© Triveni Enterprises, Lucknow (India)
ISSN : 0254-8704
http : //www.geocities.com/j_environ_biol/

J. Environ. Biol.
29(6), 831-836 (2008)
j_environ_biol@yahoo.com

Decolorisation of synthetic dyes and textile wastewater using *Polyporus rubidus*

Poonam Dayaram and Debjani Dasgupta*

Department of Biochemistry, The Institute of Science, Mumbai - 400 032, India

(Received: July 05, 2006; Revised received: April 15, 2007; Re-revised received: May 06, 2007; Accepted: May 26, 2007)

Abstract: *Effluent from textile industries were treated with enzyme from white rot fungi isolated from outskirts of Mumbai and identified as Polyporus rubidus in our laboratory. Decolorisation of 4 Reactive dyes commonly found in the effluents such as Reactive bue, Reactive orange, Ramazol black and Congo red was examined by treatment with enzyme from Polyporus rubidus. Treatment of effluent was done in a laboratory scale bioreactor constructed with laccase immobilized Na-alginate beads. Greater than 80% of dyes were degraded within 5 days under stationary incubation conditions. The enzyme had a maxmimum activity of 17.1U after 3 days and was found to be secreted extracellularly by Polyporus rubidus. In this study the Polyporus rubidus has been reported for the first time to have laccase activity offering a promising possibility to develop an easy and cost effective method for degradation of dangerous dyes.*

Key words: *Biodegradation, Decolorisation, White rot fungus, Textile dye, Bioreactor*
PDF of full length paper is available with author ***(*drdebjanidasgupta@yahoo.com)***

Introduction

Wastewater from textile industries pose a threat to the environment as large amount of chemically different dyes are used for various industrial applications such as textile dyeing and a significant proportion of these dyes enter the environment *via* wastewater. The presence of even very low concentrations of dyes in effluent is highly visible and degradation products of these textile dyes are often carcinogenic (Kim *et al.*, 2003). Further, the adsorption of light by these textile dyes creates problems for photosynthetic aquatic plants and algae (Singh and Singh, 2006). Earlier studies have shown that many reactive dyes are not degraded in ordinary aerobic sewage treatment processes and that they can be discharged unaffected from the treatment plant (Carliel *et al*., 1996; Panswad and Luangdilok, 2000). These reactive dyes are highly water-soluble polyaromatic molecules, which means they do not adsorb to solids and are prevalent in high concentration in the effluents (Ganesh *et al*., 1994).

Currently, textile effluents are treated by physico-chemical methods that are often quite expensive. In addition these methods do not generally degrade the pollutant, thereby causing an accumulation of the dye as sludge creating a disposal problem. Currently available technologies have been reviewed by Robinson (Robinson *et al.*, 2001), and therefore special attention is given to biological processes because they are cost effective and environmentally friendly. Removing dyes in aerobic conditions is mainly achieved by adsorbing the dyes on bacteria, rather than oxidation. Some anaerobic textile wastewater treatment methods have been developed at a laboratory scale and have shown to remove color efficiently (Kapdan *et al.,* 2000) but the anaerobic treatment of some azo dyes may result in the formation of toxic aromatic amines. Further more, there may be a risk of reverse colorization when anaerobic degradation products are exposed to oxygen (Brown and De Vito, 1993; Knapp and Newby, 1995).

While studying degradation of organic molecules, textile dyes may be regarded as a poorly chosen example, due to their resistance to external factors. Many dyes are also resistant to UV light and therefore constitute a challenging group of chemicals while designing degradation processes. A key feature in the degradation processes, at least in the aerobic ones, involves generation of activated oxygen forms that can carryout the initial attack on the stable structure of the dye (Hu, 2001; Wong and Yuen, 1996).

A great number of white rot fungi have been shown to excrete extra cellular enzymes like lignin peroxidase, mn-peroxidase and laccase (Hatakka, 1994). These enzymes catalyse the formation of activated oxygen so that the process of attack on the stable structure of dyes can be initiated. The lack of selectivity of these enzymes towards the aromatic compounds attacked is the basis for using white rot fungi for treating textile dyes.

Many white rot fungi *e.g. Phanerachaete chrysosporium, Ganoderma* sp WR.1, *Trametes trogii, Irpex lacteus, Dichomitus squalens, Pycnoporus strains etc.* have been intensively studied in connection with their ligninolytic enzyme production and their decolorisation ability (Borchert and Libra, 2001; Revankar and Lele, 2007; Mechichi *et al*., 2006; Maxima and Costa-Ferreira, 2004; Susla *et al*., 2007; Singh *et al.,* 2007; Demir *et al.,* 2007). White rot fungi like *Phanerachaete chrysosporium* has also been reported to treate Vinasses a highly hazardous environmental pollutant (Potentini and Rodriguez-Malaver, 2006).

In this study, the potential of white rot fungi, *Polyporus rubidus* to produce laccase enzyme and its capacity to decolorize higher concentration of several dyes such as Reactive blue, Reactive orange, Ramazol black and Congo red along with effluents from textile units has been investigated.

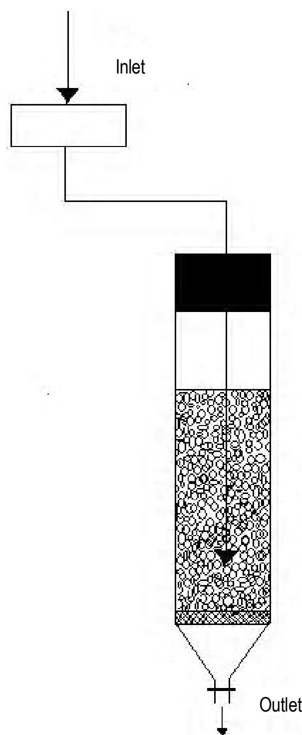

Fig. 1: The design of the laboratory bioreactor (60x3 cm) filled with laccase immobilized sodium alginate beads

Materials and Methods

Micro-organism: The fungus used in this study was isolated from the suburbs of Mumbai, India. It was taxonomically identified as *Polyporus rubidus* (Bakshi, 1971).

Chemicals: Synthetic dyes were obtained from Raymonds Textile company and other chemicals used in fungal cultures, enzyme assays including 2, 6, dimethoxyphenol were purchased from Sigma.

Storage of organism: The fungus was stored on Potato Dextrose agar plate. New plates were inoculated once a month to ensure viability of the isolates.

Production of enzymes: The medium for cultivation of *Polyporus rubidus* contained 4.5% (wt/vol) wheat bran, 1.5% yeast extract, 1% glucose, 0.25% NH_4Cl, 0.05% thiamine dichloride, 0.2% KH_2PO_4, $MgSO_4.7H_2O$, 0.01% $CaCl_2$ and 0.05%KCl. Tap water was used for preparation of the medium, and the pH was adjusted to 5.5 by using NaOH or HCl. Incubation was carried out at 28 $\pm$2°C on a rotary shaker (150 rpm) in cotton – plugged 250 ml Erlenmeyer flask containing 100 ml of media. Flasks were inoculated with 1cm square agar pieces from an actively growing fungus on potato dextrose agar (PDA). Cultures were harvested after 7 days, filtered and clarified by centrifugation at 8,000 x g for 20 min to remove the mycelia, and the enzyme activity assayed for further purification.

Enzyme assay: Laccase activity was determined using 2, 6, dimethoxyphenol (DMP) as a substrate as described before. The reaction mixture contained1 mM DMP in 50 mM sodium malonate (pH 4.5). The formation of 2, 2' 6, 6' dimethoxyphenoquinone (orange /brown color) at 30°C was followed spectrophotometrically at 468 nm wavelength, and laccase activity was calculated from the molar extinction co-efficient (E) of 49.6 mM cm^{-1} (de Jong *et al.,* 1992).

Microbial treatment of textile dyes and dyeing effluents: *Polyporus rubidus* was cultivated as described earlier and the mycelium was collected by filtration under aseptic conditions and washed thrice with 500 ml of sterilized D/W. A sample of 1 g (wet weight) of mycelium was incubated for 8 days with different dyes such as Reactive blue, Ramazol black, Reactive orange and Congo red with concentration ranging from 100 mgl^{-1} to 1000 mgl^{-1}. Sterile controls without inoculum were also maintained under the same conditions. Growth of the fungus was inhibited with antibiotics to determine whether the decolorisation was due to metabolic activity of the organism or due to other phenomena. After 8 days, all incubation mixtures were filtered using 0.45 μm pore size filter paper. The decolorization efficiency was determined spectrophotometrically at the absorption maximum of each dye. Adsorption of dyes to the mycelium was determined by solubilization of the dyes with water. Adsorbed dye was washed off the mycelium twice with 20 ml of water.

Enzymatic treatment of textile dyes in lab bioreactor: Eight cylindrical reactors with conical bottoms were used for continuous experiments with laccase from *Polyporus rubidus* (Fig. 1). Four of the reactors were used to study decolorization of Reactive blue, Ramazol black, Reactive orange and Congo red, while the other four were used for studying decolorisation of dyes in real textile wastewater from 4 textile units in the state of Maharashtra, India. The reactors had a liquid volume of 1 liter. They were autoclaved and then filled with laccase immobilized sodium alginate beads (Park *et al*., 2006). Bioreactors were kept at Room temperature (28$\pm$2°C) and dye solutions at different concentration were added to these bioreactors. Aliquots were collected after 24 hr and percentage color remaining was determined spectrophotometrically for the subsequent period of 5 days. The pH of the textile wastewater was set between 4-7, the optimal pH range for the fungal enzymes. Untreated effluents were also subjected to chemical analysis before pouring in bioreactor. The experiments were continued for 5 days. All reactor experiments were performed at room temperature (28 $\pm$2°C).

Results and Discussion

The ability of white rot fungi to decolorize various synthetic textile dyes has been extensively studied (Panswad and Luangdilok,2000; Libra *et al*., 2003; Binz and Canevascini,1996; Ramsay *et al*., 2005). During the past decade, white rot fungi basidiomycetes were studied in relation to their ability to degrade recalcitrant organo-pollutants (Field *et al*., 1993; Vyas *et al*., 1994; Demir, 2004). These studies were done in parallel with those on a complex enzymic system that allows these fungi to degrade lignin (Barr and Aust, 1994; Costa *et al*., 1994). In particular, the relatively non-specific nature of the lignolytic phenoloxidases makes these

Table - 1: Determination of the various parameters of the untreated effluent samples. Analysis was done as per standard procedures

Effluent samples	COD	DO	pH	Chlorides	TSS	TDS
1	600	7.12	10.43	95.14	638	2806
2	360	5.30	11	79.53	1260	6930
3	544	7.23	7.23	90.88	726	8684
4	108	7.12	7.12	32.66	58	1190

All units are in mgl^{-1} except pH
COD = Chemical oxygen demand, DO = Dissolved oxygen,
TSS = Total suspended solids, TDS = Total Dissolved Solids

fungi useful for various potential applications (Eriksson *et al*., 1990; Duarte and Costa –Ferreira, 1994) *Phanerachaete chrysosporium*, a much-studied white rot fungus, is known to be a good degrader of azo dyes (Paszczynski *et al*., 1991). Among the other fungi studied are *Trametes versicolor, Pleurotus* sp, *Bjerkandera* sp, *Ischnoderma resinosum, Irpex lactus* and *Pycnoporus cinnabarinus* which have also been successfully shown to decolorise commercial dyes such as Reactive black 5, Reactive blue 19, Reactive yellow *etc* (Roy-Arcand and Archibald,1991; Shin *et al*., 1997; Swamy and Ramsay,1999; Eichlerova *et al*., 2005; Maxima and Costa-Ferreira, 2004). Although in many of these studies model monoazo compounds were used, a wealth of information is presently available that inidicates the versatility of fungi. However, such an approach involving whole fungal cultures suffers from two major technical drawbacks. Firstly, the process is slow, typically requiring several days and secondly, the sludge volume increases due to the generation of biomass.

Enzymes represent an attractive option for dye bio-transformation for numerous reasons (Karan and Nicell, 1997). This strategy has been used in the treatment of colorants in wastewater from pulp and paper mills (Hakulinen, 1988; Bajpai and Bajpai,1994), which paved the way for applications for textile mill effluents, encouraging the search for new promising white rot fungi.

Among numerous white rot fungi screened earlier for degradation of dyes, the potential of *Polyporus rubidus* to decolorize textile dyes has not been studied so far. The aim of this study therefore was to investigate the novel ability of this rarely studied white rot fungi *Polyporus rubidus* to decolorize industrially important synthetic dyes. *Polyporus rubidus* was capable of decolorizing four commercially used dyes and untreated textile effluents collected from four textile

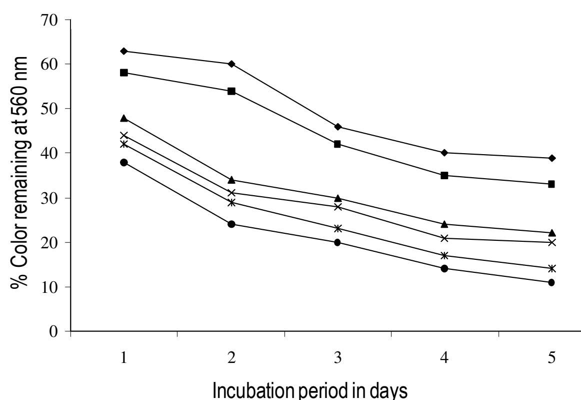

Fig. 2: Decolorisation of reactive blue by enzyme in bioreactor

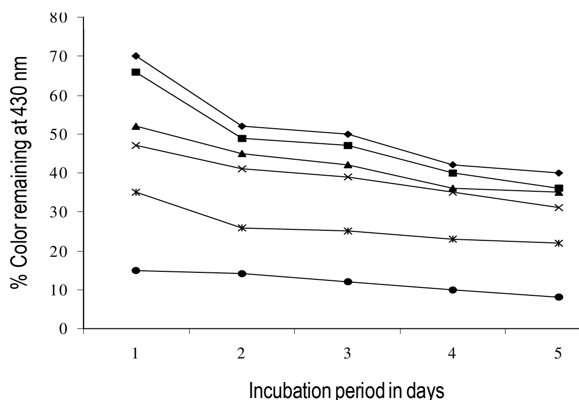

Fig. 3: Decolorisation of reactive orange by enzyme in bioreactor

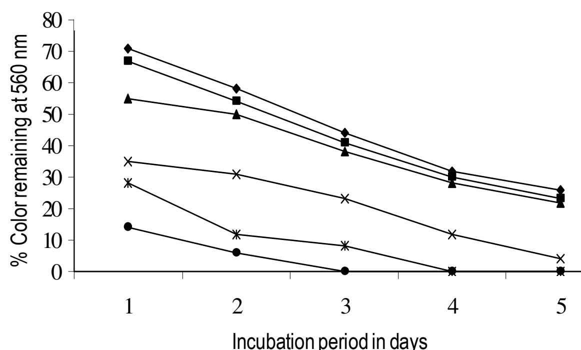

Fig. 4: Decolorisation of ramazol black by enzyme in bioreactor

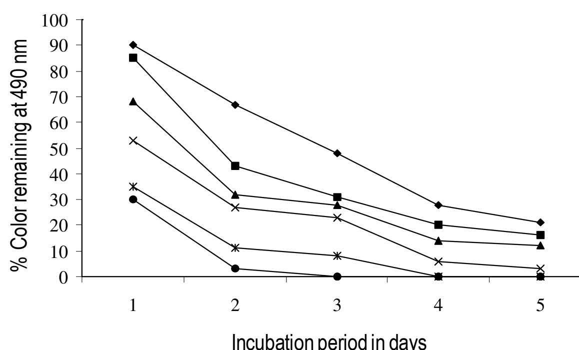

Fig. 5: Decolorisation of congo red by enzyme in bioreactor

% Colour remaining at 1000 mgl^{-1} conc. % Colour remaining at 600 mgl^{-1} conc. % Colour remaining at 200 mgl^{-1} conc.
% Colour remaining at 800 mgl^{-1} conc. % Colour remaining at 400 mgl^{-1} conc. % Colour remaining at 100 mgl^{-1} conc.

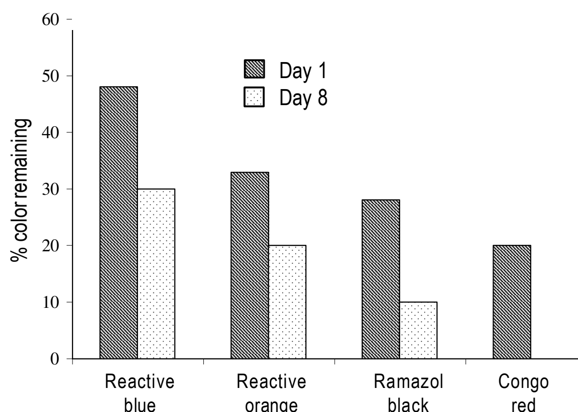

Fig. 6: Mycelial degradation of reactive blue, reactive orange, ramazol black and congo red at concentration of 100 mgl^{-1}

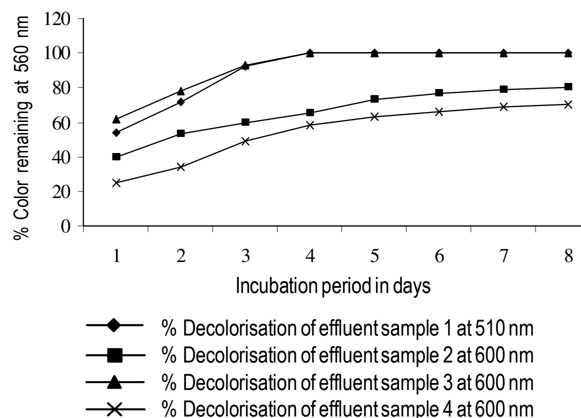

Fig. 7: Decolorisation of effluent samples by enzyme in bioreactor

units near Mumbai, in the state of Maharashtra, India. Synthetic dyes such as Reactive blue, Reactive orange, Ramazol black and Congo red were found to be decolorized by an extra cellular enzyme and also mycelium of *Polyporus rubidus*.

During our investigation it was observed that laccase appeared to constitute the major phenoloxidase activity in the culture fluid, when *Polyporus rubidus* was grown in specially designed wheat bran broth in our laboratory. Growth and enzyme production in shake flasks were superior to those found in standing cultures, and shaking conditions were therefore chosen for routine cultivation. Laccase activity in the culture fluid was detectable after 48 hr and reached a maximum of 17.1U/ml when assayed with 2,6 Dimethoxyphenol. Partially purified extracellular laccase enzyme was immobilized in bio-reactor as explained earlier.

Standard synthetic dyes such as Reactive blue with varying concentration from 1000 mgl^{-1} to 100 mgl^{-1} were introduced in the bio-reactor and the percentage color remaining monitored for five days as shown in (Fig. 2). It was observed that as the period of incubation increased the percentage color remaining decreased from an initial 100% color on the day of inoculation to 63% color on day one, 46% color on day three, 39% color on day five for 1000 mgl^{-1} concentration of dye. It was also found that for 400 mgl^{-1} concentration of dye 20% color remained after five days of incubation. However, the rate of decolorisation was significantly enhanced when the initial concentration of the dye taken was reduced. In the case of 100 mgl^{-1} concentration of Reactive blue 90% decolorisation of the dye was achieved after five days of incubation. This indicated the high efficiency of enzyme in the bioreactor. Mycelium was found to be less efficient in decolorizing the dye as compared to immobilized enzyme (Fig. 6). Vanhullel *et al.* (2007) have observed that Reactive blue 19 causes an increase in laccase activity in Pycnoporus strain 7 culture and that laccase catalyses the oxidation of Reactive blue 19 dye. A full decolorisation of the dye was not achieved with the enzyme alone, but required other factors present in the whole cell.

The percentage color remaining after five days was also monitored for Reactive orange at different concentrations as shown in Fig. 3. For 1000 mgl^{-1} of Reactive orange, 40% color remained after five days incubation, as was also seen in the case of Reactive blue. However, 92% decolorisation was obtained for 100 mgl^{-1} of this dye. Baldman and Snajdr (2006), have shown that strain X3 isolated from soil could decolorize Reactive orange 16, only after 28 days of incubation, whereas only 15-21% decolorisation was obtained with *C. dryophila, S.rugosoannulate* and *T. versicolor.*

Similarly two other dyes namely Ramazol black and Congo red were introduced at different concentrations in the bioreactor and percentage color remaining was monitored for five days as shown in Fig. (4, 5). Ramazol black at 1000 mgl^{-1} showed 26% color remaining on day 5 of incubation, whereas for 100 mgl^{-1} complete decolorisation of both the dyes was observed within 3 days of incubation. The commercial dyes were also subjected to decolorisation by mycelia as mentioned in the methods. Mycelia were found to be far less efficient than immobilized enzyme in bioreactor in the decolorisation process (Fig. 6). Murugesan *et al.* (2007), found that crude laccase 25U/ml decolorized Ramazol black-5 (50 mgl^{-1}) by 62%. However, our studies have shown that Ramazol black at 100 mgl^{-1} was completely decolorized within 3 days and our process also did not require any mediator. A recent work by Boer *et al.* (2004), have shown complete decolorisation of Congo red after 18 days of incubation by using solid state culture.

Further studies were performed under similar experimental conditions with 4 untreated effluent samples having various parameters as shown in Table (1). The effluents samples were neutralized before introducing in the bioreactor and percentage decolorisation was monitored continuously over a period of 8 days as shown in Fig. 7. It was observed that effluent sample 1 and 3 showed 100% decolorisation within four days where as 25% color remained on treatment with mycelia. Sample 2 and 4 showed 20% and 30% color remaining after five days of incubation. 72% and 60% decolorisation was observed on treatment with mycelia. The immobilized enzyme in bioreactor is far more efficient then mycelia in the decolorisation process. The enzyme was able to decolorize Congo red and Ramazol black most efficiently followed by Reactive blue and Reactive orange dyes.

Earlier studies with white rot fungi, *Trametes hirsuta, Pleurotus ostreatus,* and *Ischnoderma resinosum,* have already shown the use of redox mediator such as violuric acid and hydroxybenzotriazole (HBT) to decolorise recalcitrant dyes such as C.I Acid red 97, Acid green 26, Reactive black 5, Reactive red 22 and Reactive yellow 15 (Couto and Sanroman, 2007; Eichlerova *et al*., 2005).

From our study it was observed that ready availability of laccase from *Polyporus rubidus* and its robust characteristics helps to overcome the barriers for wastewater treatment. Our results suggested that laccase from *Polyporus rubidus* could decolorise synthetic dyes in a broad range of concentration without the need for redox mediators, provided that these dyes are water-soluble. It was also observed that immobilized enzymes tend to have higher activity and are more resilient to environmental perturbations such as pH or exposure to toxic chemical concentrations than suspension cultures. The enzyme from *Polyporus rubidus* was also found to be effective in removing color from untreated effluents. Enzymes from white rot fungi, *Phlebia tremellos* a took 14 days (Kirby *et al*., 2000), *Trametes versicolor* took 6 days, *B. adusta* took 9 days for decolorisation of textile effluents (Robinson *et al*., 2001). This study showed white rot fungi, *Polyporus rubidus* is far more efficient for decolorizing a range of industrially important textile dyes and real wastewater from such industries.

Further studies are in progress for the characterization and purification of laccase enzyme from this isolate. Future work with this isolate would concentrate upon finding the breadth of pollutants that can be further exploited to develop a cost effective bioremediation process.

Acknowledgments

The authors acknowledge DST, New Delhi, for the infrastructural facilities made available under its FIST-Programme.

References

Bajpai, P. and P.K. Bajpai: Biological color removal of pulp and paper mill wastewaters. *J. Biotechnol*., **33**, 211-220 (1994).

Bakshi, B.K.: Indian Polyporaceae. Indian Council of Agricultural Research Publication, New Delhi (1971).

Baldrain, P. and J. Snajdr: Production of ligninolytic enzymes by litter – Decomposing fungi and their ability to decolorize synthetic dyes. *Enzymes Microbial Technol*., **39**, 1023-1029 (2006).

Barr, D.P. and S.D. Aust: Mechanisms white rot fungi use to degrade pollutants. *Environ. Sci. Technol*., **28**, 78-87 (1994).

Binz, T. and G. Canevascini: Differential production of extracellular laccase by the Dutch elm disease pathogens *Ophiostoma ulmi* and *O. novoulmi*. *Mycol. Res.,* **100**, 1060-1064 (1996).

Boer, C.G., L. Obici, C.G.M. de Souza and R.M. Peralta: Decolorisation of Synthetic dyes by solid-state cultures of *Lentinula* (*Lentinus*) *edodes* producing manganese peroxidase as the main ligninolytic enzyme. *Bioresource Technol*., **94**, 10 7-112 (2004).

Borchert, M. and J.A. Libra: Decolorization of reactive dyes by the white rot fungus,*Trametes versicolor* in sequencing batch reactors. *Biotech. Bioeng*., **75**, 313-321 (2001).

Brown, M.A. and S.C. De Vitro: Predicting azo dye toxicity. *Crit. Rev. Environ. Sci. Technol*., **23**, 249-324 (1993).

Carliell, C.M., S.J. Barclay and C.A. Buckley: Treatment of exhausted reactive dye bath effluent using anaerobic digestion: Laboratory and full scale trials. *Water SA*., **22**, 225-233 (1996).

Costa-Ferreira, M., P. Ander and J. Duarte: On the relationship between cellobiose dehydrogenase and cellobiose:quinone oxidoreductase under conditions where [^{14}C] DHP is mineralized by whole cultures of *Phanerochaete chrysosporium*. *Enzyme Microb. Technol*., **16**, 771-776 (1994).

Couto, S.R. and M.A. Sanroman: The effect of violuric acid on the decolorization of recalcitrant dyes by laccase from *Trametes hirsuta*. *Dyes and Pigments*, **74**, 123-126 (2007).

de Jong, E., F.P. de Vries, J.A. Field, R.P. Vander Zwan and J.A.M. de Bont: Isolation and screening of basidiomycetes with high peroxidative activity. *Mycol. Res.,* **96**, 1098-1104 (1992).

Demir, G.: Degradation of toluene and benzene by *Trametes versicolor. J. Environ. Biol*., **25**, 19-25 (2004).

Demir, Goksel, H. Kurtulus Ozcan, Nese Tufekci and Mehmet Borat: Decolorization of Remazol Yellow RR Gran by white rot fungus *Phanerochaete chrysosporium*. *J. Environ. Biol*., **28**, 741-745 (2007).

Duarte, J. and M. Costa-Ferreira: Aspergilli and lignocellulosics: Enzymology and biotechnological applications. *FEMS Microbiol. Rev*., **13**, 377-386 (1994).

Eichlerova, I., L.Homolka, L. Lisa and F. Nerud: Orange G. and remazol brilliant blue R decolorisation by white rot fungi *Dichomitus squalens, Ischnoderma resinosum* and *Pleurotus calyptratus*. *Chemosphere*, **60**, 398-404 (2005).

Eriksson, K.E., R.A. Blanchette and P. Ander: Microbial and enzymatic degradation of Wood and Wood Components. Berlin: Springer-Verlag. p. 416 (1990).

Field, J.A., E. de Jong, G. Feijoo-Costa and J.A.M. de Bont: Screening for ligninolytic fungi application to the biodegradation of xenobiotics. *Trends Biotechnol*., **11**, 44-49 (1993).

Ganesh, R., G.D. Boardman and W.C. Tincher: Fate of azo dyes in sluges. *Water Res.,* **28**, 1367-1376 (1994).

Hakulinen, R.: The use of enzymes for wastewater treatment in the pulp and paper industry – A new possibility. *Water Sci. Technol*., **20**, 251-262 (1988).

Hatakka, A.: Lignin modifying enzymes from selected white rot fungi : Production and role in lignin degradation. *FEMS Microbiol. Rev*., **13**, 125-135 (1994).

Hu, T.L.: Kinetics of azo reductase and assessment of toxicity of metabolic products from azo dye by *Pseudomonas luteola*. *Water Sci. Technol*., **43**, 261-269 (2001).

Kapdan, I.K., F. Kargi, G. McMullan and R. Marchant: Effect of environmental conditions on biological decolorization of textile dyestuff by *C. versicolor*. *Enzyme Microb. Technol*., **26**, 381-387 (2000).

Karan, J. and J.A. Nicell: Potential applications of enzyme in wastewater treatment. *J. Chem. Tech. Biotechnol*., **69**, 141-153 (1997).

Kirby, N., R. Marchant and G. McMullan: Decolorisation of synthetic textile dyes by *Phlebia tremellosa. FEMS Microbiol. Letters*, **188**, 93-96 (2000).

Kim, S., C. Park, T.H. Kim, J. Lee and S.W. Kim: COD reduction and decolorisation of textile effluents using a combined process. *J. Biosci. Bioeng*., **95**, 102-105 (2003).

Knapp, J.S. and P.S. Newby: The microbiological decolorization of an industrial effluent containing a diazo-linked chromophore. *Water Res*., **29**, 1807-1809 (1995).

Libra, J.A., M. Borchert and S. Banit: Competition strategies for the decolorisation of a textile reactive dye with the white rot fungi *Trametes versicolor* under non-sterile conditions. *Biotech. Bioeng*., **82**, 736-744 (2003).

Maxima, C. and M. Costa Ferreira: Decolourisation of reactive textile dyes by *Irpex lacteus* and lignin modifying enzymes. *Proc. Biochem*., **39**, 1475-1479 (2004).

Mechichi, T., N. Mhiri and S.Sayadi: Remazol brillant blue R decolorisation by the laccase from *Trametes trogii. Chemosphere*, **64**, 998-1005 (2006).

Murugesan, K., I.H. Nam, Y.M. Kim and Y.S. Chang: Decolorisation of reactive dyes by a thermostable laccase produced by *Ganoderma*

lucidum in solid state culture. *Enzyme Microbial Technol.,* **40**, 1662-1672 (2007).

Park, C., B. Lee, E.J. Han, J. Lee and S. Kim: Decolorization of acid black 52 by fungal immobilization. *Enzyme Microbial Technol.*, **39**, 371-374 (2006).

Panswad, T. and W. Luangdilok: Decolorisation of reactive dyes with different molecular structures under different environmental conditions. *Water Res.*, **34**, 4177-4184 (2000).

Potentini, M.F. and A.J. Rodriguez-Malaver: Vinasse biodegradation by *Phanerochaete chrysosporium*. *J. Environ. Biol.*, **27**, 661-665 (2006).

Paszczynski, A., M.B. Pasti, S. Goszczynski, D.L. Crawford and R.L.S. Crawfard: New approach to improve degradation of recalcitrant azo dyes by Streptomyces sp and *Phanerochaete chrysosporium*. *Enzyme Microb. Technol.*, **13**, 378-384 (1991).

Ramsay, J.A., W.H.W. Mok and Y.S. Luv: Decolorisation of textile dyes by alginate-immobilized *Trametes versicolor. Chemosphere.*, **61**, 956-964 (2005).

Revankar, M.S. and S.S. Lele: Synthetic dye decolorization by white rot fungus, *Ganoderma* sp *WR-1. Bioresource Technol.*, **98**, 775-780 (2007).

Robinson, T., B. Chandran and P. Nigam: Studies on the production of enzymes by white rot fungi for the decolorisation of textile dyes. *Enzyme Microbiol. Technol.*, **29**, 575-579 (2001).

Robinson, T., G. McMullan, R. Marchant and P. Nigam: Remediation of textile dyes in textile effluent: A critical review on current treatment technologies with a proposed alternative. *Bioresource, Technol.*, **77**, 247-255 (2001).

Roy-Arcand, L. and F.S. Archibald: Direct dechlorination of Chlorophenolic compounds by laccase from *Trametes* (Coriolus) *versicolor*. *Enzyme Microb. Technol.*, **3**, 194-203 (1991).

Shin, K.S., I.K. Oh and C.J. Kim: Production and purification of Remazol Brilliant Blue R decolorizing peroxidase from the culture filtrate of *Pleurotus*. *Appl. Environ. Microbiol.,* **63**, 1744-1748 (1997).

Singh, K.D. S. Sharma, A. Dwivedi, P. Pandey, R.L. Thakur and V. Kumar: Microbial decolorization and bioremediation of melanoidin containing molasses spent wash. *J. Environ. Biol.,* **28**, 675-677 (2007).

Singh, V.K. and J. Singh: Toxicity of industrial wastewater to the aquatic plant *Lemna minor* L. *J. Environ. Biol.*, **27**, 385-390 (2006).

Susla, M., C. Novotny and K. Svobodova: The implication of *Dichomitus squalens* laccase isoenzyme in dye decolorisation by immobilized fungal cultures. *Bioresource Technol.*, **98**, 2109-2115 (2007).

Swamy, J. and J.A. Ramsay: The evaluation of white rot fungi in the decolorisation of textile dyes. *Enzyme Microb. Technol.*, **24**, 130-137 (1999).

Vyas, B.R.M., S. Bakowski, V. Sasek and M. Matucha: Degradation of anthracene by selected white rot fungi. *FEMS Microbiol. Ecol.*, **14**, 65-70 (1994).

Vanhulle, S., E. Enaud, M. Trovaslet, N. Nouaimeh, C.M. Bols, T. Keshavarz, T. Tron, G. Sannia and A.M. Corbisier: Overlap of laccases /cellobiose dehydrogenase activities during the decolourisation of anthraquinonic dyes with close chemical structures by *Pycnoporus strains*. *Enzyme Microbial Technol.*, **40**, 1723-1731 (2007).

Wong, P.K. and P.V. Yuen: Decolorisation and biodegradation of methyl red by *Klebsiella pneumoniae RS-13. Water Res.*, **30**, 1736-1744 (1996).