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Application of white-rot fungi for biodegradation of refractory organic compounds—a review

Maria Łebkowska*, Monika Załęska-Radziwiłł

Faculty of Environmental Engineering, Department of Biology, Warsaw University of Technology, Nowowiejska 20, 00-653 Warsaw, Poland
Email: maria.łebkowska@is.pw.edu.pl

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ABSTRACT

The article presents an overview of literary sources on the use of fungi that cause white rot for removal of organic pollutants from water, wastewater and wastewater sludge. The study characterizes enzymes produced by these fungi, and the methods and conditions for their culture. The article includes some examples of the use of fungi for treatment of wastewater from paper, textile, alcohol and food production, among others, and for biodegradation of dyes, hydrocarbons, phenols, chlorophenols, nitrotoluen and pesticides. It discusses some technological parameters of devices used for elimination of pollutants from wastewater with the use of white-rot fungi. The article highlights the need to extend research work from lab scale to semi-technical scale.

Keywords: White-rot fungi; Industrial wastewater; Biodegradation

1. Introduction

White-rot fungi (WRF) belong to the group of Basidiomycota (*Basidiomycetes*). They produce the following enzymes: laccases (Lcc), Mn-peroxidases (MnP) and lignin peroxidases (LiP). Lacasse-glycoprotein, which contains copper atoms, catalyzes the oxidation of lignin to phenolic compounds by reducing molecular oxygen to water. In the presence of redox mediators, such as 3-hydroksyanthranilic acid and N-hydroxybenzotriazole, it has also the ability to oxidize non-phenolic aromatic substances. LiP is an exoenzyme which catalyzes the oxidation of aromatic substrates to aryl free radicals. MnP is an extracellular enzyme which oxidizes a variety of phenolic substrates. WRF are involved in biodegradation not only of lignin, but also dyes, PAHs, chlorophenols, nitrotoluens, hydrocarbons, polychlorinated biphenyls and dioxins, and pesticides [1]. They can be used for delignification of wood and decolourization of pulp in the paper industry, for discolouring textile wastewater, for purification of distillery and brewery wastewater, as well as wastewater containing phenol derivatives and pesticides. WRF grow in media containing glucose, xylose, yeast extract, malt extract, starch and dextrins. Their increase in biomass requires basic salts of nitrogen and phosphorus. The optimum pH is 4–5 and the temperature 25–35˚C.

*Corresponding author.

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Thermotolerant ligninolitic fungi showing the activity of Lcc, lipase and protease were discovered in Mexico by Cruz Ramirez et al. [2].

Liang et al. [3] researched the effect of phosphorus concentration on growth and production of the enzyme MnP in *Phanerochaete chrysosporium*. They found that at 10 g/L of glucose, the optimal concentration of KH_2PO_4 was 2 g/L. With the presence in the medium of 20 g/L of glucose, 2 g/L of ammonium tartrate and 1 g/L of KH_2PO_4 they noticed some loss of colour of the synthetic dyes poly B-411, reactive black 5, reactive orange 16 and remazol brilliant blue R (RBBR) at the concentration of 100 mg/L depending on the enzyme production by the fungi. The highest decolourization activity was shown by *Collybia dryophila* and *Stropharia rugosoannulata* [4]. Studies on the induction of Lcc, MnP and LiP demonstrated that it generally occurs with lowering the content of nutrients, especially nitrogen. Leung and Pointing [5] found that high nitrogen concentration inhibited the production of ligninolitic enzymes. The authors proved that the presence of glucose and xylose as sources of carbon was followed by decolourization of the dye poly R 478 by 10 representatives of WRF. The production of Lcc by *Cerrena unicolor* occurred after exhaustion of the carbon and nitrogen sources (glucose and L-asparagine), whereas participation of nutrients in the production of biomass was necessary [6].

Some amino acids and vitamins have a stimulatory or inhibitory effect on the production of Lccs. Dhawan and Kuhad [7] found that DL-methionine, DL-tryptophan, glycine and DL-valine intensify the production of the enzyme by *Cyathus bulleri*, as well as biotin, riboflavin and pyridoxine. L-cysteine monohydrochloride inhibited the production of Lcc by the fungi.

The Production of Lccs is regulated by metal ions such as copper (II) and iron (III). Fonseca et al. [8] discovered that fungi *Ganoderma applanatum*, *Peniophora* sp., *Pycnoporus sanguineus*, *Coriolus versicolor* and f. antarctius were sensitive to stimulation with copper. Lee et al. [9] found that from a variety of inducers, such as guaiacol, $CuSO_4$, $MnSO_4$, $ZnSO_4$ and alcohols, it was copper which had an effective influence on the production of Lcc and colour removal from acid blue 350 by *Funalia trogii*. In the presence of high concentrations of metals, many WRF produce oxalate, which may neutralize their toxicity [10].

An interesting phenomenon is the increase in Lcc activity during the interaction between strains of white rot as well as between WRF and soil fungi. It was proved that the addition of *Trichoderma harzianum* to the culture of *Trametes versicolor* caused a 40-fold increase in the activity of Lcc, while introduction of other soil fungi or bacteria and soil or soil extract caused, respectively, a 2–25 and 10–15-fold increase in the enzyme activity [11].

The activity of extracellular enzymes Lcc, MnP and LiP is connected with generation of H_2O_2 and intermediate metabolites by oxidases. Free radicals, small molecules and ions initiate an "attack" on the lignin, moreover, the efficiency increases together with small distances between the cells of fungi and the lignin. Thus, it was found that fermentation of the so-called solid-state in the absence or small quantity of liquid increases the efficiency of the degradation process of the lignin as compared to the process which takes place during fermentation with water in which the substrates and microorganisms are located under the surface. This phenomenon was confirmed, among other things, in the research on the effect of ligninolitic enzymes on saccharification of wheat straw by fungi, including *Bjerkandera adusta*, *Fomes fomentarius*, *Ganoderma resinaceum*, *Irpex lacteus*, *P. chrysosporium* and *T. versicolor* [12].

2. The use of WRF for degradation of dyes and decolourization of textile wastewater

In 2001, Fu and Viraraghavan [13] published an extensive overview of data concerning decolourization of wastewater by WRF. They discussed the mechanisms for removing dyes, gave some examples of fungi decomposing colouring agents and demonstrated the possibilities and advantages of using dead fungal biomass for removal of colour from wastewater. The use of living cells of WRF requires proper operating conditions of the process, such as presence of nutrients and knowledge of pollutants and their concentrations as well as the toxicity of wastewater influent.

In the last decade, there appeared some studies showing a continued interest in the problems of biodegradation of dyes by WRF, including optimization of conditions in the discolouration of real wastewater. Balan and Monteiro [14] showed that indigo used in textile industries is decomposed within a few hours—4 d in 100% by *Phellinus gilvus*, in 94% by *Pleurotus sajor-caju*, in 91% by *P. sanguineus* and in 75% by *P. chrysosporium*, which predestines them to be used in wastewater treatment.

Discolouration of azo dye acid red 183 and antrachinone dye basic blue 22 by 115 strains of WRF was researched by Jarosz-Wilkołazka et al. [15].

Acid red 183 was more resistant to discolouration, both in solid and liquid culture. Among the isolated fungi, *Bjerkandera fumosa, Kuehneromyces mutabilis* and *Stropharia rugoso-annulata* in the static culture did not show the ability to decompose dyes, whereas in the agitated culture (180 rpm) they removed colour in 75–100% (basic blue 22) and 20–100% (acid red 183).

Two other antrachinone dyes RBBR and poly R-478 were researched in the area of their biodegradation by *B. adusta* in the liquid culture [16]. It was found that the discolouration process required the presence of an easily degradable source of carbon —glucose as a co-substrate in the process of cometabolism.

More and more research concerns the use of WRF for discolouration of industrial wastewater, including the real wastewater. Blanquez et al. [17] used *T. versicolor* for discolouration of 150 mg/L of dye grey lanaset G in the lab scale, and then on the pilot scale, in non-sterile conditions and on the pilot scale with the use of real wastewater. In the pilot scale which lasted for 3 months, the effect of discolouration was 78% on average, and in the tests with real wastewater—40–60%, which met the legal requirements for discharging of wastewater into municipal wastewater treatment plants. The process occurred in the presence of glucose as the co-substrate. *T. versicolor* and three other fungi WRF were used for discolouration of antrachinone dye reactive blue 4 and azo dye reactive red 2 in synthetic wastewater and real wastewater [18]. *T. versicolor* removed 200 mg/L of both dyes from synthetic wastewater in the presence of glucose (HRT—3 d). Discolouration of real wastewater occurred with the use of *Pleurotus flabellatus* (HRT—25 h). Anastasi et al. [19], examined 12 species of WRF in their use for degradation of 13 industrial dyes and for treatment of four model types of wastewater from textile and tanning industry. Among the fungi, it was *B. adusta* that was able to discolour and detoxicate most of the dyes in three types of wastewater. In further experiments with the use of *B. adusta*, it was found that it discoloured real textile wastewater with addition of glucose and yeast extract in the proportion 1:0.015 g/L [20]. The process was examined before and after ozonation of wastewater on the basis of chemical and toxicological determinants. The fungi caused slightly lower discolouration of wastewater than O_3, but the effect was still satisfactory. Wastewater treatment by the fungi resulted in detoxification of wastewater in the presence of one of three bioindicators—*Cucumis sativus*. They observed an increase in toxicity for *V. fischeri* i *Pseudokirchneriella subcapitata* after both types of treatment, which indicates the need for ecotoxicological examinations in the course of wastewater treatment.

3. Removal of pollutants from different types of wastewater with the use of WRF

Wastewater from an alcohol distillery is coloured and contains molasses, phenols (tannin and humic acids), melanoidins from Maillard reaction of sugars with proteins, caramel and furfural [21]. WRF such as *P. chrysosporium*, *C. versicolor* and *T. versicolor* require an easily degradable source of carbon for decolourization of the wastewater. Strong and Burgess [22] researched treatment of post-distillation wastewater from the production of wine with the use of fungi *Trametes pubescens* and enzyme—Lcc. They found a decrease in COD of 83%, in phenolic compounds of 87% and in colour of 88%. The fungal Lcc used for the treatment of wastewater caused a reduction of phenols of 61%, but the colour of the wastewater increased by 160%, indicating formation of components which give colour to wastewater.

Other fungi WRF such as *Panus tigrinus* [23] removed COD, colour and phenolic compounds from wastewater generated by pressing olive oil (initial COD was 43,000 mg/L). With higher COD in raw wastewater 85,000 mg/L or with the phenols content >1.9 g/L, some inhibition of Lcc and MnP production was observed. Lcc produced by fungi *Pycnoporus cinnabarinus* and *Coriolopsis rigida* reduced the content of phenols from olive-mill dry residues with the initial value of 26 g of phenols/dm by 73% after 20 d and phytotoxicity of tomatoes, whose biomass increased after application of olive-mill dry residue as fertilizer [24]. Ntougias et al. [25], from among 39 WRF, selected four fungi of *Ganoderma* and *Pleurotus* species for treatment of olive-mill wastewater. The fungi discoloured wastewater in 40–65% and decreased the content of phenols by 64–81%. A reduction in COD was in the low range of 12–29%. They found a 5–15-fold decrease in toxicity of wastewater expressed as inhibition of *Aliivibrio fischeri* luminescence. For degradation of refractive pesticides, such as aldicarb, atrazine and alachlor, they applied the activated-sludge process, in which bacterial biocoeonosis was enriched with WRF [26]. In the period of 14 d, they obtained the removal of 47% of aldicarb, 98% of atrazine and 62% of alachlor by means of biosorption and biodegradation with their initial concentration of 10 mg/L, addition of glucose 1 and 0.13 g/L of ammonium nitrate.

Bisphenol A is an endocrine compound used for production of polymers—polycarbonates and epoxy

resins. Three WRF *T. versicolor, Stereum hirsutum* and *Pleurotus ostreatus* grown on agar with dextrose were used for removing 4.6 mg/L of bisphenol A from water. The growth of fungi was stimulated by addition of leonardite humic acids and of compost humic acids. The result achieved was a significant degradation of bisphenol A [27].

4. Devices for treatment of wastewater with WRF

Devices used for treatment of textile wastewater with and without WRF that should be mentioned are: activated sludge, trickling filters, membrane reactors, column reactors and hybrid methods. In order to increase the efficiency of the process, immobilization of fungi is introduced [28].

The research on the treatment of wastewater containing dyes was carried out with the use of trickling filter microorganisms, which effectively removed COD reaching 36,000 mg/L in raw wastewater [29]. It was observed, however, that 30–60% of the total COD removal was due to the aeration of wastewater and the reduction of the remaining COD to biodegradation processes. The process was conducted in a continuous system and by means of SBR method which includes in a 24-h cycle the following phases: filling, reacting, settling and decanting. SBR method proved to be more efficient, and the authors suggested that method for initial treatment of wastewater.

In a reactor with a biofilm composed of bacteria and fungi in the ratio 1:51.8–1:6.8 in the presence of 0.5 g/L of glucose and 0.1 g/L of $(NH_4)_2SO_4$, the following biodegradation of dyes occurred: 50–75% of reactive black 5, 35–56% of reactive red M—3BE and 82–90% of acid red 249 with their initial concentration of 30 mg/L and a significant decrease in colour and COD [30].

Data included in the literary sources show that WRF exhibit an ability to decompose a variety of refractive dyes to a greater extent than activated sludge consisting of bacteria or mould fungi and bacteria. Novotny et al. [31] applied a two-stage treatment of textile wastewater: a trickling filter with WRF (*I. lacteus*) immobilized on polyurethane foam followed by bacterial reactors—this resulted in a decrease in colour up to 99% and in TOC up to 97%. Currently, the use of membrane reactors for treatment of wastewater containing refractory components is more and more frequent. Fungi *C. versicolor* in the presence of starch and urea removed 100 mg/L of azo dye acid orange II in a membrane reactor at HRT 1 d [32]. Similarly, *T. versicolor* degraded dyes (reactive blue 19, reactive blue 49 and reactive black 5) in culture with glucose (5 g/L) and ammonium tartrate (0.22 g/L) with the use of a membrane bioreactor and aid of nanofiltration and reverse osmosis [33].

Pakshirajan and Kheria [34] used a rotating biological contactor reactor for treatment of colour industrial wastewater. Fungi *P. chrysosporium* were immobilized on polyurethane foam and polystyrene grid. In the presence of 5 g/L of glucose, decolourization of textile wastewater amounted to 80%. COD of raw wastewater was 5,380 mg/L and its decrease in the value of this ratio occurred similarly as with colour.

Immobilization of microorganisms and the role of this process in decolourization of wastewater were studied by Qiao et al. [35]. The authors immobilized WRF by polyvinyl alcohol—sodium alginate, and on bacterial cellulose in the conditions of dynamic and static adsorption. For production of the cellulose, they used the strain *Acetobacter xylinum* on the medium with glucose, peptone, yeast extract, citric acid and ethanol. The adsorption of fungi in static conditions consisted in mixing *A. xylinum* and WRF in the medium and 30˚C culture for 5–7 d. The fungi are attached to newborn bacterial cellulose membrane. In dynamic conditions the fungi were added to the medium after producing cellulose membrane blocks with the size of 1 cm^2 and they were agitated in 30˚C at 150 rpm for 5 d. It was found that the fungi immobilized in the static conditions removed malachite green from wastewater most efficiently, in more than 93%.

Tannery dyes are difficult to remove from wastewater [36]. It was found, however, that black Dycem—a commercial dye, was degraded in 86–89% by fungi *T. versicolor* in an air-pulsed bioreactor with the air pulse frequency of 0.16 s^{-1}. The medium with the dye 150 mg/L was supplemented, among other things, with 7 g/L of glucose and 2.1 g/L of NH_4Cl. The fungi were used in the form of spherical pellets. The method of production of *T. versicolor* pellets from micellar suspension on the substrate with 2% malt extract, pH—4.5 in the period of 5 d of agitating the culture (135 rpm) in 25˚C was given by Borras et al. [37]. Olive washing wastewater was also susceptible to biodegradation by fungi *T. versicolor* [38]. Mycelial pellets were used in a bubble column bioreactor. The effect was a 65% decrease in colour, 73% decrease in COD and an 89% decrease in phenols. The authors concluded that the use of a bioreactor with capacity of 10 m^3 will enable treatment of 2 m^3/d of wastewater produced in olive pressing plants.

5. Concluding remarks

WRF are widely spread in the environment. They produce enzymes catalyzing degradation of many organic compounds resistant to biodegradation, certain types develop in nutrient deficiency, and they can also use waste materials as sources of carbon.

The use of WRF for treatment of real wastewater requires a lot of research, and above all, changing from lab scale to technical scale. There has been a shortage of studies conducted in pilot devices, in which parameters of biological processes are optimized. The results of experiments with the use of fungi for decomposition of toxic, refractive ingredients of industrial wastewater are so promising that it is worth taking some steps in order to introduce biological methods to remove these compounds from wastewater in technical conditions. Applied physical and chemical methods, such as flocculation, electroflotation, filtration, coagulation, adsorption and ozonation, are expensive and often not fully effective. Biological methods require selection of fungi and culture conditions, defining parameters of the biotechnological process with respect to a suitably chosen bioreactor and often dilution of raw wastewater due to the substantial load of organic pollutants and their harmfulness. Differences in production technologies prevent finding a unified approach that could solve the problems of wastewater disposal of various industrial plants. It seems, however, that from an economic point of view, WRF will be used in the future to eliminate pollutants from wastewater.

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