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White Rot Fungi in the Treatment of Hazardous Chemicals and Wastes

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White-Rot Fungi

Microbiological treatment of hazardous wastes has generally been associated with the use of bacteria. However, during the past decade a significant body of evidence has accumulated that demonstrates that fungi, in particular lignin-degrading or white-rot fungi, have the ability to degrade a wide range of hazardous organic compounds and thus might also be useful for treatment of materials contaminated with these compounds.

White-rot fungi are filamentous wood-decay fungi, primarily Hymenomycetous basidiomycetes but including a few ascomycetes from the order Sphaeriales, that share the ability to metabolize lignin and the polysaccharide components of wood simultaneously (Rayner and Boddy, 1988). These fungi are the major degraders of highly lignified tissues (lignin contents $\geq$ 20%) and therefore play a vital role in the recycling of photosynthetically fixed carbon (Kirk, 1983). Decay by these fungi gives wood a bleached appearance, thus the term white-rot. Over 90% of an estimated 1600 to 1700 species of wood-decay fungi in North America are white-rot fungi (Gilbertson, 1980). In North America white-rot basidiomycetes are classified in the orders Tremellales, Agaricales, and Aphyllophorales.

Pure culture studies have shown that litter-decay basidiomycetes that cause a bleaching of litter and humus, resulting in "white-rot humus," can also metabolize lignin (Hering. 1967). However, these fungi are poor degraders of wood (Kirk. 1983). Hering (1982) suggested that white-rot fungi are more efficient degraders of wood than litter-degrading fungi because they are better adapted to the environment in the interior of decaying wood. where aeration is restricted and various volatile substances may accumulate. However, to degrade lignin, litter-inhabiting fungi must posses ligninolytic systems similar to those of white-rot fungi and therefore might possess similar xenobiotic degrading abilities. Also, litter-inhabiting fungi are adapted to soil environments and thus might be more useful in treatment of contaminated soils.

The most thoroughly investigated white-rot fungus is *Phanerochaete chrysosporium* Burds. This fungus has been used extensively as an experimental organism to elucidate the physiology and biochemistry of lignin metabolism. It has also been the primary experimental organism in the study of xenobiotic metabolism by white-rot fungi. In this report we describe the lignin-degrading system of *P. chrysosporium,* give evidence for involvement of the system in the mineralization of xenobiotics, and summarize research on use of white-rot fungi to treat effluents from the kraft pulp-bleaching process and to remediate contaminated soils.

Xenobiotics Known to Be Degraded by White-Rot Fungi

Interest in using white-rot fungi for the treatment of hazardous chemicals and hazardous wastes originated from research on the use of *P. chrysosporium* and other white-rot fungi to treat kraft pulp bleach plant effluents. These effluents contain polymeric, chlorinated, heavily-oxidized degradation fragments of lignin-which are responsible for the highly colored nature of the effluents — and a complex mixture of chlorinated phenols, anisoles, and other low-molecular-weight components (Eaton et al., 1982; Huynh et al., 1985). Several white-rot fungi, including *P. chrysosporium.* were found to decolorize the effluents (Eaton et al., 1982), and *P. chrysosporium* was shown to remove low-molecular-weight chioro-organics (Huynh et al., 1985). The results of these investigations led to further studies that demonstrated that *P. chrysosporium* and other white-rot fungi are able to degrade a broad range of structurally diverse organic and chlorinated organic compounds (Table 9.1). The list of chemicals that are mineralized by white-rot fungi continues to grow and ranges from the insecticides DDT and lindane to wood-preserving chemicals, including PCP and the creosote components anthracene and phenanthrene, to chlorinated biphenyls and dioxins.

Lignin-Degrading System

Lignin, like many of the hazardous organic compounds. is a biologically recalcitrant substrate. Lignin degradation by *P. chrysosporium* is effected in part by extracellular hemoprotein peroxidases that initiate the depolymerization of the polymer (Kirk. 1987). Further metabolism of the resultant heterogeneous mixture of low-molecular-weight aliphatic and aromatic lignin fragments is intracellular (Kirk and Shimida, 1985), but virtually nothing is known about the uptake and intracellular metabolism that leads to their mineralization.

Much of what is known about the physiology of lignin degradation by *P. chrysosporium* has come from studies measuring evolution of ^{14}CO, from mineralization of [^{14}C]-labeled compounds by axenic cultures under varying conditions (reviews: Kirk and Shimida. 1985; Buswell and Odier, 1987). These studies showed that ligninolytic activity ([^{14}C]lignins - $^{14}CO2$) occurs only during sec-

Table 9.1. Xenobiotics Mineralized by White-Rot Fungi

Reference	Xenobiotics Mineralized
Bumpus et al. (1985)	1,1,1-trichloro-2,2-bis (4-chlorophenyl)ethane (DDT) Lindane 2,3,7,8-tenachlorodibenzo(*p*)dioxin 3,4,3,4'-tnchlrobiphenyl Benzo(a)pyrene
Eaton (1985)	Aroclor 1254
Arjmand and Sandermann (1985) Arjmand and Sandermann (1986)	4-Chloroaniline 3,4-Dichloroaniline Chloroaniline-lignin conjugates
Sanglard et al. (1986)	Benzo[*a*]pyrene
Burnpus and Augst (1987)	DDT
Mileski et al. (1988)	Pentachlorophenol (PCP)
Bumpus and Brock (1988)	Triphenylmethane dyes Crystal violet Pararosaniline Cresol red Bromphenol blue Ethyl violet ,Malachite green Brilliant green
Ryan and Bumpus (1989)	2,4,5-Trichlorophenoxyacetic acid
Bumpus (1989)	Phenanthrene
Huttermann et al. (1989)	Anthracene Fluoranthene Benzo[*b*]fluoranthene Benzo[*k*]fluoranthene Benzo[*a*]pyrene Indeno[*ghi*]pyrene Benzoperylen
Cripps et al. (1990)	Azo and heterocyclic dyes Orange II Tropaeolin O Congo Red Azure B
Fernando et al. (1990)	2,4,6-Trinitrotoluene (TNT)

ondary (idiophasic) metabolism and is triggered by limiting cultures for nutrient nitrogen, carbon, or sulfur (Jeffries et ai., 1981). Since wood is nitrogen-poor, nitrogen limitation is the natural situation for wood-inhabiting fungi (Cowling and Merrill, 1966). The lignin-degrading system appears in nitrogen-starved cultures whether or not lignin is present (Keyser et al., 1978). 'Therefore, for

technological uses, addition of lignin to cultures is not necessary for expression of the lignin-degrading system. However, adding lignin to cultures increases the titer of the lignin-degrading system (Faison and Kirk, 1985; Ulmer et al.l 1984). Lignin alone is an inadequate source of carbon for fungal metabolism and is mineralized by *P. chrysosporium* only in the presence of an additional carbon source such as cellulose, glycerol, or glucose.

The lignin-degrading system has a high oxygen requirement (Kirk et al., 1978; Reid and Seifert, 1982; Bar-Lev and Kirk, 1981). Ligninolytic activity occurs at much greater rates under oxygen (100%) than under air (21% oxygen) and is absent in an atmosphere of 5% oxygen in nitrogen (Kirk et al., 1978). Increasing the oxygen concentration in the culture atmosphere has a dual effect: it leads to an increased titer of the lignin-degrading system (Faison and Kirk, 1985; Bar-Lev and Kirk, 1981), and it leads to increased activity of the existing system (Bar-Lev and Kirk, 1981).

Lignin-degrading enzymes were first isolated from the extracellular culture fluid of *P. chrysosporium.* These enzymes have been identified as peroxidases and are termed lignin peroxidases (Tien and Kirk, 1983; Glenn et al., 1983). Another enzyme found in the extracellular culture fluid of *P. chrysosporium,* glyoxal oxidase (GLOX), is a H_2O_2-producing enzyme (Kersten, 1990). A number of GLOX substrates, including glyoxal and methyglyoxal, are also found in ligninolytic cultures (Kersten, 1990). The temporal correlation of GLOX, lignin peroxidase, and GLOX substrates in cultures of *P. chrysosporium* suggests a close physiological relationship among these components. Lignin peroxidases, in the presence of H_2O_2, catalyze the one-electron oxidation of alkoxybenzenes and other lignin-related compounds to the corresponding aryl cation radicals (Kersten et al., 1985). Further reactions of the cation radicals that fragment the polymer are nonenyzmatic and include cleavages of side-chain carbon-carbon and ether linkages and cleavages of aromatic nuclei (Hammel et al.l 1985, Hammel et al., 1986a). The peroxidases are nonspecific, oxidative, and extracellular, which accommodates the heterogeneity and nonstereoregularity of the interunit linkages and the nonhydrolyzability and the large size of the lignin polymer.

A second kind of peroxidase, manganese peroxidase, in the presence of H_2O_2, oxidizes Mn^{2+} to Mn^{3+}. which in turn can oxidize phenolic units in lignin (Huynh and Crawford, 1985). The roles of such oxidation and of manganese peroxidase in lignin biodegradation are not yet clear.

Evidence for Involvement of the Lignin-Degrading System in Xenobiotic Metabolism

Several lines of evidence suggest that the lignin-degrading system of *P. chysosporium* is involved in the degradation of xenobiotics. The nonspecificity of lignin peroxidases suggests that they will oxidize nonlignin aromatics. Indeed,

oxidation of polycyclic aromatic hydrocarbons and dibenzo[*p*]dioxins, including 2-chlorodibenzo[*p*]dioxin by lignin peroxidase from *P. chrysosporium,* has been reported (Hammel et al., 1986b; Haemmerli et al., 1986). Mineralization of [^{14}C]DDT was like [^{14}C]lignin, promoted in nitrogen-starved cultures and suppressed in nitrogen-sufficient cultures; also, the onset and time course of DDT and lignin mineralization seemed to coincide (Bumpus et al., 1985). Oxidation of several xenobiotics by isolated ligninase (Hammel et al., 1986b; Haemmerli et al., 1986), the mineralization of some environmental pollutants by *P. chrysosporium* only during secondary metabolism (Eaton, 1985; Bumpus et al., 1985; Bumpus and Aust, 1987), and the inhibition of mineralization by high-nutrient nitrogen levels all suggest that the ligninolytic system is partly responsible for degradation of some xenobiotics.

Even so, mineralization of DDT and PCP has also been observed in nonligninolytic cultures of *P. chrysosporium* (Kohler et al., 1988; Lin et al., 1990), leading to the suggestion that a second xenobiotic-degrading system is present (Lin et al., 1990).

Oxidation of Xenobiotic Compounds by Lignin Peroxidases

Initial work with polynuclear aromatics and dioxins demonstrated that the one-electron oxidative mechanism of lignin peroxidase, initially described for alkoxybenzenes (Kersten et al., 1983, is also applicable to certain xenobiotics (Haemmerli et al., 1986; Hammel et al., 1986b; Hammel and Tardone, 1988). Reaction of benzo[*a*]pyrene with either crude preparations of lignin peroxidase or purified lignin peroxidase fractions, in the presence of H_2O_2, resulted in the production of 1,6-, 3,6- and 6,12-quinones (Haemmerli et al., 1986). These quinones are the expected products from the one-electron oxidation followed by a nucleophilic attack by H_2O and further oxidations. Hammel et al. (1986b) demonstrated (1) that lignin peroxidase catalyzes the oxidation of dibenzo[*p*]dioxin to the cation radical, (2) via $H_2{}^{18}O$-labeling experiments that the source of quinone oxygens is H_2O, and (3) that lignin peroxidase catalyzes the oxidation of polynuclear aromatic hydrocarbons with ionization pontentials $\leq$ ~7.55 eV. Chlorinated phenols were also shown to be oxidized by lignin peroxidase, leading to the corresponding *p*-quinone (Hammel and Tardone, 1988). This reaction occurred on all tested chlorinated phenol congeners regardless of whether a chlorine was present at the *para* position; *para* chloro substituents were oxidatively dechlorinated (Hammel and Tardone, 1988).

Use of White-Rot Fungi for Treatment of Kraft Bleach Effluents

Treatments using white-rot fungi have been proposed for decolorization and detoxification of kraft pulp-bleaching effluents (Marton et al., 1969: Fukuzumi et al., 1977; Lundquist, 1977) and for decolorization of pretreated coal gasification

wastewater (George et al., 1986) and molasses wastewater (Ohmomo et al., 1985). All these treatments rely on expression of the lignin-degrading system of the fungi. Most of the work to date has been focused on the treatment of kraft pulp bleach effluents. In the following discussion we identify the nature of these effluents and the problems that their disposal presents, and then summarize the research to date on development of systems for the treatment of these effluents that employ the lignin-degrading ability of white-rot fungi.

Nature of the Effluents

After kraft pulping, residual lignin is degraded and dissolved in various sequences of bleaching and extraction stages in which chlorine, hypochlorite, chlorine dioxide, oxygen, and hydrogen peroxide are used. The most common sequence of stages used is CEDED (C = chlorine stage, E = alkali extraction stage, D = chlorine dioxide treatment), which is followed by washing to remove residual chemicals (Pellinen et al., 1988). The effluents produced during bleaching of kraft pulp are the major contributors to wastewater pollution from the pulp industry and they require treatment prior to discharge (Eriksson and Kirk, 1985). Most of the residual lignin in kraft pulp is dissolved during the first two stages: the first chlorination stage (C_1) and the first alkaline extraction stage (E_1). The effluents produced from these two stages account for 85% to 90% of the COD, 75% to 80% of the BOD, more than 95% of the color, and the major portion of the toxicity discharged by bleach plants (Belt et al,. 1981).

Effluents from the C, stage contain a large proportion of low-molecular-weight (i.e., $M_w < 1000$) components. Approximately 30% of the organically bound chlorine in C_1-stage effluents is associated with compounds with low molecular weights ($M_w < 1000$) (Kringstad and Lindstrom, 1982). Kringstad and Lindstrom (1982) divided these low-molecular-weight components into three groups comprising acidic, phenolic, and neutral compounds. The acidic group includes fatty, hydroxy, dibasic, and aromatic acids. The phenolic group, the components of which are mainly responsible for the toxicity of this effluent, is a complex mixture of chlorinated phenols, catechols, and guaicols. Methanol and various hemicellulose-derived products are quantitatively the most important components of the neutral fraction. The total quantity of chlorinated compounds in the neutral fraction is small. However, several chlorinated compounds from this fraction are mutagenic; indeed, chloroacetones, chlorinated furanone derivatives, and 2-chloropropenal are considered to be the major contributors to the mutagenicity of C_1-stage effluents (Douglas et al., 1983).

The materials responsible for the color of the E_1-stage effluent from kraft bleach plants are water-soluble, polymeric, chlorinated, oxidized lignin degradation fragments with a low content of aromatic moieties (Bennett et al., 1971, Hardell and deSousa, 1977; Sundman et al., 1981; Lindstrom and Osterberg, 1984). Approximately 95% of the chlorine of E_1 effluents is bound to organic compounds

with molecular weights larger than 1000 (Kringstad and Lindstrom, 1982). The chromophoric materials are probably biologically inactive because they are too large to penetrate cell membranes of living organisms. However, discharge of these materials to receiving waters is of concern because the brown color of the effluent may increase water temperature and decrease photosynthesis, resulting in a decrease in the dissolved oxygen concentration. Biological and chemical degradation of the polymeric materials might result in the release of low-molecular-weight, toxic, chlorinated compounds (Pellinen et al., 1988). The toxicity of E_1 effluents is largely caused by low-molecular-weight components, including 3, 4, 5-trichloroguaiacol, tetrachloroguaiacol, and dichlorodehydroabietic acid (Leach and Thakore, 1975).

Treatment of E_1-Stage Effluents: Removal of Chromogenic Materials

A process for the decolorization of E_1-stage effluents using white-rot fungi was developed jointly by researchers at the Forest Products Laboratory (FPL) and North Carolina State University (NCSU). This process called the FPL-NCSU MyCoR (Mycelial Color Removal) process is based on the biological decolorization of E_1-stage bleach plant effluents by a white-rot fungus immobilized on disks in a rotating biological contactor (RBC) (Eaton et al., 1982). Early work focused on the decolorization of E_1-stage effluents (Eaton et al., 1980; Eaton et al., 1982) and led to the patenting of the process by Chang et al. (1987). *Phanerochaete chrysosporium* was shown to decolorize the effluent by destroying both chromophoric groups and structures (Sundman et al., 1981; Yin et al., 1989) and by degrading the high-molecular-weight chlorolignins to CO_2 and inorganic chloride (Sundman et al., 1981; Pellinen et al., 1988).

Laboratory and bench-scale experiments on fungal decolorization identified several factors that are important to the MyCoR process. However, these factors would be relevant to any decolorization process that employs the lignin-degrading ability of white-rot fungi. (1) A growth stage is necessary before decolorization begins. During this stage the fungi are grown in a nitrogen-limited medium. Once the nutrient nitrogen is depleted, the fungi become ligninolytic (Keyser et al., 1978) and able to decolorize. (2) The fungi require a carbon source for decolorizaton (Eaton et al., 1980), just as they do for lignin degradation (Kirk et al., 1976). Glucose, in most cases, has been used as the growth substrate. Because addition of an easily degradable carbon source such as glucose might be too expensive in an industrial situation, cheap substrates such as the primary sludge from pulp and paper mills could be used. When primary sludges, which are rich in cellulose (*cã.* 30% to 60% cellulose), were used as growth substrate, decolorization was similar to that when cellulose (Solka-Hoc) was used as substrate (Eaton et al., 1982). Use of these sludges in the decolorization process would be a welcome alternative to their disposal via landfill. (3) When glucose is used as the growth substrate, a concentration of 2 g/L is the minimum amount needed for the fungi

to maintain effective decolorizing ability (Pellinen et al., 1988; Yin et al., 1989). Higher color removal rates are obtained at higher initial glucose concentrations (e.g., 10 g/L) but result in higher effluent COD because of the contribution of residual glucose to effluent COD (Pellinen et al., 1988). (4) The rate of fungal decolorization is influenced by the initial color concentration of the effluent (Campbell, 1983). Color is measured as absorbance of chromogenic material at 465 nm and is expressed as equivalent platinum-cobalt units (PCU). One PCU equals the color produced by 1 mg/ml platinum in the form of chloroplatinate ion (NCASI, 1971). A 1-day batch kinetic study indicated that when the initial color concentration was less than 8000 PCU, the rate of decolorization decreased exponentially with time (Yin et al., 1989). When the initial color concentration was between 8000 and 19,000 PCU, decolorization decreased linearly with time. Increasing the initial color concentration above 19,000 PCU did not result in further increases in the rate of decolorization (Yin et al., 1989).

Yin et al. (1990) investigated the decolorization and dechlorination of conventional (C_1 and E_1) softwood bleaching effluent by sequential biological treatments in which the MyCoR process was followed by bacterial treatment. They found that pretreatment of effluents with white-rot fungi, which dechlorinated and depolymerized chlorolignins. enhanced the ability of the bacteria to degrade and dechlorinate the lower-molecular-weight chlorinated organics. Ultrafiltration of the effluents prior to fungal treatment to remove most of the water and low-molecular-weight chlorinated organics decreased the cost of the sequential treatment by reducing effluent volume (Yin et al., 1990). The optimum color removal to date using the MyCoR process for softwood kraft bleach effluent that had an initial color concentration of 20,000 PCU is 50% to 60% in a 1-day batch treatment (Yin et al., 1989).

A variation of the MyCoR process, called the MYCOPOR system, is a trickling filter system in which the fungus is immobilized on foam cubes (Messner et al., 1988). The design enhances oxygen availability. A color reduction of 70% to 80% in 6–12hours is expected from this system after further optimization (Messner et al., 1990).

White-rot treatment of kraft pulp bleach effluents is a promising technology but has not yet been scaled up for industrial application.

Remediation of Contaminated Soil with White-Rot Fungi

Evidence accumulated from work on the role microorganisms play in the formation of humic materials in soils and on the binding of xenobiotic compounds in soils suggests that fungal enzymes with phenol-oxidizing activity–specifically. laccases, peroxidases, and tyrosinases–are in part responsible for the incorporation of xenobiotics into soil humic materials via enzymatic polymerization reactions (Bollag, 1983). These reactions result in the formation of xenobiotic–humic acid hybrid polymers (Bartha, 1980; Bartha et al., 1983).

Given the evidence that lignin peroxidases from *P. chrysosporium* are able to oxidize various xenobiotics and that the fungus can mineralize these compounds, several laboratory-scale investigations have been performed to test the ability of white-rot fungi to stabilize, deplete, or transform to innocuous products xenobiotic compounds in soils. For example, the amounts of fluorene, 9-fluorenone, and 1,4-naphthoquinone were decreased by 8.5%. 52%, and 94%, respectively, in a sterile soil inoculated with *P. chrysosporium* (George and Neufield, 1989). Also, inoculation of a nonsterile soil contaminated with 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and amended with ground corncobs (4 g corncobs per 1 g soil), with *P. chrysosporium* resulted in mineralization of ~ 33% of the 2,4,5-T (Ryan and Bumpus, 1989).

We have investigated the potential of using white-rot fungi for the remediation of soils contaminated with wood preservative products, particularly PCP. In a laboratory-scale study, inoculation of three sterile soils with *P. chrysosporium* resulted in a dramatic decrease (98%) in the PCP concentration in inoculated soils (Fig. 9.1) (Lamar et al., 1990). The rate of PCP depletion varied among the soils and appeared to relate to fungal growth and metabolic activity. In a separate study we found that rank in terms of growth of *P. chrysosporium* in the three soils was Marshan > Zurich > Batavia and that there was a positive correlation between fungal growth and the soil nitrogen (N) and organic carbon (C) contents (Lamar et al., 1987). Depletion of PCP by *P. chrysosporium* resulted from its conversion to nonvolatile transformation products — losses of PCP via mineralization or volatilization were negligible, and the nature of the transformation products, whether they were soil-bound or extractable was greatly influenced by soil type. For example, in the Marshan soil about 60% of the PCP depletion was due to its conversion to extractable transformation products. In a similar investigation inoculation of the Marshan soil with *P. chrysosporium* or *Phanerochaete sordida.* a litter-inhabiting white-rot fungus. resulted in an average 90% depletion of PCP about 64% of which was due to conversion via methylation to pentachloroanisole (PCA) (Lamar et al., 1990).

In the fall of 1989 we conducted a field-scale study at a site contaminated by a commercial wood preservative product that originally contained 84% mineral spirits, 1% paraffin wax, 10% alkyd varnish, and 5% technical grade PCP (4.3% PCP) (Lamar and Dietrick, 1990). Inoculation of the soil, which contained 250–400 μg/g PCP, with wood chips thoroughly colonized with either *P. chrysosporium* or *P. sordida* resulted in an overall decrease of 88% to 91% of PCP in 6.5 weeks (Fig. 9.2). This decrease was achieved under suboptimal temperatures for the growth and activity of the fungi. The large initial decrease in plots inoculated with either *P. chrysosporium* or *P. sordida* or application of chips was probably due to adsorption of PCP by the chips. A similar decree was not observed in soil from plots receiving chips plus peat. peat only, or no treatment. The lack of a large decrease in the amount of PCP in soil treated with chips plus peat may have been due to an inhibition of chip adsorption of PCP by the peat.

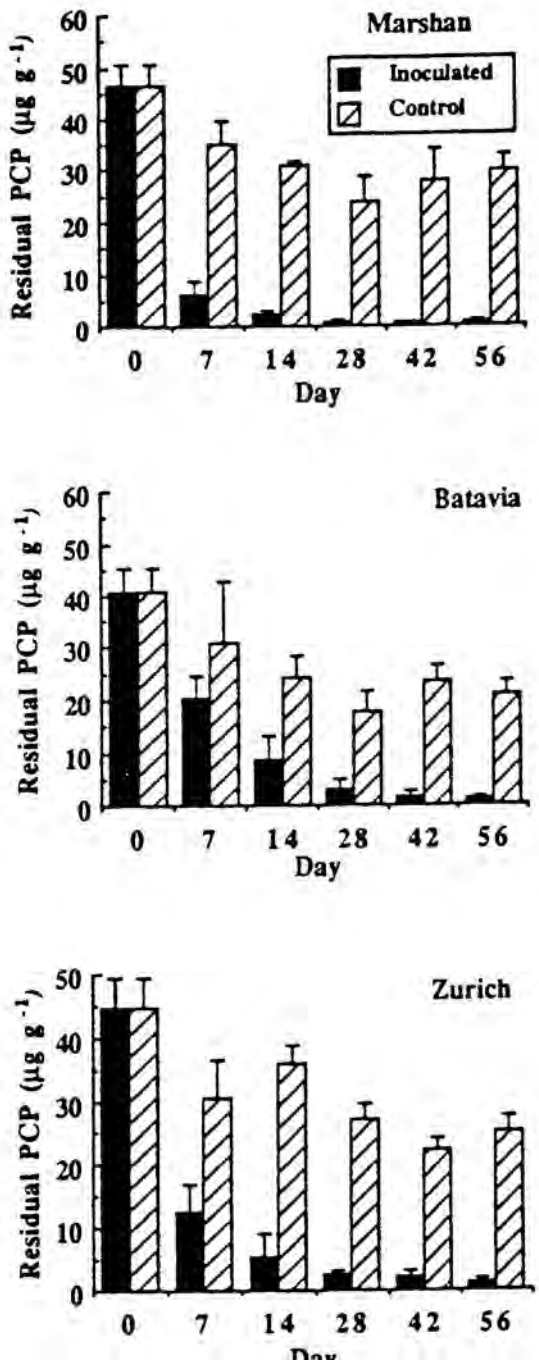

Figure 9.1. Effect of inoculation with *Phanerochaete chrysosporium* on the concentration of pentachlorophenol in three soils. Error bars represent the standard deviations of eight determinations for inoculated culturres and four determinations for control cultures for days 7–56and eight determinations for day 0.

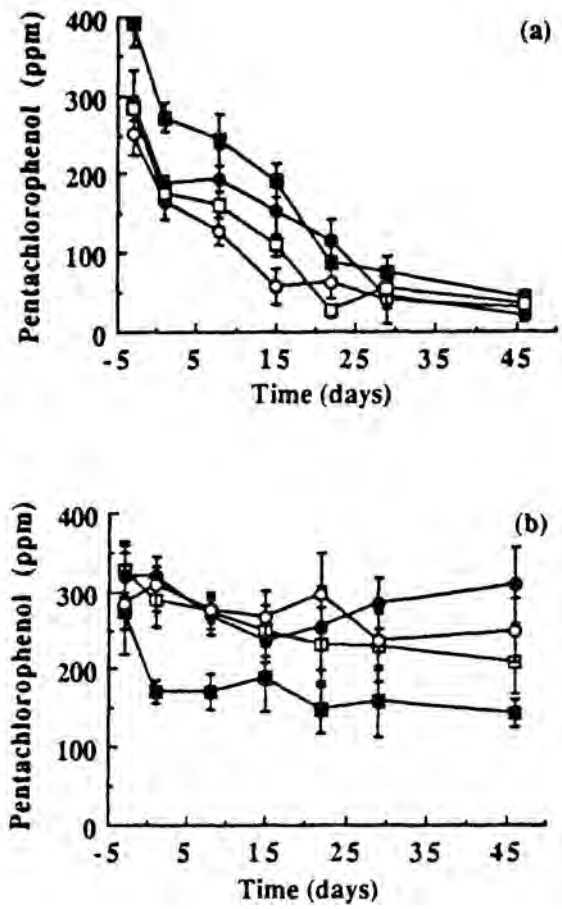

Figure 9.2. Concentration of PCP (μg/g) in (a) soil inoculated with *Phanerochaete chrysosporium* (●○) or *Phanerochaete sordida* (■□), or (b) soil receiving chips (■), chips and peat (□), peat (●), or no treatment (○).

In the field soil, 9% to 14% of the decrease in PCP was a result of methylation to PCA. Thus. methylation was not the major route of PCP depletion. Gas chromatographic analysis of sample extracts did not reveal the presence of extractable transformation products other than PCA. Thus, if losses of PCP via mineralization and volatilization were negligible. as in the laboratory studies, most of the PCP was converted to nonextractable soil-bound products. The nature of these products is not known. Bollag (1983) reported that chlorophenol–syringic acid hybrid polymers were produced when *Rhizoctonia praticola* laccase, a phenol-oxidizing enzyme,was exposed to syringic acid, a humus constituent, and chlorophenols. Similarly, oxidation of PCP in the field soil by ligninolytic enzymes of *P. chrysosporium* or *P. sordida* might have resulted in polymerization reactions, perhaps via quinonoid intermediates (Hammel and Tardone. 1988). resulting in irreversible binding to organic matter.

The stability of xenobiotic–humic acid hybrid polymers under natural conditions is not known. However, work with artificially produced humic acid–xenobiotic hybrid polymers suggests that xenobiotics bound to humic materials through enzymatic polymerization reactions are relatively stable (Dec and Bollag, 1985; Haider and Martin, 1989).

The results of these investigations indicate that the use of white-rot fungi to deplete xenobiotics like PCP in contaminated soils has potential. However, effective application of white-rot fungi in soil remediation will require a better understanding of the process of fungal xenobiotic degradation. This will involve such things as determining how fungi metabolize xenobiotics. resolving the role of the lignin-degrading system in this metabolism and identifying fungi with superior characteristics for use in bioremediation. This information can then be used to develop methods and procedures that enhance the rate and extent of fungal xenobiotic degradation. Laboratory- or bench-scale systems or treatment strategies that successfully optimize and apply the fungal degradative processes will be developed and tested in the field. Finally, new technologies must be developed or existing technologies adapted for application and maintenance of fungal treatments under field conditions.

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