

Review of environmental organopollutants degradation by white-rot basidiomycete mushrooms

Revisión de degradación de contaminantes ambientales por basidiomicetos de la pudrición blanca

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Abstract

White-rot fungi consist of a group of basidiomycetes that are able to remove lignin, cellulose, and hemicellulose concurrently at approximately equal rates. These fungi produce three enzymes commonly known as lignin-modifying enzymes (LMEs) that are responsible for the degradation of wood components. These enzymes are produced during the secondary metabolism under an obligatory aerobic process and are induced by nutrient starvation, low pH, and high concentrations of Mn. We focused this review on the source of environmental organopollutants and the role that these white-rot fungi play on the transformation or mineralization of the environmental contaminants. These recalcitrant compounds originate mainly from human contamination. White-rot fungi or their enzymes showed mineralization of many environmental contaminants such as 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane (DDT), 2, 4, 6-Trinitrotoluene (TNT); polychlorinated biphenyls (PCB's); polycyclic aromatic hydrocarbons (PAH's); wood preservatives; some synthetic dyes; and bleach-derived from paper producing plants.

Keywords: Bioremediation, environmental contaminants, lignin modifying enzymes.

Resumen

Los hongos de la pudrición blanca de la madera pertenecen a un grupo de basidiomicetos capaces de degradar lignina, celulosa y hemicelulosa en proporciones más o menos equivalentes. Estos hongos producen tres enzimas comúnmente conocidas como enzimas modificadoras de la lignina (LME's), las cuales son producidas durante el metabolismo secundario bajo un proceso estrictamente aerobio y son favorecidas por deficiencias nutricionales, bajo pH, y exceso de Mn. En esta revisión nos enfocamos en la fuente de los contaminantes ambientales más comunes y el papel que juegan los hongos de la pudrición blanca de la madera sobre la degradación de los diversos contaminantes orgánicos que afectan el medio ambiente. Estos contaminantes ambientales se originan principalmente por la acción del ser humano. Los hongos de la pudrición blanca de la madera o sus productos mostraron mineralización de varios de los contaminantes ambientales como el 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane (DDT), el 2, 4, 6-Trinitrotolueno (TNT); bifenil policlorinados (PCB's); hidrocarburos policíclicos aromáticos (PAH's); conservadores de la madera; colorantes sintéticos; y blanqueadores derivados de plantas productoras de papel.

Palabras clave: Biorremediación, contaminantes orgánicos, enzimas modificadoras de la lignina.

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Introducción

White-rot fungi consist of a group of basidiomycetes that are able to remove lignin, cellulose, and hemicellulose concurrently at approximately equal rates. Wood decay by white-rot fungi maintains its fibrous nature and loses strength gradually until it is completely degraded. Commonly a bleached and light color is observed as the wood is decomposed (Figure 1) (Alexopoulos et al., 1996). Although white-rot fungi can degrade lignin, it is extremely recalcitrant and is mineralized in an obligatory aerobic process. The oxidation of lignin generates no net energy gain; as lignin is not a substrate in the primary metabolism, and instead, lignin is degraded during secondary metabolism to access wood polysaccharides hidden within lignin-carbohydrate structures, providing energy to other organisms (Jefries, 1990).

These fungi secrete three chemically distinct extra cellular enzymes; referred to as lignin-modifying enzymes (LMEs), which are critical for lignin degradation. Two of them are glycosylated heme-containing peroxidases [lignin peroxidase (LiP, E.C. 1.11.1.14) and Mn dependant peroxidase (MnP, E.C.1.11.1.13)] and the third one is a copper-containing phenoloxidase [laccase (Lac, EC 1.10.3.2)]. The MnP enzyme catalyses an H_2O_2 -dependant oxidation of Mn^{2+} to Mn^{3+} which oxidizes phenolic components of lignin (Wariishi, Valli, and Gold, 1992). The Lac enzyme generates radicals from a low-molecular-mass re-dox mediator in an H_2O_2 -independent reaction. One mediator compound has been identified as 3-hydroxylanthranilate in the laccase-producing white-rot fungus *Pycnoporus cinnabarinus* (Bourbonnais et al., 1997), and others such as 1-hydroxybenzotriazole (HBT) and 2,2'-azino-bis-(3-ethylbenzothiazoline-6 sulfonic acid) have been reported to be laccase mediators in other fungi (Pointing, 2001). The functioning of LiP, MnP, and Lac has been studied in liquid cultures. Lignin-modifying enzyme production occurs during secondary metabolism and is induced by nutrient starvation, primarily nitrogen. The production of LiP and MnP is more efficient in high oxygen levels, but is repressed by agitation

in culture. Conversely, Lac production is favored by agitation (Leonowicz et al. 1999).

A great deal of research has been conducted on the role of white-rot fungal LMEs in bioremediation of pesticides and other organopollutants. White-rot fungi have transformed or mineralized organochlorines, organophosphates, munitions waste, polychlorinated biphenils, polycyclic aromatic hydrocarbons, wood preservatives, synthetic dyes, bleach, and pentachlorophenol (Bumpus and Aust, 1987; Gramms et al., 1999; Hawari et al., 1999; Limura et al., 1996; Lin et al., 1990; Pointing, 2001). In this review, we focus on the source and degradation or mineralization of environmental organopollutants by the white-rot fungi.

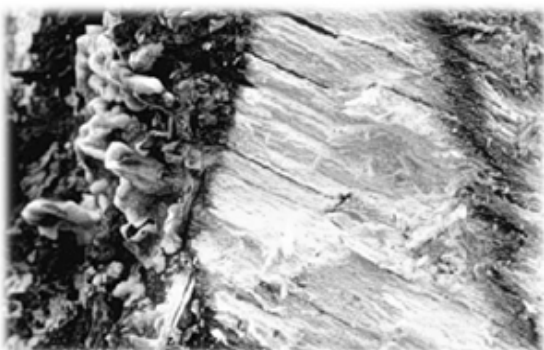
Figure 1. White rotted wood of different plants with young fruiting bodies of various species of white-rot fungi, showing the typical wood decomposition by this group of fungi (Alexopoulos et al., 1996).



Trametes versicolor growing on a beech branch.



Mycena haematopus growing on a willow log.

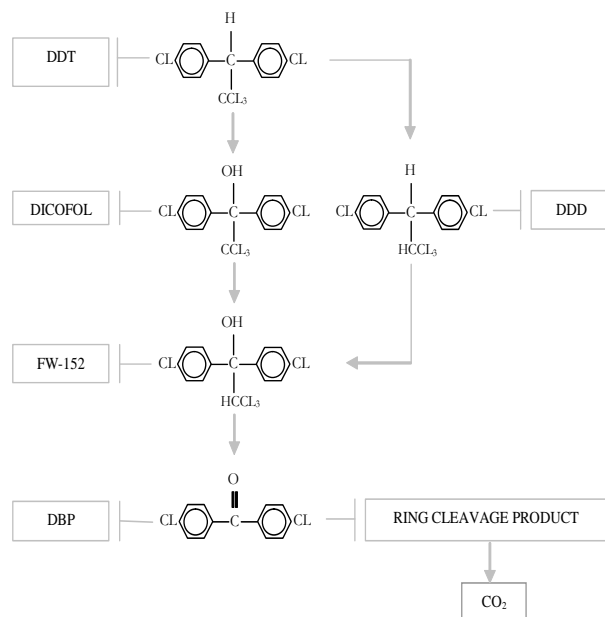


Pesticide Degradation.- Organochlorine insecticides such as 1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane (DDT), lindane and aldrins have been used in enormous quantities for many decades in agriculture and for public health. The organochlorine herbicides 2,4-dichlorophenoxy-acetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 2-methyl-4, 6-dichlorophenoxyacetic acid (MCPA) have also been widely used in agriculture with the concomitant generation of dioxins. Dioxins were components of «Agent Orange,» used extensively as defoliants during the Vietnam War and now are groundwater and soil contaminants. Although organochlorines are not being used in developed countries, their use continues in developing nations (Pointing, 2001). The organophosphorous

compounds, such as methylcarbamate and synthetic pyrethroid insecticides that were developed to replace organochlorines were toxic and persistent as well. Likewise, new herbicides such as chlorophenoxyalkanoates, triazines, and other hydrocarbons (PCBs) have also caused toxicity and persistence in the environment (Pointing, 2001).

Although DDT is a persistent environmental pollutant, it does appear to undergo slight degradation in the environment. A general pathway for DDT degradation involves reductive dechlorination, followed by further dechlorination, oxidation, and decarboxylation prior to ring cleavage (Figure 2) (Bumpus and Aust, 1987).

Figure 2. Proposed degradation of DDT by the white-rot fungus *Phanerochaete chrysosporium*. Adapted from Bumpus and Aust (1987).



Species of *Pleurotus ostreatus*, *Phelinus weirii*, and *Polyporus versicolor* have been shown to degrade DDT. They were able to mineralize 5.3-13.5% of added ^{14}C -

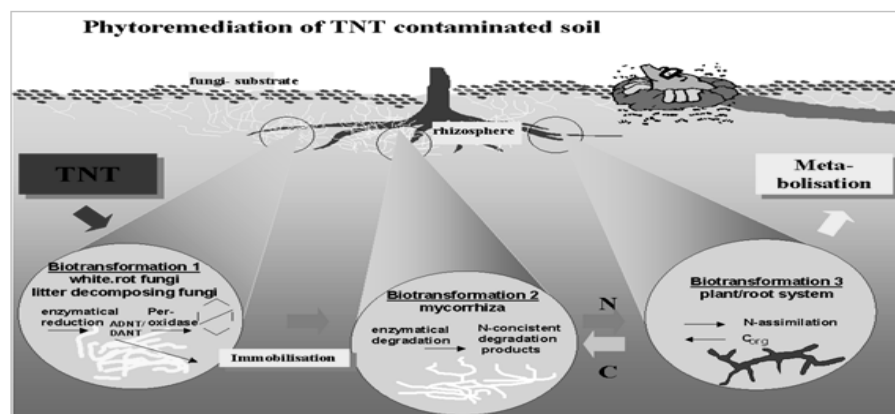
radiolabeled DDT, dicofol, and methoxychlor over 30 days under ligninolytic growth conditions (Bumpus and Aust, 1987). During DDT biodegradation, intermediate products are generated which later are degraded. It has been shown that ^{14}C -radiolabeled 1,1-dichloro-2,2-bis(4chlorophenil) ethane (DDE), a sub product of DDT, is mineralized to $^{14}\text{CO}_2$ and H_2O by *Phanaerochaete chrysosporium* (Bumpus and Aust, 1987). *Phanerochaete chrysosporium* has also shown to degrade up to 23.4% of ^{14}C -radiolabeled aldrin, dieldrin, chloridane, lindane, and mirex during 30 days of incubation (Kennedy et al., 1990). Organophosphate insecticides are not usually persistent in the environment, and are easily degraded. *Phanerochaete chrysosporium* mineralized up to 27.5% of ^{14}C -radiolabeled chloropyrifos, fonofos, and turbufos in 18 days of incubation (Bumpus and Aust, 1987). The organophosphate insecticides are less persistent in the environment than organochlorine insecticides and *P. chrysosporium* has been demonstrated to mineralize 12.2-27.5% of ^{14}C -radiolabeled chloropyrifos, fonofos, and turbufos in 18-day incubation (Bumpus et al., 1993 cited in Pointing, 2001). The chlorinated triazine herbicide 2-

chloro-4-ethylamine-6-isopropyl-amino-1, 2, 4-triazine (atrazine), which is recalcitrant in the environment has also been transformed by *P. chrysosporium* (Mougin et al., 1994 cited in Pointing, 2001) and *Pleurotus pulmonarius* (Masaphy et al., 1993 cited in Pointing, 2001) into less recalcitrant hydroxylated and N-dealkylated metabolites.

2, 4, 6-Trinitrotoluene (TNT)

At military sites, TNT wastes from munitions production and storage have been found to contaminate water, soil, and sediments. TNT has been shown to cause liver damage and anemia in humans (Pointing, 2001; Spiker et al., 1992). Currently, methods for remediation of TNT-contaminated soils are being studied. Some anaerobic bacteria can transform but not mineralize TNT (Pointing, 2001). However, recent studies have demonstrated the ability of white-rot fungi to mineralize TNT. The transformation of TNT results in the formation of the dinitrotoluenes (DNTs), which are generally not degraded immediately. Many microorganisms are able to transform TNT into DNTs, but only white-rot fungi degrade and mineralize the DNTs to CO_2 and H_2O (Hawari et al., 1999; Hodson et al., 2000). The transformation and mineralization of TNT by white-rot fungi is illustrated in Figure 3.

Figure 3. Bioremediation of 2,4,6-trinitrotoluene (TNT) contaminated soil by white-rot fungi. Adapted from Hawari et al (1999); Hodson et al. (2000).

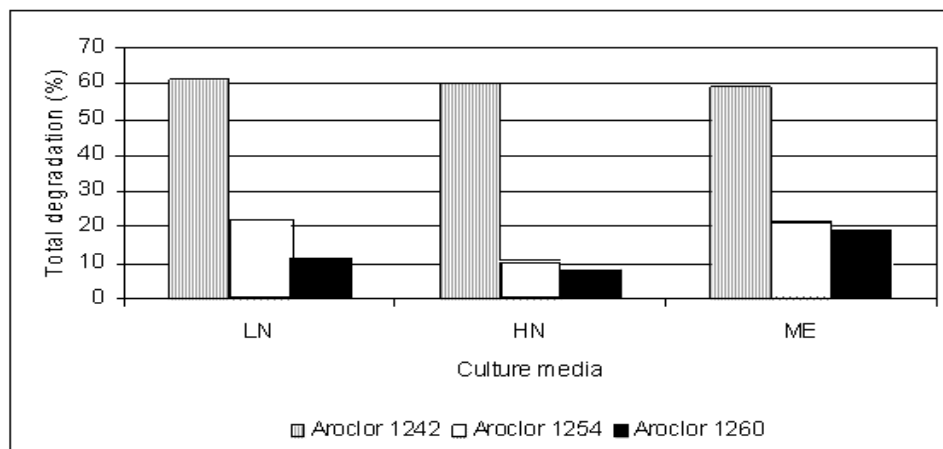


important role in the degradation of DNTs. Addition of surfactant to ligninolytic cultures of *P. chrysosporium* significantly enhanced TNT mineralization (Hodson et al., 2000). Many studies related to TNT degradation have employed relatively low TNT concentrations. *Phanerochaete chrysosporium* could germinate from spores, grow and transform at 20 ppm, but not at 100 ppm of TNT (Spiker, et al., 1992).

Polychlorinated Biphenyls (PCB's) PCBs are produced through chlorination of the biphenyl group. PCBs are a family of compounds with a wide range of industrial applications in heat transfer fluids, dielectric fluids, hydraulic fluids, flame-retardants,

solvent extenders and organic diluents. PCBs have entered into soil and sediment environments as a result of improper disposal of industrial PCB wastes and leakage of PCBs from electric transformers (Pointing, 2001; Yadav et al., 1995). Numerous studies have shown that white-rot fungi including *Corioloopsis polyzona*, *P. chrysosporium*, *Pleurotus ostreatus*, and *Trametes versicolor* were capable of PCB removal *in-vivo* (Figure 4) (Yadav et al., 1995). Studies using ¹⁴C-radiolabeled PCBs showed that *C. polyzona*, *P. chrysosporium* and *T. versicolor* were capable of PCBs elimination but the exact role of LME in this process still is not clear (Baudette et al., 2000).

Figure 4. Degradation of PCBs (Pentachlorophenol Aroclors) by *Corioloopsis polyzona*, *P. chrysosporium*, *Pleurotus ostreatus*, and *Trametes versicolor*. Adapted from Yadav et al. (1995).



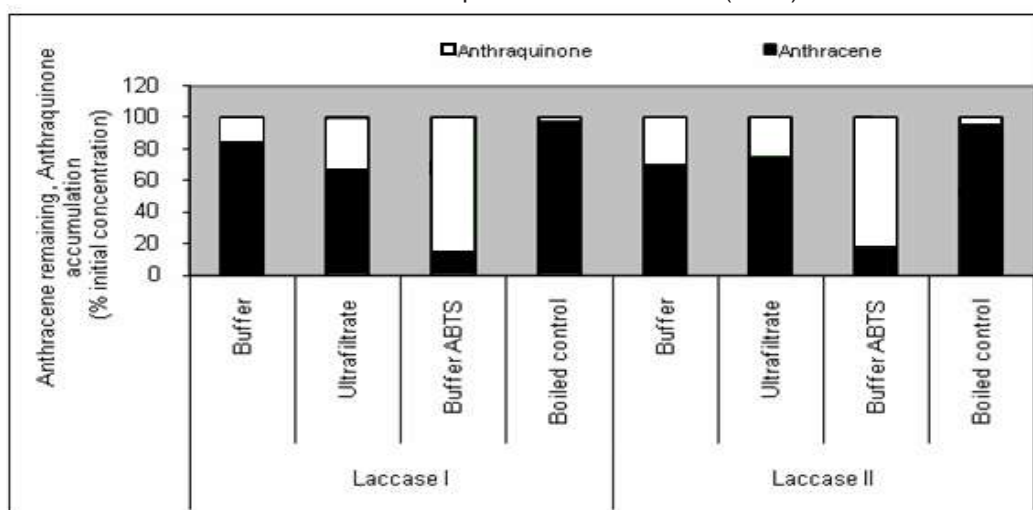
Polycyclic Aromatic Hydrocarbons (PAH's)- PAHs are benzene homologues generated from the fusion of four or more benzene rings. PAHs are highly toxic organopollutants, which are widely distributed in industrial and terrestrial environments. They originate from natural oil deposits, wood burning, vegetation decomposition, vehicle transport, waste incineration and industrial processes. Due

to their toxic effects, PAHs pose a serious health risk to animals, including humans. Many of these compounds are known to be mutagenic and carcinogenic (Collins and Ng, 1997). Several reports have shown that the white-rot fungi are the only organisms capable of PAHs degradation and that the rates of mineralization are correlated with the production of LMEs (Field et al., 1992). Extracellular preparations of LiP from *P.*

chrysosporium was one of the first LMEs capable of PAH degradation (Bumpus and Aust, 1987). Because MnP and Lac have been shown to be the most predominant LMEs during the PAHs metabolism, most research has focused on those enzymes. The MnP of *P. chrysosporium* showed activity in the oxidation of twelve 3-6 ring

PAH. In some cases the enzyme was limited by Mn^{2+} availability (Bogan et al., 1996). Results differ between investigators and fungal species. For instance, Collins et al. (1996) found that Lac of *T. versicolor* could not oxidize phenanthrene (Figure 5), but Lac of *Coriopsis gallica* could (Pickard et al., 1999).

Figure 5. Anthracene transformation in the presence of either Lac I or Lac II enzymes purified from *Trametes versicolor*. Adapted from Collins et al. (1996).



Wood Preservatives.- The organic wood preservatives creosote and pentachlorophenol (PCP) have been used extensively, although their use has been discontinued as a result of soil and groundwater contamination. Creosote is a coal-tar distillation product consisting of a highly heterogeneous PAH mixture, which includes 16 of the United States EPA priority-listed pollutants. PCP is a benzene ring with five chloride substitutions, and is listed as a priority pollutant by the United States EPA (Pointing, 2001). Decomposition of creosote by white-rot fungi is very similar to PAH degradation. However, creosote is more complex and therefore can be more toxic to fungi than PAH during degradation (Pointing, 2001). *Phanerochaete chrysosporium*

mineralized up to 50% of ^{14}C -radiolabeled PCP when grown under ligninolytic conditions. Not all species of the genus were able to mineralize PCP. Some of them were inhibited in the presence of 5 ppm of PCP; however, *Phanerochaete chrysosporium* and *P. sordida* grew in the presence of 25 ppm of PCP. A rapid reduction of PCP resulted in accumulation of the product pentachloroanisole (PCA), a quinone intermediate expected for LME-mediated oxidation (Lin et al., 1990).

Synthetic Dyes.- Synthetic dyes are chemically diverse, with those commonly used in industry divided into those of azo, triphenylmethane or heterocyclic/polymeric structures. They are used extensively in biomedical, food, plastic,

and textile industries. Synthetic dyes are not biodegradable and when discharged into the environment, they are persistent and many are also toxic (Pointing, 2001). Early studies have shown that polymeric dyes were decolorized by ligninolytic cultures of *P. chrysosporium* and that inhibitors of lignin degradation also repressed dye decolorization (Glenn and Gold, 1983). Other studies have also reported decolorization of an extensive number of azo, triphenylmethane and heterocyclic dyes by white-rot fungi (Pointing, 2001). The role of sorption in dye decolorization appears to be influenced by ligninolytic compounds. A non-ligninolytic culture of *P. chrysosporium* was found to have about 49% of total azo and heterocyclic dye bound to the mycelium (Cripps et al., 1990). Conversely, a ligninolytic culture of *P. sanguineu* accounted for less than 3% of azo and triphenylmethane dye removal (Pointing, 2001). The action of LMEs in dye decolorization has been demonstrated in several studies using purified cell-free enzymes. LiP of *P. chrysosporium* decolorized azo, triphenylmethane and heterocyclic dyes in the presence of veratryl alcohol and H₂O₂ (Cripps et al., 1990).

Bleach-Derived from Paper Producing Plants.- Billions of gallons or sometimes intensely colored waste effluents are released into the environment annually by the pulp and paper industry (Michael et al., 1991). The primary contributor to the color and toxicity of these streams is the pulp bleach plant effluent, which contains largely high molecular, modified and chlorinated lignin and its degradation products, including chlorolignins, chloro-phenols, chloro-catechols, and chloro-aliphatics (Michael et al., 1991; Pointing, 2001). There are a few reports that describe the role of white-rot

fungi in the mineralization of those pollutants. It has been shown that *P. chrysosporium* can oxidize, demethylate and dechlorinate bleach-plant effluents. Among the LMEs, MnP has been the most effective in these processes (Michael et al., 1991). It has also been demonstrated that Lac of *T. versicolor* has high activity in dechlorinating the bleach-plant effluents (Limura et al., 1996).

Conclusions

White-rot fungi are able to degrade lignin, cellulose, and hemicellulose concurrently at approximately equal rates. These fungi secrete three extra cellular enzymes (LMEs) that are critical for wood degradation. Because of their ability to biodegrade natural lignocellulosic materials and humic substances to CO₂ and H₂O, the white-rot basidiomycete mushrooms or their products are able to transform and mineralize, *in-vitro* or *in-vivo*, many environmental organopollutants, including pesticides, munitions waste, polychlorinated biphenyls, polycyclic aromatic hydrocarbons, wood preservatives, synthetic dyes, and waste materials from paper producing plants.

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