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White-rot fungi in phenols, dyes and other xenobiotics treatment – a brief review

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review

Summary

Bioremediation is an attractive technology that utilizes the metabolic potential of microorganisms in order to clean up the environmental pollutants to the less hazardous or non-hazardous forms with less input of chemicals, energy and time. White-rot fungi are unique organisms that show the capacities of degrading and mineralizing lignin as well as organic, highly toxic and recalcitrant compounds. The key enzymes of their metabolism are extracellular lignolytic enzymes that enable fungi to tolerate a relatively high concentration of toxic substrates. This paper gives a brief review of many aspects concerning the application of white-rot fungi with the purpose of the industrial contaminants removal.

Keywords: bioremediation, lignolytic enzymes, white-rot fungi, waste treatment

Introduction

There is an increasing awareness around the world regarding the environmental pollution caused by industrial wastes. Billions of hazardous pollutants are produced annually by the chemical, agricultural, oil, paper, textile and other industries. Development of efficient, cost effective and sustainable methods as well as the improvement of the existing ones is becoming more and more important.

Bioremediation is an attractive technology that utilizes the metabolic potential of microorganisms in order to clean up the environmental pollutants to the less hazardous or non-hazardous forms with less input of chemicals, energy and time (Asgher at al., 2008, Haritash and Kaushik, 2009). Implementation of fungi in the process of remediation is called mycoremediation. Fungi play vital roles in all ecosystems, regulating the flow of nutrients and energy through their mycelial networks. It can be said that they act like natural and true ecosystem engineers (Singh, 2006). Great number of fungi, however, has not been discovered yet. There are data that approximately 80 000 to 120 000 species of fungi have been described to date, although the total number of these species is estimated at around 1.5 million (Webster and Weber, 2007). Fungi are known to degrade, or cause to deteriorate a wide variety of materials and compounds. They can degrade different type of wood, stored paper, textiles, plastics, leather and various wrapping materials. They can assist in deterioration of concrete or can cause decay of wall paintings or can even attack ancient and medieval glass surfaces. Due to all of these facts, scientists have realized the possible benefits of the application of fungi in the complex areas in applied remediation engineering. Moreover, the discovery of the value of white-rot fungi in bioremediation has brought a great success in this field (Bennett, 2006; Singh, 2006). A significant progress has been achieved in the area of the white-rot fungi growth and enzyme production with the aim of enhancement the enzyme production. Molecular biology related to white-rot fungi, especially related to the extraction of genetic material (RNA and DNA), gene cloning and the construction of genetically engineered microorganisms is especially attractive and thus investigated in recent years (Gao et al., 2010.; Fan et al., 2010.). The present paper gives a brief review of the different application of white-rot fungi in the biodegradation of natural and artificial compounds presented in different industries with the aim to emphasize the importance of the biological waste treatment.

White-rot fungi

In comparison to bacteria most of fungi are robust organisms generally more tolerant to high concentrations of pollutants. It explains why they have been investigated extensively since the mid-1980s for their bioremediation capacities (Evans and Hedger, 2006; Strong, 2010; More et al., 2010). White-rot fungi (Fig. 1) constitute a diverse ecophysiological group

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comprising mostly of basidiomycetes and litter-decomposing fungi (Wesenberg et al., 2003).

Fig. 1. White-rot fungi a) *Trametes versicolor* in the nature (Singh, 2006), b) *Trametes versicolor* G-99 in the laboratory, from spores to mycelial pellets

White-rot fungi posses a great range of different enzymes such as hydrolytic enzymes (cellulase, pectinase, xylanase) and extracellular ligninolytic enzymes (lignin peroxides, manganese peroxidase and laccase) (Teerapatsakul et al., 2007). The expression pattern of these enzymes depends on the organism itself: some white-rot fungi produce lignin peroxidase and manganese peroxidase, but not laccase, while the other produce manganese peroxidase and laccase, but not lignin peroxidase (Hatakka, 1994). Therefore, among different types of white-rot fungi, some can equally decompose all of the three lignocellulose components in wood material, while some can degrade lignin and hemicellulose leaving cellulose intact (Lara et al., 2003; Bahri et al., 2006; Fang et al., 2008). Most of these enzymes are industrially important and have the great potential in processes of bioremediation, biodegradation, biopulping, degradation and detoxification of recalcitrant substances (Wesenberg et al., 2003; Tortella et al., 2008).

In the nature, photosynthetic production of wood is balanced by degrading activity of different wood-destroying organisms (Leisola and Garcia, 1989). Wood is comprised of chemical substance called lignocellulose. Lignin, cellulose and hemicellulose are the main constituent of lignocellulose materials (Grönqvist at al., 2005; Mussatto et al., 2007). The complex architecture of wood is summarized in Fig. 2. Lignin is a three-dimensional biopolymer of high molecular weight. It is the most abundant aromatic compound on the earth and an important constituent of the living terrestrial biomass. Lignin is synthesized in plants by linking together hydroxycinnamyl, coniferyl and sinapyl alcohols to give *p*-hydroxyphenol (Bahri et al., 2006).

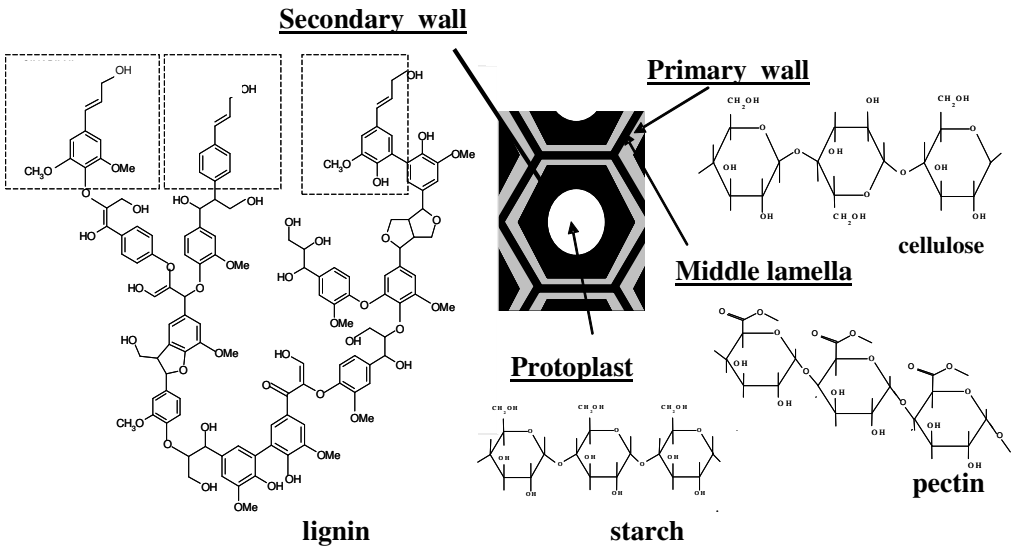

Fig. 2. Polymers in wood

White-rot fungi can degrade lignin in the way that the mycelia of the organisms penetrate the cell cavity and release lignolytic enzymes to decompose xylon to a white sponge-like mass (Gao et al., 2010). Beside lignolytic enzymes, in the process of lignin degradation many other enzymes are also involved: glyoxal oxidase, aryl oxidase, veratryl alcohol oxidase, oxalate decarboxylase, NAD-dependent formate dehydrogenase, P450 monooxygenase (Hattakka, 1994; Ander and Marzullo, 1997; Asgher, 2008). However, the main characteristic that differentiates white-rot fungi from most other microorganisms is their ability to completely mineralize all components of lignin to carbon dioxide and water (Asgher et al., 2008). Beside the role in wood degradation white-rot fungi can be applied for degradation of different industrial contaminants such as low molecular polycyclic aromatic carbohydrates, aromatic carbohydrates and chlorophenols (Harvey and Thurston, 2001; Cerniglica and Sutherland, 2001; Valentin et al., 2006; Valentin et al., 2007), textile dyes (Podgornik et al., 2001a; López et al., 2002; Wesenberg et al., 2003; Tychanowicz et al., 2004; Zille et al., 2005; Kariminiae-Hamedaani et al., 2007) pesticides (Maloney, 2001) or in recent time pharmaceuticals such as ibuprofen, clofibric acid and carbamazepine (Marco-Urrea et al., 2009) or naproxen (Rodríguez-Rodríguez et al., 2010).

The ligninolytic systems have been widely studying in last few decades mainly on *Phanerochaete chrysosporium* (Leisola, 1984; Renganathan et al., 1990; Covert et al., 1992; Linko, 1992; Barclay et al., 1993; Jaspers and Penninckx, 1996; Rivela et al., 2000; Podgornik, 2001a; Podgornik, 2001b; Podgornik, 2002; Shahvali et al., 2000; Rodríguez Cuoto, 2002). In last two decades most of the investigations were done in order to find new varieties of white-rot fungi or to study the low cost production or overproduction of desired lignolytic enzymes. Furthermore, different investigations have been done in order to study the catalytic activity of produced enzymes on different substrates. According to the literature data, beside the *Phanerochaete chrysosporium*, some of the good producers of all lignolytic enyzmes are also *Phanerochaete crassa* (Takano et al., 2006), *Pycnoporus cinnabarinus* (Sigoillot et al., 1999; Jonas et al., 2000; Geng and L., 2002; Alvarado et al., 2003; de Wilde, 2008; Gubta et al., 2010), *Pleurotus ostreatus* (Palmieri, 1997; Reddy et al., 2003; Membrillo et al., 2008) and *Bjerkandera adusta* (Moreira et al., 2001). Good laccase producers are *Trametes vesicolor* (Jang et al., 2002; Rancaño et al., 2003; Taveres et al., 2005; Revankar and Lele, 2006; Kurniawati and Nicell, 2007; Xavier et al., 2007; Thiruchelvam and Ramsay, 2007; Tišma et al., 2008; Tišma et al., 2009; Tišma et al., 2010a; Tišma et al., 2010), *Pleurotis pulmonarius* (Tychanowicz et al., 2003), *Marasmius quercophilius* (Tagger et al., 1998) and *Polysporus ostreatus* (Claus, 2003).

The importance, properties and applications of lignolytic enzymes were described by several review papers (Dashtban et al., 2010; Lundell et al., 2010; Gianfreda et al., 1999; Mayer and Staples, 2002; Burton, 2003; Claus, 2003; Claus, 2004; Rodríguez Couto and Toca-Herrera, 2006; Rodríguez Couto and Toca-Herrera, 2007; Widsten and Kandelbauer, 2008; Linko, 1992; Klaus, 2002; Hamid and Rehman, 2009; Hofrichter, 2002; Hamid and Rehman, 2009).

A lot of research has been done in the field of genetic engineering, mainly on the most investigated fungus *Phanerochaete chrysosporium* (Kasai et al., 2010). There are several investigations where genes encoding ligninolytic enzymes in the white-rot fungi have been cloned and expressed in different hosts (Wang et al., 2004)

The potential of white-rot fungi can be harnessed thanks to emerging knowledge of the physiology and morphology (Žnidaršič and Pavko, 2001; Papagianni, 2004; Žnidaršič-Plazl, 2006) of these organisms. This knowledge could be transformed into reliable and robust waste treatment processes (Wesenberg et al., 2003).

In further text a brief overview of possible application of white-rot fungi and their enzymes in bioremediation purposes is given.

Application of white-rot fungi in treatment of phenolic compounds, dyes, and other xenobiotics

Numerous literature data are related to the treatment of different types of wastewater by fungi. The latest papers about degradation of phenolic compounds and specific industry wastewater treatment processes by white-rot fungi or their enzymatic system are presented in this review. Once released in the environment, pollutants can be biodegraded or bioaccumulated, non-transformed or block as soil-bound residue, and involved in non-biological transformation or physical processes (Gianfreda et al., 2006).

Removal of phenols and related compounds by white-rot fungi

Phenols are widely distributed compounds in the nature, especially in the plants (Boudet, 2007) where they occur in the form of alkaloids, coumarins, flavanoids, terpens, tannins and lignins. They can

also be found in marine systems, produced by marine plants and animals where they can be degraded by indigenous microbial population. However, industrial production of phenols has been increasing. About 70 % of industrially produced phenols is employed in the production of resins and also used in the manufacture of plastic, biocides, disinfectans, textiles, medicines, explosives, inks, perfumes, photographic materials (Singh, 2006). Several types of industrial and agricultural wastes contain phenols (Gianfreda et al., 2003).

Pentachlorophenol (PCP) has been used since the late 1930s as a wood preservative together with its salt, sodium pentachlorophenate, due to its broad spectrum and low cost. Its esters have also been used as biocides. This generalized use has led to the contamination of many ecosystems. PCPs are currently considered as a priority product for decontamination studies according to the European Community and the American Environmental Protection Agency (Gomes Machado et al., 2005).

Huge amount of phenol-polluted waters are formed from the production of olive oil in Mediterranean region as well (Gianfreda et al., 2006). Olive mill wastewater (OMW), highly toxic effluent obtained from the extraction process by the olive oil industry, presents a major problem in the Mediterranean region. This effluent is variable in composition, but is always antibacterial and phytotoxic due to its phenolic content. The phenols responsible for the recalcitrant brownish color of OMWs are present in the residue as a mixture of monomeric aromatics and as polymerized heterogeneous pigments. The efficiency of dephenolization and decolorization of OMWs together with the production of laccase and manganese peroxidase were determined and compared by some authors showing that it can be effectively used as a pre-treatment step in a combined aerobic-anaerobic and/or physical and chemical treatment process to solve the environmental problems caused by OMW in Mediterranean olive oil-producing countries (Jaouani et al., 2003; Ergül et al., 2010).

Laconi et al. investigated the possibility of degradation of the high polyphenolic concentration of OMWs and, at the same time, they followed the production of microbial biomass that can be potentially useful as animal feed integrators. Four strains of the ligninolytic edible basidiomycete genus of *Pleurotus*, yeast strains *Saccharomyces cerevisiae* and *Kluyveromyces lactis*; the species of filamentous fungi *Oidodendron* spp. and *Penicillum* spp. were used in this study (Laconi et al., 2007).

Justino et al. reported the efficiency of three different approaches on phenols removal from OMW (Justino et al., 2009; Justino et al., 2010). They oxidized different type of phenols that were extracted from OMW by a) biological treatment with two fungal species *Trametes versicolor* and *Pleurotus sajor*, b) enzymatic treatment by laccase, and c) chemical treatment by photo-Fenton oxidation. Phenols were removed more efficiently by photo-Fenton treatment than by biological or enzymatic treatments which indicates that additional investigations should be done in order to improve the biological approach.

D'Annibale et al. investigated the effect of submerged fermentation parameters, such as agitation and aeration, on growth and/or performance (i.e. lignin modifying enzymes production) of *Panus tigrinus*. The results of this study show that the up-scaling of OMWs treatment by *P. tigrinus* CBS 577.79 should be carried out in reactors providing good oxygen transfer with minimal shear effects such as the bubble column bioreactor. Moreover, due to the low aeration necessary for good mixing and mass-transfer the use of a simple bubble column bioreactor would have a positive impact on process costs (D'Annibale et al., 2006).

Phenolic compounds are part of coking wastewaters from the coke industry which are produced in the process of coking and coal liquefaction. Coking wastewaters contains complex inorganic and organic contaminants (ammonia, cyanide, thiocyanide, polycyclic nitrogen-containing aromatics, oxygen- and sulfur-containing heterocyclics and acyclic compounds) which are refractory, highly concentrated, toxic, mutative and carcinogenic (Lu et al., 2009). Lu et al. improved the process of phenolic compounds removal from the coking wastewater by the application of immobilized *Phanerochaete chrysosporium*.

Beside the possibility of the use of whole cells in the process of phenolic compound removal, considerable effort was done by different authors in the field of the application of pure enzymes. Bollag et al. successfully removed different chlorophenols presented alone or in combination of two or three by the pure laccase from *Trametes villosa* (Bollag et al., 2003.), D'Acunzo et al. removed different phenols by laccase with and without presence of different mediators (d'Acunzo et al., 2002). Lots of efforts are done on kinetics and modeling of phenol (Kurniawati and Nicell, 2006; Kurniawati and Nicell, 2007; Kurniawati and Nicell, 2008), catechol (Aktaş and Tanyolaç, 2003a; Aktaş and Tanyolaç, 2003b), pyrogallol (Güreşir et al., 2005), 1-naphtol (Aktaş et al., 2001) and L-DOPA (Tišma et al., 2008) oxidation catalyzed by laccase.

Improvements in the long-term application of enzymes and thereby a reduction in treatment costs,

could be also accomplished through the selection of an appropriate reactor configuration. Application of white-rot fungi in the purpose of removal of phenols and related compounds is summarized in Table 1.

Table 1. White-rot fungi used in treatment of phenols and related compounds

White-rot fungi	Waste treated	References
Pleurotus sp.	Olive mill wastewater	Laconi et al., 2007
Trametes versicolor and *Pleurotus sajor*	Olive mill wastewater	Justino et al., 2009; Justino et al., 2010
Panus tigrinus	Olive mill wastewater	D'Annibale et al., 2006
Phanerochaete chrysosporium	Coking wastewaters	Lu et al., 2009
Trametes villosa	Wastewater containing chlorophenols	Bollag et al., 2003
Poliporus pinsitus	Oligomeric polyphenol compounds	D'Acunzo et al., 2002

Removal of polycyclic aromatic hydrocarbons (PAHs) by white-rot fungi

Polycyclic aromatic hydrocarbons (PAHs) are a class of ubiquitous xenobiotic environmental pollutants. The common sources of PAHs in environment include natural as well as anthropogenic. Natural sources are forest and rangeland fires, oil seeps, volcanic eruptions and exudates from trees. Anthropogenic sources of PAH include burning of fossil fuel, coal tar, wood, garbage, refuse, used lubricating oil and oil filters, municipal solid waste incineration and petroleum spills and discharge (Haritash and Kaushik, 2009). PAHs consist of three or more benzene rings fused in linear, angular or cluster arrangements which are thermodynamically stable. Based on the structure and mechanisms of activation, they exhibit toxic, mutagenic and carcinogenic properties. Improper disposal methods and inadequate control of these materials have created widespread contamination in soil groundwater and surface water. Bioremediation is shown to be effective for soils contaminated with low-molecular-weight PAHs (Singh, 2006). In last two decades the knowledge about the removal of PAHs by fungi is limited in comparison to that of bacteria. It is reported that some of white-rot fungi can efficiently degrade naphthalene, acenaphthene, antracene, phenanthrene, fluorine, fluoranthene, chrysene, pyrene benzantrancene, benzopyrene (Singh, 2006), chlorobenzene (Wang et al., 2008).

Considerable efforts have been done with the aim of fungal bioremediation of different types of pesticides and polycyclic aromatic hydrocarbons by some authors (Eggen and Sveum, 1998; Eggen and Majcherczy, 1999; Valentín et al., 2006, Quintero et al., 2007; Acevedo et al., 2011). Clemente et al. investigated degradation of PAH by thirteen deuteromycete ligninolytic fungal strains and found that the degree of degradation varies with a variation of lignolytic enzymes.

Maximum degradation of naphthalene (69 %) was observed by the strain 984 having manganese-peroxidase activity, followed by strain 870 (17 %) showing lignin peroxidase and laccase activities. Phenanthrene degradation of 12 % was observed with strain 870 with manganese-peroxidase and laccase activities. A good level of degradation of anthracene (65 %) was observed by the strain 710 (Clemente et al., 2001). Boyle et al. found that white-rot fungi growing in soil doesn't degrade significant amounts of PAHs. However, in liquid culture they degrade many PAHs (Boyle et al., 1998). The effect of nitrogen as nutrient was also assessed because nitrogen sources are frequently added during bioremediation. On the other hand nitrogen can inhibit the lignin-degrading system of white-rot fungi (Higson, 1991). Among hundreds of white-rot fungi displaying lignolytic activity, *Phanerochaete chrysosporium*, *Bjerkandera adusta* and *Pleurotus ostreatus* have been extensively studied. Intermediate compounds as quinones, hydroxyl- and dihydroxy-PAH have been isolated, but it is not clear whether they accumulate as dead-end products. Accumulation of PAH-quinones was reported in liquid cultures of *Phanerochaete chrysosporium* and *Bjerkandera adusta* and in soil by *Pleurotus ostreatus* (Haritash and Kaushik, 2009).

Application of white-rot fungi in the purpose of polycyclic aromatic hydrocarbons removal is summarized in Table 2.

Table 2. White-rot fungi used in treatment of polycyclic aromatic hydrocarbons

White-rot fungi	Waste treated	References
Pleurotus ostreatus	PAHs aged creosote contaminated soil	Eggen and Sveum, 1998; Eggen and Majcherczy, 1999
Bjerkandera adusta, Irpex lacteus and *Lentinus tigrinus*	PAHs in forest and salt marsh soils	Valentín et al., 2006
Bjerkandera adusta	hexachlorocyclohexane (HCH) isomers present in a spiked soil	Quintero et al., 2007
Phanerochaete chrysosporium and *Pleurotus pulmonarius*	aromatic hydrocarbons in an aged contaminated soil containing high concentrations of heavy metals	Boyle et al., 1998
Phanerochaete chrysosporium	xenobiotics in soil	Higson, 1991

Decolorization of dyes by white-rot fungi

The textile industry is the most frequent user of synthetic dyes. Dyes can be classified according to their structure (particularly the nature of the chromophore) or the method of application (Knapp et al., 2006). *Phanerochaete chrysosporium* was the first identified fungus able to degrade polymeric synthetic dyes. Up to date most investigations on dyes degradation have been performed by this fungus (Shahvali et al., 2000; Podgornik et al., 2001a; Singh et al., 2009; Singh and Pakshirajan, 2010; Nilsson et al., 2006; Faraco et al., 2009; Lucas et al., 2008; Levin et al., 2010).However it has been shown that it was not the best one (Lucas et al., 2008). A lot of efforts were done with some other species such as *Coriolus versicolor* (Hai et al., 2006; Nilsson et al., 2006; Erkurt et al., 2007; Asgher, 2009), *Pleurotus ostreatus* (Nilsson et al., 2006; Erkurt et al., 2007; Faraco et al., 2009; Lu et al., 2008) *Trametes trogii* and *Trametes villosa* (Levin et al., 2008; Levin et al., 2010). It has been proved that the mechanism of the oxidative enzymes in the dyes decolorization is strain dependant. For a fast screening of numerous fungal strains for their ability to decolourise textile dyes, a microtitre plate-based method was developed recently (Lucas et al., 2008).

Vijaykumar et al. isolated a novel fungus *Cladosporium cladosporioides* from coal sample and used it in the process of decolorization of five different azo and triphenylmethane dyes like acid blue 193, acid black 210, crystal violet, reactive black B(S) and reactive black BL/LPR both on solid and in liquid broth medium (Vijaykumar et al., 2006). Azo dyes such as DR-80 are important colorants and constitute the largest class of dyes for application not only in textile, but also in paper, leather, gasoline, foodstuffs and cosmetics industries (Sing et al., 2009). Shahvali et al. investigated the effect of different parameters (size of inoculum, temperature, carbon source) on decolorization of textile wastewaters using *Phanerochaete chrysosporium*. *Phanerochaete chrysosporium* was able to decolorize textile effluents with efficiency of up to 97 % (Shahvali et al., 2000). With the aim of decolorization of cotton bleaching effluent, Zhang et al. successfully developed a continuous fluidized-bed bioreactor (Zhang et al., 1998).

Singh et al. investigated decolourization of Direct Red 80 (DR-80) with *Phanerochaete chrysosporium* MTCC 787 by employing sequential design of experiments. Media components for growing the white-rot fungus were first screened using Plackett-Burman design and then optimized using response surface methodology (RSM), which resulted in enhancement in the efficiency of dye removal by the fungus. At the RSM optimized levels of the media constituents, *Phanerochaete chrysosporium* showed complete (100 %) dye decolorization efficiency due to its maximum LiP activity (Sing et al., 2009).

Nilsson et al. investigated the removal of two dyes, Reactive Blue 4 and Reactive Red 2 by different white-rot fungi *Phanerochaete chrysosporium*, *Trametes versicolor*, *Pleurotus ostreatus* and *Pleurotus sajor-caju*, in a reactor systems with immobilized mycelia (Nilsson et al., 2006).

Capabilities of the fungi *Phanerochaete chrysosporium* and *Phanerochaete ostreatus* and of free and immobilized laccase mixtures from *Phanerochaete ostreatus* on industrial dye wastewaters have been demonstrated by Faraco et al. Wastewater model containing dyes with complex trisazo-, polyazo- and stilbene- type structures was decolorized by *Phanerochaete chrysosporium* (about 45 % decolourization in only 1 day of treatment). The acid wastewater model was decolorized by *Phanerochaete ostreatus* (60 % decolorization in 7 days). Based on the discharged amounts, economic relevance and representativeness of chemical structures of the contained dyes, models of acid,

direct and reactive dye wastewaters from textile industry have been defined (Faraco et al., 2009). Decolourization of two azo dyes, Direct Red-80 and Mordant Blue-9 by *Phanerochaete chrysosporium* was investigated both individually and in mixtures in batch shake flasks. The profile of enzyme activities and dye decolourization by the fungus, in both single and mixed dye systems, suggested that fungal peroxidase enzymes (MnP and LiP) play a strong role in these dyes decolourization (Singh and Pakshirajan, 2010). Chander and Arrora have shown that *Dichomitus squalens*, *Daedalea flavida*, *Irpex flavus* and *Polyporus sanguineus* are better decolorizers of laboratory dyes than the much studied *Phanerochaete chrysosporium*. The fungal-based biocleaning systems have been suffering from drawback of adsorption, thus, in order to overcome this limitation, the cell free enzyme extracts obtained from fungal cultures have been used (Chander and Arrora, 2007). Application of white-rot fungi in the purpose of different dyes removal is summarized in Table 3.

Table 3. White-rot fungi used in treatment of dyes

White-rot fungi	Dye(s)	References
Phanerochaete chrysosporium	Anthraquinone dyes	Lucas et al., 2008
Phanerochaete chrysosporium	Azo dye	Shahvali et al., 2000
Phanerochaete chrysosporium	Indigo carmine	Podgornik et al., 2001a
Phanerochaete chrysosporium	Direct Red-80	Sing et al., 2009
Phanerochaete chrysosporium, *Coriolus versicolor*, *Pleurotus ostrea tus* and *Pleurotus sajor-caju*	Reactive Blue 4 and Reactive Red 2	Nilsson et al., 2006
Phanerochaete chrysosporium and *Phanerochaete ostreatus*	Direct Blu 71, Direct Red 80, Direct Yellow 106, Reactive Blue 222, Reactive Red 195, Reactive Yellow 145, Reactive Black 5, Acid Blue 62, Acid Yellow 49, Acid Red 266	Faraco et al., 2009
Coriolus versicolor	A synthetic waste water with Poly S119 dye	Hai et al., 2006
Pleurotus ostreatus, *Coriolus versicolor* and *Funalia trogii*	Remazol Brillant Blue Royal and Drimaren Blue	Erkurt et al., 2007
Coriolus versicolor	Crescent Textile Industry (CRT), Itmad Textile Industry (ITT), Megna Textile Industry (MGT) and Ayesha Textile Industry (AST) effluents	Asgher, 2009
Pleurotus ostreatus	Acid Orange 7, Acid Orange 8 and Mordant Violet 5	Lu et al., 2008
Cladosporium cladosporioides	Acid blue 193, acid black 210, crystal violet, reactive black B(S) and reactive black BL/LPR	Vijaykumar et al., 2006
Dichomitus squalens, *Daedalea flavida*, *Irpex flavus* and *Polyporus sanguineus*	Coracryl dyes (black, pink, violet, red) Reactive dyes (yellow, orange and red) Rathiodal dyes (scarlet)	Chander and Arrora, 2007

Decolorization of industrial effluents by white-rot fungi

The application of white-rot fungi in large-scale waste treatment, however, has been impeded owing to the lack of an appropriate reactor system capable of coping with rather slow fungal degradation, loss of the extracellular enzymes and mediators with discharged water, and excessive growth of fungi. In this context, a feasible system may be envisaged by coupling the excellent degradation capability of the white-rot fungi with the inherent advantages of a membrane bioreactor (MBR), yielding reduced excess sludge production.

Accomplishment of excellent stable pollutant removal (99 % color and 97 % TOC removal), using *Coriolus versicolor* along with the alleviation of the membrane fouling problem by employing a reasonable chemical cleaning dose is however a novel and attractive system (Hai et al., 2006).

Asgher et al. in their work presented the screening of *Coriolus versicolor* IBL-04 on five effluents of different industries. Optimization of different process parameters for Arzoo Textile Industry (ART) effluent decolorization showed that manganese peroxidase (MnP) was the lignolytic enzyme present in the culture filtrates, while lignin peroxidase (LiP) and laccase were undetectable

(Asgher et al., 2009). From the other hand, Erkurt et al. showed that laccase was the only enzyme responsible for decolorization of Remazol Brillant Blue Royal (RBBR) and Drimaren Blue CL-BR by *Pleurotus ostreatus*, *Coriolus versicolor* and *Funalia trogii* (Erkurt et al., 2007).

Three sulphonated phenylazonaphthol dyes with similar molecular structures, Acid Orange 7, Acid Orange 8 and Mordant Violet 5 were selected and degraded by the white-rot fungus *Pleurotus ostreatus* (Lu et al., 2008).

The pulp and paper industry is one of the primary users of wood resources in the world. Pulp and paper industries produce annually over 2800 billions' lives of colored, toxic and intensely colored waste effluents, causing severe water pollution wastewater (Jaspers and Penninckx, 1996). This kind of effluent, usually called black liquor, has a high level of chemical oxygen demand. The primary contributors to the color and toxicity of these effluents are high-molecular-weight lignin and its derivatives (Wu et al., 2004). No information on the exact chemical nature of the chromophores in bleaching effluent exists. However it is likely that the chromophores are different to some extent as wood has a very high lignin content (which is removed during the treatment) and cotton does not have it. In cotton bleaching, absorable organically bound halogens are produced when chlorine is used in the process (Zhang et al., 1998). Although currently available methods, such as chemical oxidation, reverse osmosis and adsorption, are highly efficient, they suffer some disadvantages. The limitations include high cost, limited applicability, high energy input, and usually these treatments may result in the production of toxic by-products (Hamid and Rehman, 2009).

Over the past 20 years, pollution caused by pulp black liquor has been seriously increased. Black liquor contributes only 10–15 % of the total wastewater, but accounts for nearly 80 % of color, 30 % of the biochemical oxygen demand and 60 % of the chemical oxygen demand of the total pollution load of pulp and paper mill effluent.

Different approaches have been used in order to solve this problem. Shararri et al. used bagasse effluent collected from the wastewater collection tank from pulp and paper industry for the biotreatment with *Phanerochaete chrysosporium* (Shararri et al., 2010). Jaspers and Penninckx investigated a possible adsorption of color and toxic chloroderivatives of lignin absorable organically bound halogens that are essentially formed in bleaching process (the Kraft process) when using preformed pellets for inoculation. They showed that depending of the conditions of incubation, the pellets of the fungus can strongly absorb color and absorable organically bound halogens from the Kraft beach plant effluent (Jaspers and Penninckx, 1996). A repeated batch operation is developed for the treatment of alkaline pulp black liquor, through a process of biological acidification and precipitation of lignin using brown-rot fungus *Fomitopsis* sp. IMER2 (Ma et al., 2008).

Wu et al. used individually different white-rot fungi, *Phanerochaete chrysosporium*, *Pleurotus ostreatus*, *Lentinus edodes*, *Trametes versicolor* and strain S22 grown on a porous plastic media to treat black liquor from a pulp and paper mill. Three white-rot fungi, *Phanerochaete chrysosporium*, *Pleurotus ostreatus* and S22, showed high capacity for lignin degradation at pH 8.0-11.0, suggesting that the white-rot fungi were able to grow and degrade lignin even under strong alkaline conditions (Wu et al., 2005).

Distillery wastewater is produced as a result of distillation of ethanol produced in the fermentation of carbohydrates. For every liter of produced ethanol, up to 20 L of sillage is generated. Therefore, in a typically distillery, over a half million liters of sillage is generated every day. The composition of mollases sillage depends on the source of raw material used for the alcohol production. For an example, the composition of sugar beet and sugarcane molasses sillage includes various organic compounds, like acetic acid, lactic acid, glycerol and reducing sugars.

Alcohol distillery wastewater (ADW) is a dark brown colored wastewater whose color components disrupt the flow of penetration of sun rays in surface waters, which in turn reduces the photosynthetic activity and is detrimental to aquatic life. The ADH water is recalcitrant due to the presence of melanoides. ADW has antioxidant properties and decolorization of ADW is also recalcitrant to normal biological wastewater treatments. Certain physical, chemical and thermal methods have been used but they are unsuitable for commercial application. On the other hand highly suitable treatment, such as anaerobic digestion, involves high installation and implementation costs.

In the 1990s, several researchers used white-rot fungi in the decolorization of distillery effluent. It appeared that decolorization was attributed to the secretion of extracellular lignolytic enzymes. The advantage of the use of white-rot fungi, beside the decolorization, is production of by-product – a single-cell protein (Singh, 2006). In last decades, different approaches have been used. Pant and Adholeya investigated the production of lignolytic enzymes by fungi isolated from distillery effluent and effluent contaminated soils by cultivation of the isolated fungi on wheat straw and corncob powder as substrates. Among all

isolated species, maximum decolorization of effluents from a cane molasses based distillery (86.33 %) was achieved by *Pleurotus ostreatus* (Pant and Adholeya, 2007).

Many scientists have been working in the field of isolation of the new strains. Chairattanamanokorn et al. found new *Pycnoporus coccineus* strains FPF 00062506, FPF 97062901, FPF 97091303, and FPF 98063001 that have the potential for ADW decolorization at thermophilic conditions (Chairattanamanokorn et al., 2005).

Strong recently published a paper where wastewater from distillation of fermented marula fruit was treated by four white-rot fungi (*Trametes pubescens* MB 89, *Ceriporiopsis subvermispora*, *Pycnoporus cinnabarinus* and *Phanerochaete chrysosporium*) in shake flasks experiments at pH 5.0 with no additional carbon or nitrogen supplements. This study has shown that it is possible to biologically treat Amarula distillery wastewater, a wastewater that has a high COD and high phenolic compound concentration, using white-rot fungus *Trametes pubescens* MB 89 and to obtain a high removal efficiency of COD and phenolic compounds (Strong, 2010).

Application of white-rot fungi in treatment of industrial effluents is summarized in Table 4.

Table 4. White-rot fungi used in decolorization of industrial effluents

White-rot fungi	Industry	References
Coriolus versicolor	textile	Hai et al., 2006
Pleurotus ostreatus, *Coriolus versicolor* and *Funalia trogii*	textile	Erkurt et al., 2007
Coriolus versicolor	textile	Asgher, 2009
Pleurotus ostreatus	textile	Lu et al., 2008
Phanerochaete chrysosporium	pulp and paper	Sharari et al., 2010
Phanerochaete chrysosporium	pulp and paper	Jaspers and Penninckx, 1996
**Fomitopsis* sp. IMER2.	pulp and paper	Ma et al., 2008
Phanerochaete chrysosporium, *Pleurotus ostreatus* and S22	pulp and paper	Wu et al., 2005
Pleurotus ostreatus	food	Pant and Adholeya, 2007
Pycnoporus coccineus	food	Chairattanamanokorn et al., 2005
Trametes pubescens MB 89	food	Strong, 2010
Pleurotus sp.	food	Laconi et al., 2007
Trametes versicolor and *Pleurotus sajor*	food	Justino et al., 2009; Justino et al., 2010
Panus tigrinus	food	D'Annibale et al., 2006
Phanerochaete chrysosporium	coke	Lu et al., 2009

Conclusions

White-rot fungi possess complex and efficient lignolytic enzyme system. They have been successfully applied in treatment and decomposition of different phenolic compounds, dyes and other xenobiotics on the laboratory level. However, a lot of work is needed to be done to explore the potential of white-rot fungi in dye decolorization and for removal of hazardous chemicals on industrial scale. The paper underlines the importance of exploring potential of new strains in the process of bioremediation in order to expand the pool of existing biocleaning and biobleaching white-rot fungi.

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