



Industrial and environmental applications of white-rot fungi

Rodríguez-Couto S^{1,2}

¹ Ceit-IK4, Water & Health Division, 20018 Donostia-San Sebastian, Spain, srodriguez@ceit.es

² IKERBASQUE, Basque Foundation for Research, María Diaz de Haro 3, 48013, Bilbao, Spain

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Abstract

White-rot-fungi (WRF) are the only organisms able to degrade the whole wood components (i.e. lignin, cellulose and hemicellulose). This ability is due to the secretion of extracellular non-specific ligninolytic enzymes during their secondary metabolism usually triggered by nutrient exhaustion. The non-specificity of these enzymes enables them to transform a great variety of recalcitrant and hazardous pollutants such as polycyclic aromatic hydrocarbons (PAHs), pesticides, fuels, alkanes, polychlorinated biphenyls (PCBs), explosives and synthetic dyes. In addition, their extracellular nature allows WRF to access non-polar and insoluble compounds. This makes WRF very appealing for their application to different industrial and biotechnological processes. Also, new potential commercial products and processes from the fungal treatment of lignocellulosic materials may arise. The implementation of such applications would contribute to the establishment of a more sustainable industry and the development of a circular economy.

Key words – biotechnology – degradation – lignin – ligninolytic enzymes – valorisation

Introduction

White-rot fungi (WRF), so-named because of the whitish colour of the delignified wood, are the only known organisms able to mineralise the recalcitrant and bulky heteropolymer lignin (Figure 1). This ability is due to the secretion of extracellular non-specific enzymatic complexes during their secondary metabolism (Wesenberg et al. 2003), usually under limited nutrient availability (C:N ratio) with nitrogen being the limiting nutrient for fungal growth in most wood and soils (Kirk & Farrell 1987). These enzymatic complexes mainly consist of lignin peroxidases (LiPs, EC 1.11.1.14), manganese-dependent peroxidases (MnPs, EC 1.11.1.13) and laccases (benzenediol:oxygen oxidoreductases, EC 1.10.3.2) together with accessory enzymes (Ruiz-Dueñas & Martínez 2009). The non-specificity of these enzymes enables them to transform a great variety of persistent chemicals with a structure similar to lignin (Mansur et al. 2003). Furthermore, their extracellular nature allows the fungi to access non-polar and insoluble compounds (Levin et al. 2003). This makes WRF very attractive for different industrial and biotechnological applications such as the production of biofuel from plant biomass, biopulping, biobleaching and the degradation of recalcitrant environmental pollutants.

WRF are ubiquitous in nature, particularly in hardwood forests as hardwood (e.g. birch and aspen) is more susceptible to the attack of WRF than softwood (e.g. spruce and pine) (Blanchette et al. 1990). WRF can degrade all wood components (i.e. cellulose, hemicellulose and lignin) or preferentially lignin. The former are named simultaneous or non-selective WRF and the latter

selective WRF. The selective WRF are of special bioindustrial interest, since they remove lignin leaving the valuable cellulose intact (Dashtban et al. 2010). There are also WRF that cause both types of white-rot attack within one substrate (Blanchette 1984, Blanchette et al. 1985, Adaskaveg & Gilbertson 1986).

The mechanisms on how WRF degrade lignin are not fully understood but the fungal strain, the origin of lignocellulose and the culture conditions play a major role in the process (van Kuijk et al. 2015). Also, individual fungi can considerably vary their ability to degrade specific substrates under the same environmental conditions (Eriksson et al. 1990).

Potential applications of white-rot fungi

Bioremediation of environmental pollutants

One of the main environmental problems facing the world nowadays is the pollution of soil, water and air by toxic chemicals. Most of these chemicals are known to be carcinogenic and mutagenic posing a serious hazard to the ecosystem and human beings. Therefore such compounds have to be removed before entering into the environment. However, the in-use techniques for the treatment of these type of compounds are rather costly, time-consuming, mostly ineffective and sometimes generate hazardous sub-products (Grassi et al. 2011). Consequently, alternative methods to remove these hazardous recalcitrant compounds are needed. In this sense, the use of WRF represents a promising approach.

Due to the similarity between the chemical structure of lignin and those of many recalcitrant pollutants, such as polycyclic aromatic hydrocarbons (PAHs), pesticides, fuels, alkanes, polychlorinated biphenyls (PCBs), explosives and synthetic dyes (Figure 2), the use of WRF for the degradation of such pollutants has been considered (Paszczynski et al. 1991). This feature is the greatest advantage of using WRF in bioremediation, since a mixture of different pollutants is usually found in most polluted sites (Mester & Tien 2000). Also, WRF can bear a broad range of environmental conditions and, in addition to this, they can use lignocellulose for growth making them suitable for inoculation into polluted soils. Moreover, WRF can exert a positive effect on the growth of the indigenous micro-organisms facilitating the degradation of recalcitrant pollutants.

The first studies on pollutant degradation by WRF were performed with the white-rot fungus *Phanerochaete chrysosporium* (Figure 3A), which has become the model organism for lignin degradation studies (Bumpus et al. 1985). Since then, other species of WRF with promising ability to degrade recalcitrant pollutants have been described, including species belonging to the genera *Pleurotus*, *Bjerkandera*, *Corioloropsis*, *Phlebia* and *Trametes* (Rodríguez et al. 2004). In particular, the non-selective white-rot-fungus *Trametes versicolor* (Figure 3B) has been repeatedly used in assays as a WRF representative (Blanchette & Burnes 1988).

The biotransformation of pollutants by WRF entails different processes started either by the ligninolytic enzymes or the mycelial-bound redox system that produce free radicals, which can then perform either another enzyme-catalysed oxidation or non-enzymatic transformations *via* enzyme combustion. However until whole pollutant mineralisation, the use of different toxicity tests are needed to ensure the safety of the by-products formed (Jurado et al. 2011).

The ability of WRF to remove recalcitrant pollutants from wastewater has shown to be a good alternative to traditional wastewater treatment technologies. In addition, WRF have shown promising potential for the bioremediation of industrially-contaminated soils (Borràs et al. 2010; Anasonye et al. 2015). However, nowadays bioremediation on a commercial scale uses prokaryotes with comparatively few recent attempts to use WRF despite their clear advantages for bioremediation over bacteria (Table 1). In addition, WRF treatments would expand the substrate range of current treatments by degrading pollutants that prokaryotes cannot (Pointing 2001). Nevertheless, the use of WRF in bioremediation presents the following drawbacks: long growth cycle, requiring nitrogen limiting conditions, long hydraulic retention time (Banat et al. 1996, Saratale et al. 2009) and low pH for optimum enzyme activity (Doble & Kumar 2005) which make the maintenance of WRF in bioreactors problematic. Additionally, several operational problems,

such as formation of mycelia aggregates, electrode fouling and clogging, can made necessary the removal of fungal biomass from the bioreactors after short operation periods (Karthikeyan & Sahu 2014). Also, despite different authors have reported the potential of WRF to treat industrial wastewater, there are few studies at bioreactor scale operating in continuous mode and under non-sterilised conditions. Therefore, the application of WRF at industrial scale remains as a technical challenge.

Pulp and paper industry

During pulp and paper production, it is necessary to separate the cellulose fibres from lignin. This is performed by using mechanical or chemical methods. In chemical pulping, lignin is solubilised by chemicals resulting in a brown residual material that must be removed to produce white paper. For this, elemental chlorine has been used for a long time but currently delignification with oxygen and hydrogen peroxide is being used. However, they are less efficient in achieving a high degree of brightness than the chlorine reagents.

The treatment of wood chips with ligninolytic fungi prior to conventional pulping methods (mechanical, chemical or a combination of both) is named biopulping. WRF have been considered as potentially useful agents for biopulping because they reduce not only energy consumption but also chemicals, thus, being environmentally-friendly in contrast with the conventional pulping. In addition, biopulping not only removes lignin but also some of the wood extractives, thereby reducing the pitch content and effluent toxicity (Ali & Sreerishnan 2001).

The biological delignification of wood by WRF was first considered by Lawson & Still (1957) at the West Virginia Pulp and Paper Company Research Laboratory (now Westvaco Corp.) (Akhtar et al. 1998). Since then, many researchers have studied the potential use of WRF in pulping processes and pilot mill trials have been started in the last decades (Farrell et al. 1997, Breen & Singleton 1999, Scott et al. 2002, Masarin et al. 2009). The efficiency of fungal pre-treatment utilising different lignocellulosic materials has been described and several patents have been published, the one based on the use of *Ceriporiopsis subvermispora* being the most optimised one (Gutiérrez et al. 2001). This species has also proven to be very competitive on both softwoods and hardwoods (Ferraz et al. 2007).

The pre-treatment of wood chips by WRF has shown to improve the effectiveness of kraft pulping and paper brightness (Fonseca et al. 2014). Therefore it can be considered as a possible alternative to chemical pulping since, in addition to this, it requires simpler equipment and produces an effluent with reduced BOD.

Valorization of lignocellulosic wastes

The accumulation of huge amounts of lignocellulosic wastes from human activity is considered a serious environmental problem (Dias et al. 2010). The major constituent of lignocellulosic materials is cellulose followed by hemicellulose and lignin (Figure 4). Cellulose and hemicellulose are macromolecules built from different sugars, whereas lignin is an aromatic polymer synthesised from phenylpropanoid precursors. The composition and proportions of these components vary between plants (Sánchez et al. 2009). Lignin degradation by using chemical and physical methods is a process neither environmentally-friendly nor economical. The use of WRF is being considered as an attractive alternative to transform these wastes into value-added products.

Production of relevant metabolites

Different lignocellulosic wastes have been used as support-substrates for the production of different metabolites of industrial or commercial interest by WRF, generally under solid-state fermentation (SSF) conditions. The use of such wastes not only reduces considerably de production costs but also helps to alleviate the economic and the environmental problems caused by their disposal. Although most of the produced metabolites are ligninolytic enzymes (Rodríguez-Couto & Sanromán 2005) other value-added products such as organic acids are also obtained (Table 2). In addition, recently the application of bioactive compounds produced by WRF to the food and

pharmaceutical industry has impelled the search for novel bioactive compounds of fungal origin (Wong et al. 2010). Moreover, their production has become an important field in modern biotechnology. Thus, the white-rot-fungus *Ganoderma lucidum* has been reported to produce several bioactive compounds with high therapeutic value (Paterson 2006). Also, recently the white-rot fungus *Cerrena unicolor* has shown to exhibit antiviral, immunomodulatory and anticancer activities (Mizerska-Dudka et al. 2015).

Bioethanol

Biofuel production from renewable sources has received increased interest in recent years as an alternative to the use of fossil fuels in many countries. Lignocellulosic biomass, mostly from agricultural and forestry wastes, is rich in carbohydrates and widely available, thus, providing attractive feedstocks for ethanol production. To maximise the use of carbohydrates from the biomass a pre-treatment process is required. The current in use technologies are costly hampering the commercialisation of bioethanol (Mosier et al. 2005). This has impelled the search for alternative processes such as those based on WRF. Thus, several WRF, such as *Phanerochaete chrysosporium*, *Pleurotus ostreatus*, *Trametes versicolor*, *Cyathus stercoreus* and *Ceriporiopsis subvermispora*, have been studied for the pretreatment of different lignocellulosic wastes (Wan & Li 2012; Knežević et al. 2013). However, despite that the use of WRF offers the following advantages over the current thermal or chemical pre-treatment processes: simpler techniques, low energy requirements, no or reduced waste streams, reduced downstream processing costs and no or reduced inhibitors to ethanol fermentation (Keller et al. 2003; Nigam & Pandey 2009), substantial holocellulose (cellulose and hemicellulose) loss and long pre-treatment times are the main drawbacks of this process. So to ensure a cellulose-rich but highly delignified biomass for biofuel production, highly selective lignin degraders are preferred.

Ruminant feed

Cellulose and hemicellulose in most lignocellulosic wastes are highly linked to lignin which makes them difficult to digest by animals (Arora & Sharma 2009). This hampers the use of such wastes by rumen microbes and currently chemical and/or physical treatments are used to degrade lignin (Chaturvedi & Verma 2013). In search for alternative treatments to the use of chemicals or expensive physical treatments, the use of WRF is seen as a very attractive alternative. In particular to convert lignocellulosic wastes into ruminant feeding, selective lignin degraders are the preferred WRF since they left cellulose intact and, thereby, keep the energy value of such wastes. However, only small laboratory scale experiments involving singles substrates or fungal species have been conducted so far. Further optimisation is needed to develop an alternative treatment able to compete with the conventional treatments (van Kuijk et al. 2015).

Outlook

The practical use of WRF for biotechnological applications holds great potential. However before this can become a reality, progress related to process optimisation and cost reduction is needed. Searching for novel micro-organisms, taking advantage of the enormous microbial diversity existing in aquatic and terrestrial environments, is also required. The ocean is an enormous reservoir of untapped micro-organisms.

The advantages and disadvantages of using WRF or their enzymes in biotechnological applications should be evaluated before attempting industrial-scale operations. In comparison to fungal biomass, enzymes are still more expensive to produce at an industrial scale in spite of their potential for scaling up through gene technologies. However with the increasing advances in enzyme immobilisation technologies, efficiency in enzyme reusing both in amount and activity is probably to be greater than that of fungal biomass.

Table 1 Advantages of using white-rot-fungi (WRF) over bacteria for bioremediation (Maloney 2001).

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- (i) They use inexpensive and easily available lignocellulosic materials as a nutrient source
 - (ii) They can tolerate relatively high concentrations of pollutants due to their extracellular degradation system
 - (iii) They are able to survive in the presence of several xenobiotics that may be toxic to other microorganisms
 - (iv) They are able to degrade a mixture of chemicals thanks to their non-specific free-radical-based degradation mechanism
 - (v) They do not need pre-conditioning to a particular pollutant
 - (vi) They can tolerate a wide range of environmental conditions
 - (vii) The rate of degradation or biotransformation of a pollutant is proportional to its concentration and, so, the solubility of the pollutant is not important
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Table 2 Examples of different value-added metabolites produced by white-rot fungi grown on lignocellulosic wastes under solid-state fermentation conditions.

White-rot fungus	Lignocellulosic waste	Product(s)	Reference
<i>Fomes fomentarius</i>	Granary waste (small grains, husks and straw, and straw of barley, oats, rye and wheat)	Crude protein	Hatakka & Pirhonen 1985
<i>Nematoloma frowardii</i>	Wheat straw	Manganese peroxidase, organic acids	Hofrichter et al. 1999
<i>Phanerochaete chrysosporium</i> , <i>Phlebia radiata</i>	Corn cob	Protease	Cabaleiro et al. 2002
<i>Dichomitus squalens</i> , <i>Phanerochaete sanguinea</i> , <i>Trametes ochracea</i> , <i>Trametes versicolor</i>	Spruce wood chips	Oxalic acid	Mäkelä et al. 2002
<i>Physisporinus rivulosus</i>	Spruce wood chips	Manganese peroxidase, laccase, oxalic acid	Hakala et al. 2005
<i>Ceriporiopsis subvermispora</i>	<i>Pinus taeda</i> wood chips	Xylanase	Milagres et al. 2005
<i>Trametes hirsuta</i>	Grape seeds	Laccase	Rodríguez-Couto et al. 2006
<i>Bjerkandera adusta</i> , <i>Pycnoporus sanguineus</i>	Oak and cedar sawdust, rice husk, corn stubble, wheat straw, <i>Jatropha</i> seed husk	Cellulase, xylanase	Quiroz-Castañeda et al. 2011
<i>Cerrena unicolor</i>	Oat husks	Manganese peroxidase, laccase	Moilanen et al. 2015
<i>Coriolopsis gallica</i>	Sawdust waste	Laccases	Daâssi et al. 2016

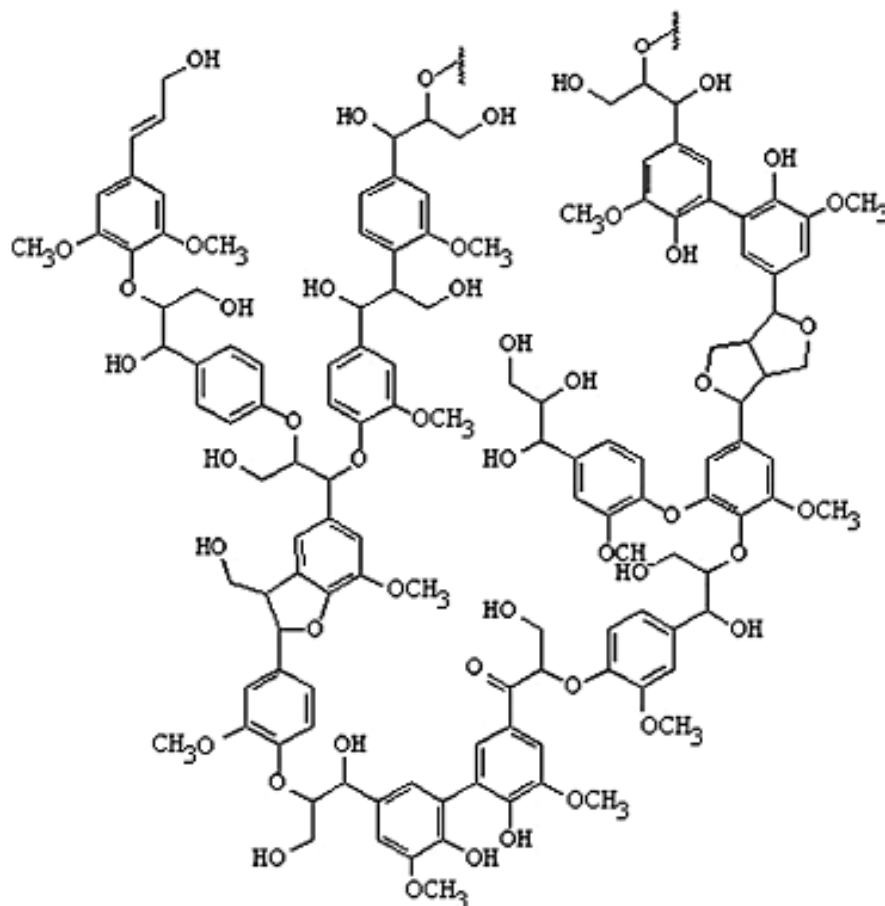
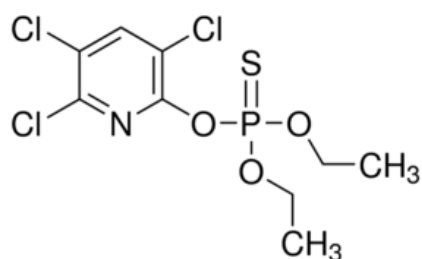
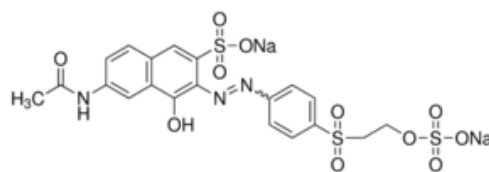


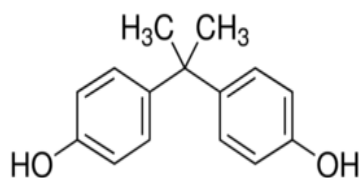
Fig. 1 – Schematic structure of a lignin molecule (source: [www.research.uky.edu/.../green energy.html](http://www.research.uky.edu/.../green%20energy.html)).



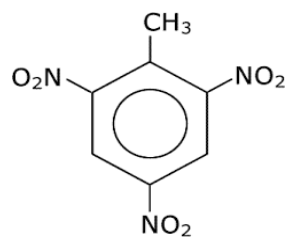
Chlorpyrifos



Reactive Orange 16



Bisphenol A



2, 4, 6-trinitrotoluene

Fig. 2 – Different environmental pollutants degraded by the white-rot-fungi.



A



B

Fig. 3 – Pictures of the white-rot fungi *Phanerochaete chrysosporium* (A; source <https://microbewiki.kenyon.edu/>) and *Trametes versicolor* (B; source <http://www.wisconsinmushrooms.com/>) as grown in nature.

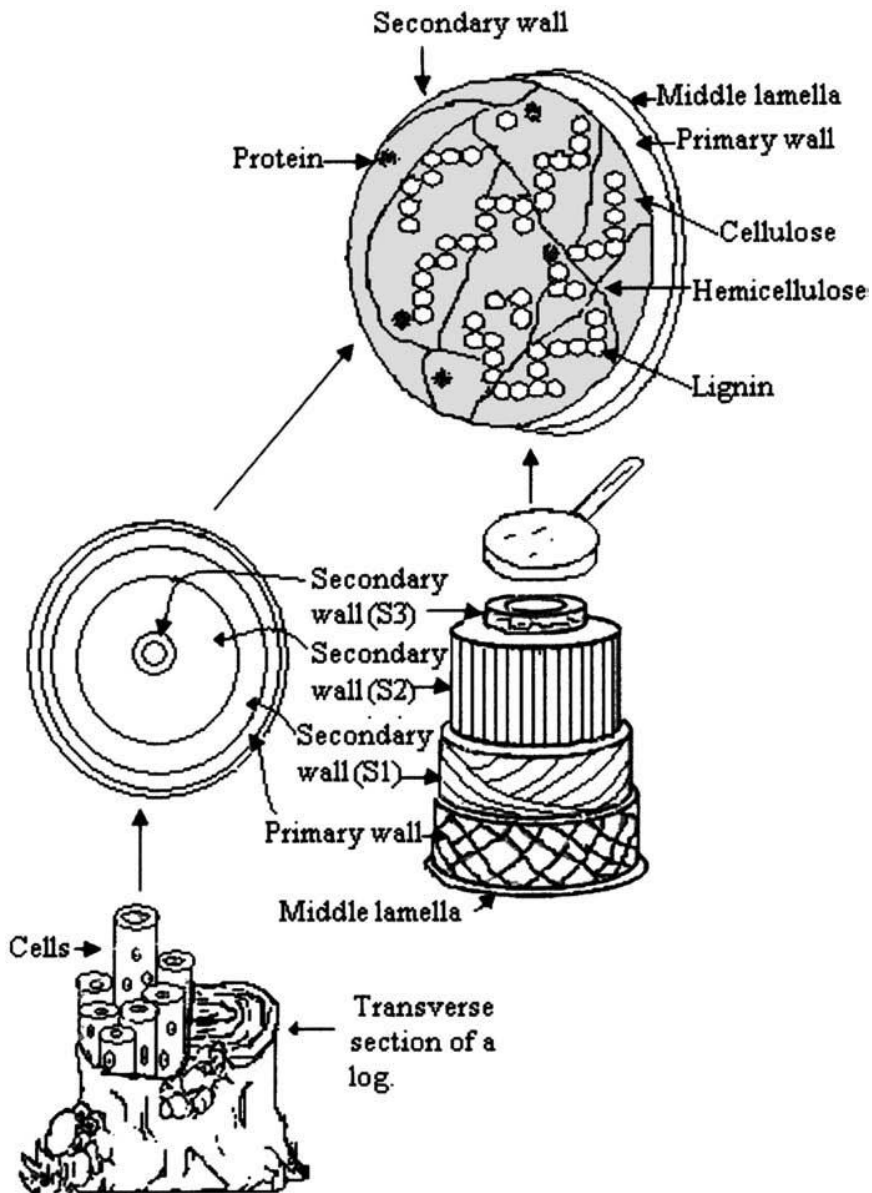


Fig. 4 – Major components of wood.

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