 1-hydroxybenzotriazole oxidizes acenaphthene and acenaphthylene to 1,2-acenaphthenedione and 1,8-naphthalic acid. The role of natural mediators, including phenols and aromatic amines, in the degradation of PAHs by laccase is now beginning to be unveiled³⁷. The white-rot fungi *Pleurotus ostreatus* and *Bjerkandera* oxidize PAHs like phenanthrene, anthracene, fluorene, pyrene and

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**Fig. 6—Reduction of TNT by plasma membrane redox potential (Modified from: Reference 28)**
Free radicals by an electron transfer process might prise off a biphenyl molecule substituted with one to enhance the degradation of the aromatic, π-π interactions, and quinoid molecules from plant material; and (3) the covalent coupling of both reactants by laccases to generate reactive oxygenates. WRF are capable of oxidative destruction by cellobiose dehydrogenase, an enzyme that catalyzes oxidation of HADTs leading to the formation of corresponding nitrosodinitrotoluenes and coupling products.

The significant solubility of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in water and its limited adsorption to clay particles in soil is a matter of concern. Cultures of P. chrysosporium reportedly degraded RDX (70%) in 24 days. Single electron oxidation of RDX by MnP may destabilize the explosive sufficiently to undergo ring cleavage. RDX degradation by cellobiose dehydrogenase under anaerobic conditions has suggested that WRF might also employ reductive mechanisms. The warfare agent_Yerpite (bis (2-chloroethyl) sulphide) is completely mineralized by T. versicolor, but the role of ligninolytic enzymes system is not known. The ability of ligninolytic fungi to degrade a wide range of xenobiotics, e.g., TNT, RDX, HMX, provides a strong rationale for research and development of field applications. These fungi provide treatment systems that will operate in situ in large areas of contaminated soils (land-farming in combination with bioaugmentation and biostimulation by straw and wood chips). However, the potential toxicity of the products remains to be evaluated.

Nitroaromatics and explosives

The soil and ground water contamination by nitroaromatic explosives 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) is a worldwide problem that started following intensive military activities in World War I. TNT, its metabolites, and related compounds exhibit considerable toxicity to humans, animals, and microorganisms. The biological decomposition of TNT and related compounds is limited by the fact that the bulky and electron-negative nitro substituents of TNT present steric constraints and reduce the electron density of the aromatic ring, impeding electrophilic attack by oxygenases. WRF are capable of oxidative destruction and mineralization of the aromatic nucleus of TNT.

Ligninolytic fungi stabilize TNT and its biotransformation products with the soil humic compounds in three major steps—(1) the transformation of nitroaromatic compounds; (2) the formation of phenolic and quinoid molecules from plant material; and (3) the covalent coupling of both reactants by laccases and peroxidases. Recently, ninety-one fungal strains were tested and most were found capable of biodegrading TNT. Mineralization of TNT is dependent on the organism's physiological state being either ligninolytic or non-ligninolytic. The initial products from TNT biotransformation with non-ligninolytic cultures of P. chrysosporium were nitroso-toluene (NS), o-hydroxyamin-4,6-dinitrotoluene (o-HADNT), p-hydroxyamino-2,4-dinitrotoluene (p-HADNT) and mono- and dinitrophenol (ADN and DANT). Secondary products including azo-, azoxy, phenolic, and acylated (acylated and formylated) derivatives were detected.

Ligninolytic fungi are more efficient than bacteria in mineralizing TNT. LiP or MnP generate reactive free radicals by an electron transfer process might enhance the degradation of the aromatic ring of TN. Stahl and Aust have proposed the involvement of a membrane-bound reduct system correlated with the proton secretion system in the reduction of TNT. Michels and Gottschalk have reported that the reductive activity is intracellular and depends on NADPH. Regardless of the site where TNT is reduced, further degradation and mineralization of TNT by P. chrysosporium occurs only when cultures are ligninolytic, implying that LIP, MnP and/or other enzymes of the ligninolytic system further transforms the reduced products of TNT. In vitro MnP system is able to mineralize a mixture of reduction products from [14C]-ring-labelled TNT as well as ADNTs, 4-HADNT and 2,6-DANT suggesting the cleavage of the aromatic nucleus to CO₂. The transformation and mineralization of reduced derivatives of TNT by MnP is significantly enhanced in the presence of cysteine or reduced glutathione (GSH), implying Mn(III)-GSH or Mn(II)-GS- as the ultimate oxidants. It indicates that thiols and unsaturated fatty acids are the only enhancers of the MnP-catalyzed mineralization process. LiP catalyzes oxidation of HADTs leading to the formation of corresponding nitrosodinitrotoluenes and coupling products.

Halo-organics

Polychlorinated biphenyls (PCBs)—PCBs comprise of a biphenyl molecule substituted with one to ten chlorine substituents. PCBs persist in the envi-
environment and bioaccumulate because of their toxic, mutagenic, chemically inert and lipophilic nature. The ligninolytic cultures of WRF P. chrysosporium B. adusta, Pl. ostreatus and T. versicolor degrade congeners of PCB commercial mixtures Aroclor 1242, 1254 and 1260. However, there does not exist any correlation between the biodegradative ability and activities of known ligninolytic enzymes. Yadav et al. have described PCB degrading ability of P. chrysosporium under both ligninolytic and non-ligninolytic conditions. WRF mineralise PCBs to CO₂ in liquid media as well as in soil but not so efficiently. Differences in the extent of PCB transformation and mineralisation by WRF may be attributed to the differences in the composition of the ligninolytic enzyme system and mechanisms employed for the degradation. Generally, PCB degradation efficiency decreases with higher degree of chlorination. Future research needs to identify the ligninolytic enzymes involved, the degradation mechanism and reaction products.

Chlorophenols—Chlorophenols are toxic and highly persistent priority pollutants. These compounds are used as wood preservatives, pesticides and precursors of herbicides. Laccase, LiP and MnP carry out initial oxidation of chlorophenols and metabolites formed during chlorophenol degradation, such as hydroquinones and methylated derivatives. The oxidation of chlorophenols by such enzymes results in the formation of phenoxyl radicals that spontaneously lead to the formation of quinones and di- and oligomeric coupling products. MnP directly but partially mineralizes 2,4-dichlorophenol (2,4-DCP) in the cell free system. 2,4-DCP is also known to be oxidised by LiP. P. chrysosporium efficiently mineralizes several chloro-, methyl- and ethyl-substituted benzene under non-ligninolytic conditions. 2,4-DCP and 2,4,6-trichlorophenol are efficiently mineralized under ligninolytic conditions. The availability of nutrient carbon and oxygen, presence of extracellular ligninolytic enzymes and the bioavailability of chlorophenols are important factors influencing the degradative process. Reductive dechlorination reactions are involved in the degradation of 2,4,6-trichlorophenol by P. chrysosporium and several other white rot fungi.

Pesticides

PCP, a broad spectrum biocide, is converted to 2,3,5,6-tetrachloro-2,5-cyclohexadiene-1,4-dione by LiP. Soil cultures of P. chrysosporium o-methylate PCP. LiP or MnP oxidatively dechlorinate PCP to tetrachloro-1,4-benzoquinone. The quinone is further reduced to tetrachlorodihydrobenzene, which undergoes four successive dechlorinations to produce 1,4-hydroquinones and further hydrolyzed to form 1,2,4-trihydroxybenzene. Alternatively, tetrachloro-1,4-benzoquinone is converted either enzymatically or non-enzymatically to 2,3,5-trichlorohydroxybenzene, which undergoes successive reductive dechlorinations to produce 1,2,4-trihydroxybenzene. The final product undergoes ring cleavage with subsequent degradation to CO₂. Key intermediate tetrachlorohydroxybenzene is readily degraded under both nitrogen-sufficient and nitrogen-limiting conditions, suggesting that other enzymes are involved in PCP degradation pathways. Extracellular peroxidases are involved only in the initial oxidation of PCP to tetrachlorobenzoquinone.

Ligninolytic cultures of P. chrysosporium, Pl. ostreatus, Phellinus weirii and Polyporus versicolor mineralise ¹⁴C-DDT resulting in the formation of several metabolites and ¹⁴CO₂. The intermediates identified are DDD, FW-152, dicofol and 4,4’-dichlorobenzophenone. One study has shown that ¹⁴C-radiolabelled 1,1-dichloro-2,2-bis(4-chlorophenyl)ethene (DDE), extremely toxic and persistent DDT, was mineralized to ¹⁴CO₂ by P. chrysosporium. Purified LiP and MnP in a multi-step pathway involving sequential oxidation, reduction and methylation reactions remove the two CI atoms and carry out ring cleavage and mineralization. P. chrysosporium mineralises aldrin, dieldrin, heptachlor, chlordane, lindane and mirex. It also degrades organochlorine herbicides 2,4,5-T and 2,4-D. The herbicide 2-chloro-4-ethylamine-6-isopropyl- amino-1,3,4-triazine (atrazine) is transformed by P. chrysosporium and Pl. ostreatus to hydroxylated and N-dealkylated metabolites. P. chrysosporium also degrades organophosphate insecticides chloropyrifos, fonofos and terbufos.

Synthetic dyes

Synthetic dyes are extensively used in textile, paper, photography, cosmetic and leather industries. These dyes include several structural varieties such as acidic, reactive, basic, disperse, azo, diazo, anthraquinone-based and metal-complex dyes. Interest in pollution potential of synthetic dyes has primarily been prompted by concern over their possible toxicity and...
carcinogenicity. Most of the dyes are highly resistant to microbial attack. Therefore, it is hard to remove them from effluents by means of conventional biological wastewater treatments, such as activated sludge. The decolourization of dyes by WRF has been first reported by Glenn and Gold [26]. *P. chrysosporium* decolourizes polymeric dyes, azo and heterocyclic dyes [8,25]. Involvement of lignin-degrading enzymes in the decolourization of dyes has been investigated by several workers [33,34]. Podgornik *et al.* [75] have studied decolourization of 50 structurally different dyes by extracellular LiP of *P. chrysosporium*. They have shown that decolourization can proceed by either a uniform decrease in the absorbance through the spectrum or formation of a new transient absorption peak. Differential expression of LiP isoforms by different taxa or culture conditions may result in variable dye-decolourizing ability.

Decolorization of dyes by ligninolytic enzymes is an oxidative process that can result in complete degradation of the dye molecule to CO₂ and H₂O [66]. LiP catalyzes oxidative decolourization of methylene blue [60]. MnP and laccase preferentially convert dyes which carry a phenolic substituent in para-position to the azo bond and additionally methyl- or methoxy-substituents in 2- or 2,6-position in relation to hydroxy-group [7]. MnP of *B. adusta* and *P. eryngii* that catalyse dye decolourization, are unusual as they catalyze Mn²⁺-independent reaction [78]. MnP decolourizes Poly R-478 [84]. Laccase from *Pycnoporus cinnabarinus* decolourizes complex, industrially relevant azo dyes such as Reactive Blue 5 and Direct Blue 1 [60].

Recently, Jarosz-Wilkolazka *et al.* [81] have screened 115 fungi of different physio-ecological group for their ability to decolorize azo and heterocyclic dyes, concluding that anthraquinone dyes are decolourized easier and faster by fungi than azo dyes. Cultures of *Chrysosporium lignorum* decolourize polymeric dyes Poly R-478 and Poly S-119 both in the static conditions as well as immobilized in a bioreactor [85]. Laccases from *T. versicolor* and *P. chrysosporium* were found to decolorize several dyes efficiently both in the presence and absence of redox-mediator 1-hydroxybenzotiazole, which improved and facilitated the decolorization [83]. A bioreactor packed with *P. chrysosporium* immobilized on polymethane foam presented good stability with high decolorization percentagew of a hardly biodegradable dye Poly R-478 [84]. Recently Selvam *et al.* [85] have reported the decolorization of azo dyes and dye industry effluents by *Thelephora* spp. Our group recently have reported the decolorization of sulfonphthalein dyes by ligninolytic enzymes of different WRF [86,87]. Involvement of degradative mechanism other than ligninolytic enzymes(s) for dye decolorization has also been studied. Pasti and Crawford [88] have proposed plasma membrane redox system of WRF for dye decolorization. Kirby [89] has evidenced that such a mechanism is involved in the decolourisation of Remazol Black B by strains of *P. chrysosporium* and *T. versicolor*. Vyas and Moltor [90] have reported decolourization of RBBR by a novel enzyme produced by *P. ostreatus*, which is different from MnP, LiP, VAO and laccase. The activity has been named RBBR oxygenase. They have reported that dye decolorization by WRF undergoes sequential changes of blue dye to colorless through a rainbow of intermediates also shown by other researchers [91]. It is also proposed from such observations that decolourization is not a single step reaction and intermediates are involved during complete decolourization.

Due to the inherent complexity of both the dye molecules and enzymatic machinery involved, the degradative pathways utilized by WRF have not so far been elucidated. Conneely *et al.* [92], have attempted to elucidate the degradation pathway of copperphthalocyanine dyes by *P. chrysosporium*. Dye is readily degraded and both free copper and organo copper breakdown products are found in culture supernatants. Spadaro and Renganathan [93] have reported that the oxidation of non-sulfonated azo dyes [1-(4'-acetamidophenylazo)-2-naphthol] by LiP from *P. chrysosporium* results in the formation of 1,2-naphthaquinone and acetylamide. Two groups have reported degradation of different sulfonated azo dyes by crude and purified peroxidase preparations, respectively [71,72]. Decolorization of polymeric dyes has been proposed as a useful screening method for ligninolytic activity. Field *et al.* [94], have revealed good correlation between PAH degradation and poly R-478 decolorization rates. RBBR and polymeric dyes are, therefore, being used for measuring ligninolytic activities [95,96]. Decolorization of such dyes correlates with the initiation of lignin degradation and reflects a combined effect of peroxidases and H₂O₂-producing oxidases [72]. Therefore, the dye decolorization studies are being used as a possible, easily usable and inexpensive alternative to radiolabelled lignins and other xenobiotics in biodegradation studies.
Summary
The mechanisms described in the present study make WRF technology unique among more established methods of bioremediation. The unusual general advantages for pollutant degradation. Ability of WRF to degrade structurally diverse xenobiotic organopollutants is demonstrated in number of experiments both in liquid media and under soil conditions. Additionally, various species of fungi that produce MnP, LiP, laccase and other enzymes may permit the use of numerous indigenous or introduced fungi for remediation purpose. Despite valuable basic knowledge on the mechanisms of pollutant biodegradation, bioremediation has not yet been accepted as a routine treatment technology and environmental industry is wary in applying bioremediation. Thus, more extensive and intensive research efforts one needs for searching and exploiting new fungal species and improvement of practical application to establish mycoremediation as an effective and reliable bioremediation technology.

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References
33 Hammel K E, Green B & Gai W Z, Ring fission of Anthracene by a eukaryote, Proc Natl Acad Sci (USA), 88 (1991) 10605.


68 Yadav J S & Reddy C A, Mineralization of 2,4-dichlorophenoxyacetic acid (2,4-D) and mixtures of 2,4-D and 2,4,5-trichlorophenoxyacetic acid by *Phanerochaete chrysosporium*, *Appl Environ Microbiol.*, 59 (1993) 2904.


88 Pasti M B & Crawford D L, Relationships between the abilities of *Streptomyces* to decolorize the anthra-type dyes.


