Chapter 5
Potential of White-Rot Fungi to Treat Xenobiotic-Containing Wastewater

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5.1 Introduction

Industrial effluents from different industries contain a high load of pollutants, which could cause detrimental effects to the ecosystem if they are released without pre-treatment. Most of these compounds are xenobiotics i.e., strange to the biosphere, and are resistant to the biodegradation by the indigenous micro-organisms. In addition, most of them are harmful to living beings including humans. Therefore, they have to be removed before being released into the environment. However, the physico, chemical and physico-chemical in-use techniques for the treatment of wastewater fail in degrading such compounds resulting in their accumulation in the environment, posing a hazard to the plants, animals and humans. Consequently, alternative methods to remove xenobiotic compounds from wastewater are needed. The use of biological degradation is seen as an economic and ecological alternative to remove hazardous compounds from wastewater. Among them, the use of white-rot fungi (WRF) represents a promising approach.

WRF have the unique ability to degrade the bulky, heterogeneous and recalcitrant polymer lignin (Fig. 5.1). This ability is due to the secretion of an extracellular non-specific enzymatic complex during their secondary metabolism (idiophasic), usually under nitrogen depletion. This enzymatic complex is mainly composed of lignin peroxidases (LiPs), manganese-dependent peroxidases (MnPs), versatile peroxidases (VPs) and laccases together with accessory enzymes (mostly H_2O_2-generating oxidases and dehydrogenases) (Mester et al. 2004).

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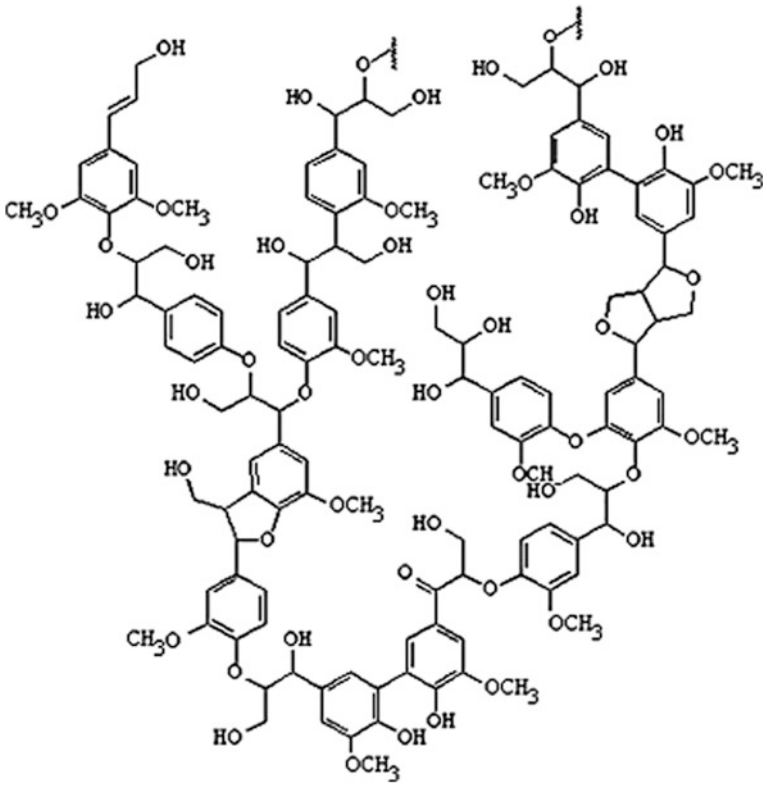

Fig. 5.1 Schematic structure of a lignin molecule. *Source* http://www.research.uky.edu/odyssey/winter07/green_energy.html

The ligninolytic enzymes secreted by the WRF have wide substrate specificity and are able to degrade a wide variety of complex molecules and even a mixture of them. This ability has driven the interest in the development of biotechnology processes based on WRF in the past couple of decades. However, studies dealing with the treatment of real wastewater are scarce. Therefore, in this chapter the latest research on xenobiotic removal from real wastewater by WRF is reviewed.

5.2 White-Rot Fungi

WRF are filamentous wood-degrading fungi, ubiquitous in nature. Most WRF belong to the Basidiomycota *phylum* (Polyporales and Agaricales orders) and together with some related litter-decomposing fungi are the only organisms able to mineralise lignin efficiently (Kirk and Cullen 1998; Hatakka 2001a, b).

Some WRF as grown in nature are shown in Fig. 5.2. The fungus *Phanerochaete chrysosporium* (order Thelephorales) was the first white-rot fungus studied and has become a model fungus for lignin biodegradation studies. The name white-rot derives from the bleached appearance of the wood attacked by these fungi due to the removal of the dark coloured lignin (Fig. 5.3). They grow mostly on hardwoods e.g., birch and aspen, although certain species grow on softwoods such as spruce and pine (Blanchette 1995). Some WRF degrade all wood components (i.e., cellulose, hemicellulose and lignin) simultaneously whereas others degrade lignin selectively. The former are called simultaneous or non-selective white-rot degraders and the latter selective white-rot degraders. The selective white-rot degraders are very interesting from a biotechnological point of view, since they remove lignin leaving the valuable cellulose intact (Dashtban et al. 2010). Simultaneous white-rot occurs mainly on hardwoods, whereas selective white-rot occurs both on hardwood and softwood. The typical characteristics of selective and simultaneous white-rot types are summarised in Table 5.1.

Fig. 5.2 Pictures of the white-rot fungi *Phanerochaete chrysosporium*. *Source* http://botit.botany.wisc.edu/toms_fungi/may97.html, *Trametes* versicolor. Photo Copyright © Michael Wood http://www.mykoweb.com/, *Pleurotus ostreatus.* Photo Copyright © Fred Stevens http://www.mykoweb.com/ and *Bjerkandera adusta.* Photo Copyright © Michael Wood http://www.mykoweb.com/

Fig. 5.3 Photograph of wood attacked by a white-rot fungus. *Source* www.bio.miami.edu/dana/pix/whiterot.jpg

The same mechanism that gives these fungi the potential to degrade lignin also allows them to degrade a wide variety of recalcitrant pollutants. Hence, the WRF are promising and attractive candidates for the bioremediation of xenobiotic compounds.

The mechanism used by the WRF to degrade pollutants gives them several advantages (Christian et al. 2005). For example:

- The WRF are able to mineralise a wide variety of toxic xenobiotics and complex mixtures as their enzymatic system is non-specific, non-stereoselective and based on free radicals.
- The WRF are ubiquitously found in nature.
- The WRF are able to oxidise low soluble compounds at high concentrations due to the extracellular nature of their main enzymatic system.
- The ligninolytic system of the WRF is triggered by nutrient limitation; hence, they do not need any pre-conditioning of the target pollutant.
- The WRF can degrade very low pollutant concentrations to non-detectable levels.
- The WRF can be cultivated on inexpensive substrates like agro and forestry wastes as well as in liquid media and in soil.
- The WRF also produce oxygen radicals (e.g., OH·) which are able to oxidise biomolecules such as proteins and DNA, and help to destroy microbes.
- The WRF are able to adjust the pH of their surrounding environment using the plasma membrane-dependent redox system.

The above-mentioned advantages helped to generate much interest in the development of technologies based on WRF for the biodegradation of hazardous and recalcitrant pollutants.

Table 5.1 Typical characteristics of selective and simultaneous white-rot

	Selective white-rot	Simultaneous white-rot	References
Degraded cell wall components	Initial stages of decay: Hemicellulose and lignin Later stages: Hemicellulose, cellulose and lignin	Cellulose, hemicellulose and lignin	Adasgavek et al. (1995); Fackler et al. (2006)
Anatomical features of decayed wood	Middle lamella dissolved Adjacent wood cells separated	Eroded cell walls, degradation beginning from the secondary wall proceeding to middle lamella	Blanchette (1995)
Lignin loss	Lignin loss diffusive throughout wood cell wall without major degradation of polysaccharides	Lignin loss together with wood cell wall polysaccharides starting progressively from lumen	Blanchette (1995)
Representatives	*Ceriporiopsis subvermispora, Phlebia radiata, Pleurotus* spp., *D. squalens, Ganoderma austral, Phlebia tremellosa, P. cinnabarinus, Phellinus pini*	*Phanerochaete chrysosporium, Fomes fomentarius, Phellinus, robustus, Trametes versicolor, Irpex lacteus, Heterobasidium annosum*	Blanchette (1995); Otjen et al. (1987); Nishida et al. (1988); Martínez et al. (2005)

5.3 Enzymatic System of WRF

In addition to lignin, WRF can oxidise a wide variety of organic compounds with structural similarities to lignin including soil humic substances (Hofrichter et al. 1998), organic pollutants (Tuomela and Hatakka 2011a, b) and synthetic dyes (Glenn and Gold 1983).

WRF usually produce one or more ligninolytic enzymes in different combinations according to which they can be divided into four groups (Hatakka 1994; Tuor et al. 1995; Nerud and Misurcova 1996): (i) laccase, LiP and MnP-producing, (ii) laccase and at least one of the peroxidases, (iii) laccases only and (iv) peroxidases only.

The ligninolytic enzymes most frequently found in the WRF are laccases and MnPs, and the least, LiPs and VPs. The ligninolytic enzymes can act jointly or separately but accessory enzymes (glyoxal oxidase, aryl alcohol oxidase, pyranose 2-oxidase, cellobiose dehydrogenase, etc.) are required to complete the process of lignin or xenobiotic degradation. In addition, intracellular cytochrome P450 monooxygenases as well as low-molecular mass oxidants such as hydroxyl radicals and chelated Mn^{3+} have also shown to be involved in the degradation of lignin and many xenobiotics (ten Have and Teunissen 2001; Hammel et al. 2002; Subramanian and Yadav 2009; Taboada-Puig

et al. 2011). Recently dye-decolourising peroxidases (DyPs), involved in the decolouration of high redox potential synthetic dyes and non-phenolic lignin model compounds (Liers et al. 2010), and aromatic peroxygenases (APOs), involved in the catalysis of oxygen transfer reactions resulting in the cleavage of ethers (Hofrichter et al. 2010; Liers et al. 2011), have been found to be part of the ligninolytic system of the WRF. The main ligninolytic enzymes, their substrates and reactions are summarised in Table 5.2.

It is worth pointing out that although a white-rot fungus species can potentially secrete laccase, MnP and LiP, a particular strain may not secrete all of them. Thus, for instance *Trametes versicolor* generally produces all the three enzymes (i.e., laccase, MnP and LiP) but laccase may be predominant in certain strains (Yang

Table 5.2 Ligninolytic enzymes and their main reactions (Hatakka 2001a, b; Harms et al. 2011; Tuomela and Hatakka 2011a, b; Lundell and Mäkelä 2013)

Enzyme and abbreviation	Cofactor	Substrate, mediator	Reaction	Occurrence in fungi
Laccase (EC 1.10.3.2)	O_2	Phenols, mediators e.g., hydroxybenzotriazole or ABTS	Phenols are oxidised to phenoxyl radicals; other reactions in the presence of mediators	Basidiomycota and Ascomycota, in most white-rot fungi and litter-degrading fungi
Lignin peroxidase (EC 1.11.1.4), LiP	H_2O_2	Veratryl alcohol	Aromatic ring oxidised to cation radical	Basidiomycota only in few white-rot fungi
Manganese peroxidase (EC 1.11.1.13), MnP	H_2O_2	Mn, organic acids as chelators, thiols, unsaturated fatty acids	Mn(II) oxidised to Mn(III); chelated Mn(III) oxidises phenolic compounds to phenoxyl radicals; other reactions in the presence of additional compounds	Basidiomycota, common in white-rot fungi and litter-degrading fungi
Versatile peroxidase (EC 1.11.1.16), VP	H_2O_2	Mn, veratryl alcohol, compounds similar to LiP and MnP	Mn(II) oxidised to Mn(III), oxidation of phenolic and non-phenolic compounds, and dyes	Basidiomycota, only in *Pleurotus* sp., *Bjerkandera* sp. and *Trametes versicolor*
Dye-decolourising peroxidase (EC 1.11.1.19), DyP	H_2O_2	Antraquinonic dyes	Oxidation of organic compounds; decolouration of Reactive Blue 5	Basidiomycota and Ascomycota

Table 5.3 Characteristics of the main ligninolytic enzymes (Dashtban et al. 2010; Sigoillot et al. 2012; Liers et al. 2014)

Enzyme	Molecular mass (kDa)	Isolectric point (pI)	Glycosylation	Redox potential (eV)	Localization
Laccase	54–80	3–4	Yes (10–20 %)* N-glycosylated	0.4–0.8	Mostly extracellular
LiP	35–48	3.1–4.7	Yes (up to 20–30 %) N-glycosylated	1.2 (at pH 3.0)	Extracellular
MnP	38–62.5	2.9–7.1	Yes (4–18 %) N-glycosylated	0.8 (at pH 4.5)	Extracellular
VP	40–45	3.4–3.9	Yes	>1	Extracellular
DyP	40–67	3.5–4.3	Yes (9–31 %)	1.1–1.2	Extracellular

*in some cases they can reach up to 49 %

et al. 2013). In addition, the secretion of specific enzymes may also depend on the culture conditions including the composition of the growth medium.

The characteristic of the main ligninolytic enzymes are presented in Table 5.3.

5.3.1 Lignin Peroxidases

Lignin peroxidases (EC 1.11.1.14, 1,2-bis(3,4-dimethoxyphenyl)propane-1,3-diol: hydrogen-peroxide oxidoreductase, family 2 at http://www.cazy.org, LiPs) were first discovered in the white-rot fungus *Phanerochaete chrysosporium* in the mid-1980s. They are considered as true ligninases since they directly catalyse lignin oxidation. LiPs are glycoproteins and contain an iron protoporphyrin IX (heme) as a prosthetic group.

LiPs catalyse the monoelectronic and H_2O_2-dependent oxidation of a wide variety of aromatic compounds through a multistep reaction. These reactions induce the formation of aryl cationic radicals, which further undergo many non-enzymatic reactions generating a number of end products such as glycolate and oxalate. Both the catalytic cycle (Fig. 5.4) and the enzymatic intermediates are similar to those of the other peroxidases. Veratryl alcohol enhances the action of LiP on many substrates, including lignin (Hammel et al. 1993), by acting as a mediator (Harvey et al. 1986) or by protecting the enzyme against inactivation by H_2O_2 (Wariishi and Gold 1989).

5.3.2 Manganese-Dependent Peroxidases

Manganese-dependent peroxidases (EC 1.11.1.13, Mn(II)-hydrogen-peroxide oxidoreductase, family 2 at http://www.cazy.org, MnPs). The first extracellular MnP

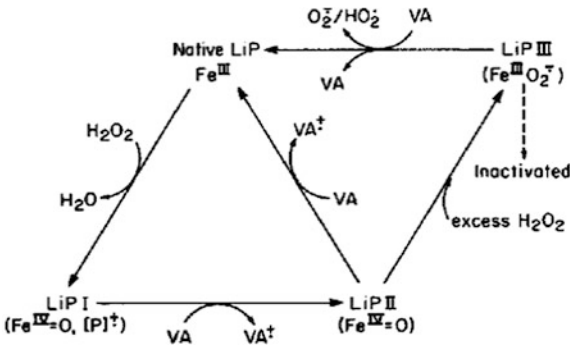

Fig. 5.4 The catalytic cycle of lignin peroxidase (LiP); $VA^{\cdot+}$: veratryl alcohol cation radical. Reprinted from FEBS Letters 243, Copyright Wariishi and Gold (1989), with permission from Elsevier Ltd., UK

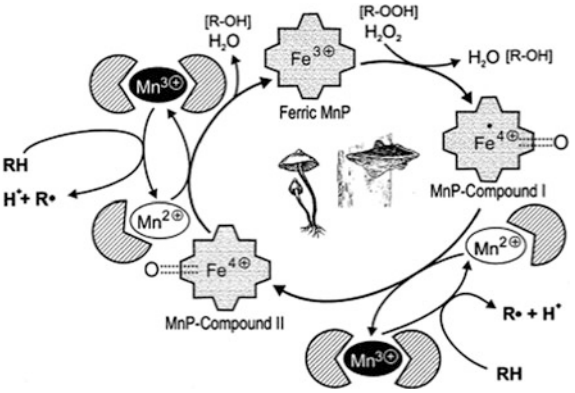

Fig. 5.5 The catalytic cycle of manganese-dependent peroxidase (MnP). Reprinted from Enzyme and Microbial Technology 30, Copyright Hofrichter (2002), *Review* lignin conversion by manganese peroxidase (MnP), 454–466, with permission from Elsevier Ltd., UK

was purified from *P. chrysosporium* and its expression and production showed to be regulated by the presence of Mn(II) in the culture medium (Bonnarme and Jeffries 1990). The catalytic cycle of MnP (Fig. 5.5) is essentially the same as for LiP with the exception that Mn(II) is necessary to complete the cycle.

5.3.3 Laccases

Laccases (EC 1.10.3.2, p-diphenol:oxygen oxidoreductases, lignin oxidases family 1, http://www.cazy.org/Auxiliary-Activities.html) are multi-copper-containing oxidases which catalyse the four-electron reduction of O_2 to water coupled with the oxidation of various organic substrates. They are widely distributed in nature and are found in plants, fungi, bacteria (Dwivedi et al. 2011) and a few insects (Xu 1999).

Laccases cannot directly oxidise all substrates either because of their large size, which hinders their introduction into the enzyme active site, or because of their particular high redox potential. However, it was shown that in the presence of low-molecular weight organic compounds acting as electron transfer mediators,

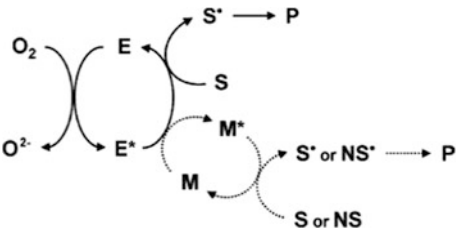

Fig. 5.6 Catalytic cycle of the laccase enzyme; E: native laccase; E^*: oxidised laccase; S: substrate; S·: substrate radical; NS: non-substrate; NS·: non-substrate radical; P: end products; O_2: oxygen; O^{2-}: divalent oxygen; M: mediator; M^*: oxidised mediator. Reprinted from Enzyme and Microbial Technology 41, Copyright Kurniawati and Nicell (2007), with permission from Elsevier Ltd., UK

laccases were also able to oxidise non-phenolic structures (Bourbonnais and Paice 1990; Call and Mücke 1997). The first step of the laccase mediator system (LMS) is the oxidation of the mediator by the laccase enzyme. Then, the oxidised mediator oxidises the bulky or high redox potential substrate. Thus, the mediator acts as an electron shuttle between the substrate and the enzyme (Galli and Gentili 2004; Widsten and Kandelbauer 2008).

Figure 5.6 represents the catalytic cycle of laccase. In typical interactions of laccase with a substrate, the catalytic site of laccase abstracts electrons from the substrate and releases an oxidised product. When a mediator is present, the mediator can be oxidised by laccase and further oxidises another compound that is either a substrate or a non-substrate of laccase resulting in the formation of oxidised product(s) and the mediator regeneration (Banci et al. 1999).

5.3.4 Versatile Peroxidases

Versatile peroxidases (EC 1.11.1.16, hybrid peroxidases, polyvalente peroxidases, family 2 at http://www.cazy.org, VPs) share catalytic properties of both LiP and MnP (Dosoretz and Reddy 2007; Hofrichter et al. 2010). Thus like MnPs, they have high affinity for Mn(II) and catalyse the oxidation of Mn(II) to Mn(III) and oxidise both phenolic and non-phenolic substrates in the absence of Mn(II) like LiPs.

VPs seem to be produced only by fungi from the genera *Pleurotus*, *Bjerkandera* and *Lepista* (Heinfling et al. 1998; Mester and Field 1998; Ruiz-Dueñas et al. 1999; Zorn et al. 2003) and maybe also by *Panus* and *Trametes* species (Martinez 2002; Lisov et al. 2003). In Fig. 5.7 the catalytic cycle of VP is depicted (Pérez-Boada et al. 2005).

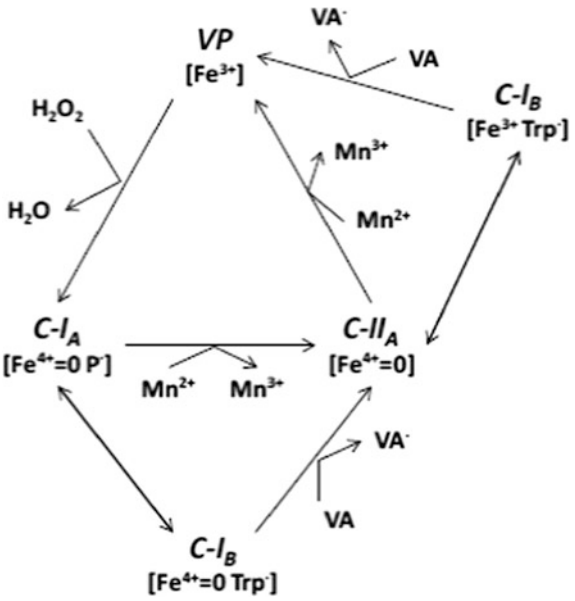

Fig. 5.7 The catalytic cycle of versatile peroxidase. Reprinted from Journal of Molecular Biology 354, Copyright Pérez-Boada et al. (2005), with permission from Elsevier Ltd., UK

5.3.5 Dye-Decolourising Peroxidases

Dye-decolourising peroxidases (DyP-type peroxidases; EC 1.11.1.19, DyPs) are glycoproteins having one heme as a cofactor and require H_2O_2 for all enzyme reactions, indicating that they function as peroxidases. They are named after their ability to oxidise a wide range of synthetic dyes, in particular, anthraquinonic dyes, which are poorly oxidised by other peroxidases (Kim and Shoda 1999; Passardi et al. 2005; Sugano 2009). In addition, they function under lower pH conditions than other peroxidases. A very important characteristic of DyPs is that they have a free position for the H_2O_2 binding (Petrides and Nauseef 2000).

Typical peroxidase substrates degraded by DyPs are, for example, 2,2′ azinobis-(3-ethylbenzthiazoline-6-sulphonate and phenolic compounds. DyPs have also been reported to cleave β-carotene and other carotenoids as well as oxidise methoxylated aromatics such as veratryl alcohol and non-phenolic β-O-4 lignin model compounds (van Bloois et al. 2009; Zelena et al. 2009; Liers et al. 2010). However, their physiological function still remains unclear.

5.4 Xenobiotics Degraded by WRF

The ability of the WRF to degrade xenobiotic compounds comes from their ability to degrade lignin, since it resembles the chemical structure of many xenobiotics (Fig. 5.8). Thus, the same mechanisms that give the WRF the ability to degrade

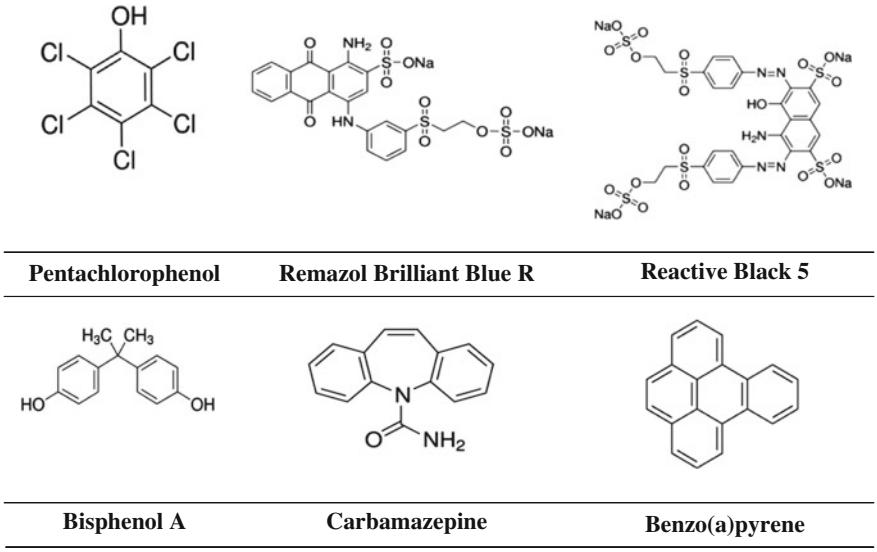

Fig. 5.8 Chemical structures of different xenobiotic compounds

lignin can be used to degrade a wide variety of recalcitrant pollutants. Under ligninolytic conditions, many xenobiotics are oxidised and mineralised to different extents by the WRF (Field et al. 1993).

Several reviews about environmental pollutant degradation by the WRF have already been published (Bumpus et al. 1985; Reddy 1995; Raghukumar et al. 2008; Pointing 2001; Reddy and Mathew 2001; Wesenberg et al. 2003; Chang 2008; Pinedo-Rivlla et al. 2009; Majeau et al. 2010). However, there are few reports focused on the application of WRF in the treatment of real wastewater. In this section, recent reports on xenobiotic removal from real wastewater are reviewed (Table 5.4).

5.4.1 Pharmaceuticals[1]

Accinelli et al. (2010) studied the potential of *P. chrysosporium* entrapped in granular bioplastics to remove different pharmaceutical compounds (i.e., the antiviral drug oseltamivir and the antibiotics erythromycin, sulfamethoxazole and ciprofloxacin) from a municipal wastewater treatment plant (WWTP). It was found

[1]For further information on fungal treatment of wastewater containing pharmaceutical products, please refer to Chap. 6—*Fungal bioremediation of emerging micropollutants in municipal wastewaters* and Chap. 8—*Mycoremediation of organic pollutants: principles, opportunities and pitfalls*.

Table 5.4 Degradation of real wastewater containing different xenobiotic compounds by different white-rot fungi in the past years

White-rot fungus	Wastewater source	Xenobiotic	Removal	Reference
Pharmaceuticals				
Phanerochaete chrysosporium	Municipal WWTP (Italy)	Oseltamivir (Tamiflu)	>50 % in 30 days	Accinelli et al. (2010)
		Erythromycin	>80 % in 5 days	
		Sulfamethoxazole	>50 % in 5 days	
		Ciprofloxacin	>70 % in 5 days	
P. chrysosporium	Municipal WWTP (Germany)	Carbamazepine (1 mg/L)	60 % in 100 days	Zhang and Geissen (2012)
T. versicolor	Urban (Spain)	Pharmaceutical compounds	50 %	Cruz-Morató et al. (2013)
T. versicolor	Hospital (Spain)	Pharmaceutical and endocrine disrupting compounds	83.2 % (sterile) in 8 days; 53.3 % (non-sterile) in 8 days	Cruz-Morató et al. (2014)
T. versicolor	Hospital (Spain)	Iopromide	87 % (sterile), 65.4 % (non-sterile) in 8 days	Gros et al. (2014)
		Ofloxacin	98.5 % (sterile), 99 % (non-sterile) in 8 days	
Textile wastewater				
Bjerkandera adusta	Textile (Italy)	Dyes	Up to 84 % during 10 cycles	Anastasi et al. (2010)
B. adusta	Textile (Italy)	Dyes	40 % in 24 h	Anastasi et al. (2011)
P. chrysosporium	Textile (India)	Dyes	84 % in 6 days	Sangeeta et al. (2011)
T. pubescens	Textile (Italy)	Dyes	76 % decolouration in 24 h, COD reduction and toxicity removal (flasks); 30 % decolouration (bioreactor)	Anastasi et al. (2012)

(continued)

Table 5.4 (continued)

White-rot fungus	Wastewater source	Xenobiotic	Removal	Reference
Bjerkandera sp.	Textile (Colombia)	Dyes Everzol Black EDR and Everzol Black EDG	65 % (sterile) and 40 % (non-sterile) in 8 days	Osorio-Echavarría et al. (2012)
P. chrysosporium	Textile (India)	Dyes	80 % (5 g/L glucose); 83 % (10 g/L glucose)	Pakshirajan and Kheria (2012)
Curvularia lunata	Textile (Brazil)	Indigo dye	95 % (non-aerated) and 93 % (aerated) in 10 days	de Miranda et al. (2013)
P. chrysosporium			95 % (non-aerated) and 98 % (aerated) in 10 days	
Pleurotus floridanus	Textile (India)	Dyes	71.2 % colour, 80.5 % COD	Sathian et al. (2013)
B. adusta	Textile effluent from a WWTP (South Korea)	Industrial dyes	71–92 % in 3 weeks	Choi et al. (2014)
Ganoderma sp. En3	Textile (China)	Indigoid and sulphur dyes	85.1 % in 8 days	Ma et al. (2014)
Combination of *P. floridanus, G. lucidum* and *T. pubescens*	Textile (India)	Dyes	71.3 % colour and 79.4 % COD (HRT 5 days)	Sathian et al. (2014)
Olive mills				
Trametes versicolor	Olive mill (Italy)	Phenolics (277 mg/L)	60 % colour, 72 % phenols (shaken flasks) in 216 h; 65 % colour, 89 % phenols (reactor, continuous) in 192 h	Cerrone et al. (2011)

(continued)

Table 5.4 (continued)

White-rot fungus	Wastewater source	Xenobiotic	Removal	Reference
Ganoderma spp.	Olive mill (Greece)	Phenolics (4.9 mg/mL)	40–46 % colour, 64-67 % phenolics in 20 days	Ntougias et al. (2012)
Pleurotus spp.			60–65 % colour, 74–81 % phenolics in 20 days	
Pleurotus ostreatus	Olive mill (Italy)	Polyphenols (5 g/L)	70 % in 4-7 days (batch); 42–68 % for 5 cycles (batch with biomass recycling and nutrient addition)	Olivieri et al. (2012)
Wastewater from other sources				
Trametes pubescens	Distillery (South Africa)	Phenolics (866 mg/L)	86 % in 2 days	Strong (2010)
Ceriporiopsis subvermispora			57 % in 2 days	
Pycnoporus cinnabarinus			69 % in 2 days	
P. chrysosporium			<40 % in 2 days	
P. chrysosporium	Pulp and paper mill (India)		83 % colour in 96 h	Gomathi et al. (2012)
P. ostreatus	Petrochemical (Italy)	Mixture of 2-NSA (2-naphthalene sulfonic acid) polymers	70 % (20–24 % adsorbed by fungal biomass) in 40 days	Palli et al. (2014)

WWTP Wastewater treatment plant
HRT Hydraulic retention time

that the antibiotics were more readily removed by *P. chrysosporium* than the antiviral drug (Table 5.4). DNA analysis showed that fungal growth was mainly confined to the bioplastic carriers making it easy to insert the fungus to the polluted site.

Zhang and Geissen (2012) studied the degradation of carbamazepine in an effluent from a municipal WWTP by *P. chrysosporium* immobilised on polyether foam in a novel plate bioreactor. Carbamazepine (1 mg/L) was removed by 60 % in

100 days of continuous operation provided that additional glucose and nitrogen were supplied.

Cruz-Morató et al. (2013) reported for the first time the degradation of pharmaceutical compounds (PhACs) in urban wastewater by *T. versicolor* pellets in a batch fluidised-bed bioreactor operating under non-sterile conditions where 50 % of the detected PhACs was removed. In addition, a considerable reduction in toxicity was achieved after the fungal treatment. In the following study, Cruz-Morató et al. (2014) reported the removal of PhACs and endocrine disruptor compounds (EDCs) from hospital effluents under sterile and non-sterile conditions using the same approach. They found that the overall load removal was 83.2 % under sterile and 53.3 % under non-sterile conditions after 8 days of treatment. In addition, toxicity tests showed the reduction of wastewater toxicity after the fungal treatment.

Gros et al. (2014) studied the degradation of the X-ray contrast agent iopromide (IOP) and the antibiotic ofloxacin (OFLOX) in hospital wastewater by *T. versicolor* in a 10-L fluidised-bioreactor. They found that within 8 days, IOP and OFLOX were degraded by 87 and 98.5 % respectively, under sterile conditions, and by 65.4 and 99 % respectively, under non-sterile conditions. In addition, toxicity of the treated wastewater was reduced after the fungal treatment.

5.4.2 Textile Wastewater[2]

Anastasi et al. (2010) reported the ability of *Bjerkandera adusta* to treat wastewater from a textile factory in a fixed-bed reactor operated in continuous mode. This fungus was able to decolourise the effluent up to 84 % during 10 cycles under non-sterile conditions. In addition, the chemical oxygen demand (COD) and the toxicity were effectively reduced after the fungal treatment. Subsequently, Anastasi et al. (2011) tested the capacity of the same fungus to degrade wastewater from a textile industry after a secondary treatment and found that the fungal treatment decolourised the effluent by 40 % in 24 h. Further, they (Anastasi et al. 2012) showed that fungal treatment with *Trametes pubescens* followed by activated sludge of wastewater from a cotton dyeing industry led to very good results in terms of decolouration (76 % in 24 h), COD reduction and toxicity removal. However, the scale-up in a 5-L moving-bed bioreactor (working volume 2 L) with *T. pubescens* immobilised on 2 cm^3 cubes of polyurethane foam (PUF) led to lower decolouration (30 %). Therefore, optimisation of the reactor technology is needed before fungal treatment could be successfully applied.

Sangeeta et al. (2011) studied the decolouration of textile wastewater by *P. chrysosporium* in shaken flasks and found that the decolouration of raw

[2]Additional information on treatment of dye using fungi is presented in Chap. 4—*Application of biosorption and biodegradation function of fungi in wastewater and sludge treatment.*

wastewater was negligible. Nevertheless, when wastewater was diluted with medium containing glucose and other nutrients, the decolouration considerably increased (84 % in 6 days).

Osorio-Echavarría et al. (2012) reported the decolouration of textile wastewater by the white-rot fungus anamorph R1 of *Bjerkandera sp*. under sterile and non-sterile conditions. The former led to a decolouration percentage of 65 % in 8 days, whereas the latter led to a decolouration percentage of 40 % for the same period of time. The decolouration under non-sterile conditions was mainly due to dye adsorption onto fungal mycelium since the pH increased affecting both the fungus and the ligninolytic enzymes. They found that the presence of high concentration of salts (i.e. NaCl and Na_2CO_3) in the wastewater favoured the decolouration process. This indicates that the fungus anamorph R1 of *Bjerkandera sp*. is able to grow under hypersaline conditions. This makes this fungus advantageous for the treatment of industrial effluents with high salt concentrations such as those from the textile industries.

Pakshirajan and Kheria (2012) investigated the continuous treatment of textile wastewater by *P. chrysosporium* in a rotating biological contactor reactor operating at an HRT of 48 h. The fungus was able to decolourise the effluent by more than 64 % when diluted with media containing glucose. Maximum decolouration efficiencies of 83 and 80 % were attained with 10 and 5 g/L of glucose respectively.

de Miranda et al. (2013) investigated the decolouration of a textile effluent by the white-rot fungi *Curvularia lunata* and *P. chrysosporium* in static bioreactors under aerated and non-aerated conditions. The effluent was almost totally decolourised within 10 days under both conditions. However, the effluent treated by *P. chrysosporium* contained a mutagenic byproduct from indigo biodegradation that was not found in the effluent treated by *C. lunata.* This indicates that different degradation pathways are used by different ligninolytic fungi and that degradation is not always accompanied by detoxification.

Sathian et al. (2013) studied the decolouration of textile wastewater by *Pleurotus floridanus* in batch culture. After optimisation of different parameters, the fungal treatment achieved 71.2 % decolouration and 80.5 % COD reduction. Furthermore, in studying the ability of the white-rot fungi *Coriolus versicolor*, *P. floridanus*, *Ganoderma lucidum* and *T. pubescens* to decolourise textile wastewater in pure and mixed cultures, Sathian et al. (2014) found that the combination of *P. floridanus*, *G. lucidum* and *T. pubescens* led to the best results (87.2 % decolouration) and this combination was used subsequently in a sequential batch reactor (SBR). When operating at the optimised conditions, a decolouration percentage of 71.3 % and a COD reduction of 79.4 % could be obtained.

Choi et al. (2014) investigated the ability of the white-rot fungi *B. adusta*, *Ceriporia lacerata*, *Phanerochaete calotricha* and *Porostereum spadiceum* to decolourise an untreated textile effluent from a WWTP. They found that only *B. adusta* was able to decolourise the effluent significantly (71–92 % in 3 weeks). In addition, wastewater toxicity decreased after fungal *B. adusta* treatment. These results highlight again the different degrading abilities of different fungal species. Ma et al. (2014) reported that *Ganoderma* sp. En3, a white-rot fungus isolated from

a forest in China, was able to decolourise indigo jean dyeing wastewater from a textile factory up to 85.1 % in 8 days.

5.4.3 Olive Mills

Cerrone et al. (2011) evaluated the white-rot fungi *Panus tigrinus*, *Funalia trogii* and *T. versicolor* to treat olive washing wastewater (OWW) and found that *T. versicolor* performed well, reducing colour, COD and phenols by 60, 72 and 87 %, respectively, in 216 h. Also, only this fungus grew well in a bubble-column bioreactor (working volume 1 L) and the treatment of OWW in continuous operation reduced colour, COD and phenols by 65, 73 and 89 %, respectively, after 192 h.

Ntougias et al. (2012) studied the treatment of olive mill wastewater (OMW) by different strains belonging to the *Ganoderma* and *Pleurotus* genera and found that the *Ganoderma* spp removed 40–46 % colour and 64–67 % phenolics and the *Pleurotus* spp removed 60–65 % colour and 74–81 % phenolics within 20 incubation days. This indicates that different fungal species exhibit different degrading abilities.

Olivieri et al. (2012) studied the removal of polyphenols in raw OMW by *P. ostreatus* under controlled non-sterile conditions in flasks and in an internal loop airlift bioreactor (ILAB) operating in batch with biomass recycling and in continuous culture. Biomass recycling with nutrient addition was the most effective configuration, removing 42–68 % of polyphenols for 5 cycles. The continuous treatment in the ILAB was effectively performed provided that OMW was previously aerated to avoid oxygen consumption by endogenous micro-organisms.

5.4.4 Wastewater from Other Sources

Strong (2010) studied the treatment of Amarula distillery wastewater by *T. pubescens*, *Ceriporiopsis subvermispora*, *Pycnoporus cinnabarinus* and *P. chrysosporium*. *T. pubescens* was found to be the most efficient fungus in phenolic removal (86 %) followed by *P. cinnabariunus* (69 %) and *C. subvermispora* (57 %) within 2 cultivation days. However, *P. chrysosporium* removed less than 40 % of the phenolics for the same time period. In addition, *T. pubescens* was also very effective in removing colour and reducing COD. Therefore, this study showed the possibility to treat an effluent containing high COD and high phenolic concentration using the white-rot fungus *T. pubescens*.

Gomathi et al. (2012) reported high decolouration (83 % in 96 h) of a pulp and paper mill effluent by *P. chrysosporium* entrapped in calcium alginate when 1 % sucrose and 1 % ammonium chloride were added to the effluent.

Palli et al. (2014) assessed the ability of *P. ostreatus* to remove 2-naphthalenesulfonic acid polymers (2-NSAP) from petrochemical wastewater. In the presence of an adequate carbon source, the fungus was able to remove about 70 % of the oligomers in 40 days, from which about 20–24 % was adsorbed by the fungal biomass. Furthermore, respirometric tests showed a considerable increase of the BOD/COD ratio (from 9 % up to 57 %) after the fungal treatment which confirmed that the fungus did not mineralise the NSAP but increased their biodegradability.

5.5 Concluding Remarks

WRF hold an enormous potential for the biodegradation of a great variety of xenobiotic compounds due to the secretion of enzymatic complexes with broad substrate specificity. Different WRF show different biodegrading abilities for different xenobiotic compounds mainly due to their different physiology, culture and/or environmental conditions and nature of enzymes secreted. Also, the characteristics of the ligninolytic enzymes from different WRF sources differ considerably.

Despite the promising results reported so far, in order to assess the true technical potential of WRF to biodegrade xenobiotics, more studies under real industrial conditions are needed. However, most studies using real wastewater were performed required some pre-conditioning of wastewater (dilution, pH adjustment, sterilisation, addition of nutrients).

Detailed characterisation of the intermediates and metabolites produced during biodegradation as well as toxicity tests should also be carried out to measure the detoxification of the fungal treated wastewater and prevent accumulation of toxic byproducts. Although some studies regarding the metabolic pathway of xenobiotic degradation by WRF have been performed there is still a gap in the degradation mechanisms of xenobiotics by WRF and their ligninolytic enzymes.

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