

Production and Purification of Laccase from *Stereum ostrea* and its Ability to Decolorize Textile Dyes

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ABSTRACT

Because of its broader specificity to oxidise both phenolic and nonphenolic lignin-related compounds as well as highly recalcitrant pollutants, laccase was optimally produced on medium with 0.02% guaiacol by growth of *Stereum ostrea* for 4 days in submerged culture. Inclusion of guaiacol in the medium enhanced laccase production by 7-fold. Extracellular laccase was purified up to 70-fold from the culture filtrate by a two-step protocol – ammonium sulphate (80% w/v) and Sephadex G-100 column chromatography. The purified enzyme, which was characterized for its kinetic properties, had an apparent molecular mass of 66 kDa. The optimal pH and temperature were found to be 6.0 and 40°C, respectively. The K_m and V_{max} values for the substrate guaiacol were found to be 13.25 mM and 255 η kat/mg of protein, respectively. The effect of inhibitory compounds, EDTA, SDS and arginine on laccase activity was determined. With SDS, the percentage inhibition increased as the concentration of SDS decreased from 5 to 0.5%. The purified enzyme decolorized textile dyes up to 90%. The decolorization ability of *S. ostrea* laccase suggests that this enzyme could be used for decolorization of industrial textile dyes.

Keywords: decolorization, guaiacol, lignolytic enzyme, oxidoreductases, textile effluents

Abbreviations: ABTS, 2,2'-azino-bis-(3-ethylbenzthiazoline-6-sulfonate); APS, Ammonium persulphate; EDTA, Ethylene diamine tetra acetate; SDS, Sodium dodecyl sulphate; TEMED, Tetra methyl ethylene diamine

INTRODUCTION

Exploration for efficient and green oxidation technologies has increased interest in the use of enzymes to replace conventional non-biological methods. Among the different oxidant enzymes, laccases have been the subject of study due to their importance in environmental protection, where enzymatic catalysis could serve as a more environmentally benign alternative than the currently used chemical processes (Baldrian 2006; Riva 2006; Rodriguez Couto and Toca Herrera 2006, 2007). Laccases (benzenediol: oxygen oxidoreductase, EC 1.10.3.2) are defined in the Enzyme Commission (EC) nomenclature as oxidoreductases, which oxidize diphenols and related substances and use molecular oxygen as an electron acceptor (Viswanath *et al.* 2008). In contrast to most enzymes, which are generally very substrate-specific, laccases act on a surprisingly broad range of substrates, including diphenols, polyphenols, different substituted phenols, diamines, aromatic amines, benzenethiols, polyphenols and even some inorganic compounds (Yaropolov *et al.* 1994; Solomon *et al.* 1996; Rosana *et al.* 2007). Presently, laccases are of particular interest with regard to potential applications, because of their capacity to oxidize a wide range of industrially relevant substrates.

The textile industry plays an important role in the Indian economy. It contributes to 20% of industrial production, 9% of excise collection, 30% of export revenue, and 18% of employment in the industrial sector. The apparel industry is one of India's largest foreign exchange earners, accounting for 12% of the country's total exports. Since global trade in textile and clothing is expected to reach US\$ 600 billion in 2010 from the 2004 level of US\$ 356 billions (Confederation of Indian Industry, 2004; <http://www.ciionline.org>), there is an urgent need to augment our textile production capacity. At the same time, it is very es-

sential that the environmental problems associated with industrial development are properly addressed to sustain both industrial as well as economic growth. The chemical reagents used in the textile industries are very diverse in chemical composition, ranging from inorganic compounds to polymers, organic products and dyes (Banat *et al.* 1996; Claus *et al.* 2002). Due to their chemical structure, dyes are resistant to fading on exposure to light, water and different chemicals and most of them are difficult to decolorize due to their synthetic origin. Government legislation is becoming more and more stringent, especially in more developed countries, regarding the removal of dyes from industrial effluents (O'Neill *et al.* 1999). Concern arises, as several dyes are made from known carcinogens such as benzidine and other aromatic compounds (Baughman and Perenich 1988). Most currently existing processes to treat dye wastewater are ineffective and not economical (Cooper 1995). Therefore, the development of processes based on laccases seems an attractive solution due to their potential in degrading dyes of diverse chemical structure (Blanquez *et al.* 2004; Hou *et al.* 2004) including synthetic dyes currently employed in the industry (Rodriguez Couto *et al.* 2005).

The use of laccase in the textile industry is rapidly increasing, since, besides its use to decolorize textile effluents, laccase is used to bleach textiles and even to synthesize dyes (Setti *et al.* 1999). *Flavodon flavus* decolorized several synthetic dyes like Azure B, Brilliant Blue R in low nitrogen medium (Raghukumar 2000). Partial decolorization of two azo dyes and complete decolorization of two triphenylmethane dyes (bromophenol blue and malachite green) was achieved by cultures of *Pycnoporus sanguineus* producing laccase as the sole phenoloxidase (Pointing and Vrijmoed 2000). *Trametes hirsuta*, and a laccase purified from the fungus, were able to degrade triarylmethan, indi-

goid, azo and athraquinonic dyes used in dyeing textiles (Abadulla *et al.* 2000b) as well as 23 industrial dyes (Bollag *et al.* 2003). The demand for removal of synthetic dyes released from textile industries using laccase is being increased tremendously (Abadulla *et al.* 2000b; Zille *et al.* 2003; Baldrian and Snajdr 2006; Casieri *et al.* 2008). Therefore, searching for potential laccases to cope with this demand is an important task in the area of dye degradation.

Stereum ostrea (Blume & T. Nees:Fr.) Fr. also known as false turkey-tail fungus is a white-rot fungus belonging to family Stereaceae and common in hardwood forests. The present study is the first report demonstrating the decolorization of dyes by *Stereum ostrea* laccase. In view of its importance in decolorization of dyes, laccase was purified from submerged culture of *S. ostrea* and characterized in the present study. The purified enzyme was evaluated for its potential activity towards the decolorization of textile dyes.

MATERIALS AND METHODS

Organism

Stereum ostrea (Blume & T. Nees:Fr.) Fr. was kindly supplied by Prof. M. A. Singaracharya, Department of Microbiology, Kakatiya University, Andhra Pradesh, India.

Dyes and characterization

The reactive dyes Remazol black-5, Remazol blue-19 and Remazol orange-16 were provided by a local textile industry, Dharmavaram, Anantapur district of Andhra Pradesh, India. Chemical structures of the used dyes are specified in Fig. 1. Spectrophotometric scanning was performed and the maximum and minimum wavelengths identified were 590 and 455 nm, respectively.

Culture conditions

The fungal culture *S. ostrea* was maintained on medium containing (g/l): 3.0 peptone, 10.0 glucose, 0.6 KH_2PO_4 , 0.001 ZnSO_4 , 0.4 K_2HPO_4 , 0.0005 FeSO_4 , 0.05 MnSO_4 , 0.5 MgSO_4 , 20.0 agar (pH 6.0). Fungal mycelial suspension was prepared by adding 2 ml of sterile distilled water to the freshly (7 days) grown slants of *S. ostrea*. The homogenized mycelial suspension was used as inoculum.

Laccase production

The medium containing (g/l): 3.0 peptone, 10.0 glucose, 0.6 KH_2PO_4 , 0.001 ZnSO_4 , 0.4 K_2HPO_4 , 0.0005 FeSO_4 , 0.05 MnSO_4 , 0.5 MgSO_4 and 0.02% guaiacol (HIMEDIA, Mumbai, India) as inducer for laccase production (pH 6.0) was used for the production of laccase by *S. ostrea*. Homogenized mycelial suspension was inoculated in 50 ml of above medium in 250 ml Erlenmeyer flasks. These flasks were incubated in a gyratory shaker (ORBITEK, Chennai, India) (200 rpm) at 30°C and all the flasks with growing culture of *S. ostrea* were withdrawn at different time intervals to measure laccase activity.

Estimation of laccase activity

Enzyme activity was assayed at 30°C by using 10 mM guaiacol in 100 mM acetate buffer (pH 5.0) containing 10% (v/v) acetone. The changes in absorbance of the reaction mixtures containing guaiacol were monitored at 470 nm ($\epsilon = 6,740 \text{ M}^{-1} \text{ cm}^{-1}$) for 5 min of incubation. The enzyme activities were calculated using an extinction coefficient of $6,740 \text{ M}^{-1} \text{ cm}^{-1}$ and expressed as Katal (1 mol of substrate conversion/s) (Das *et al.* 1997).

Laccase purification

S. ostrea was grown on the above described medium for four days. After four days of growth the culture supernatant of *S. ostrea* was filtered through Whatman No. 1 filter paper. Solid ammonium sulphate (16.8 g/30 ml) was added to the supernatant and vortexed at room temperature for 5 min and kept at 4°C overnight. The precipitate

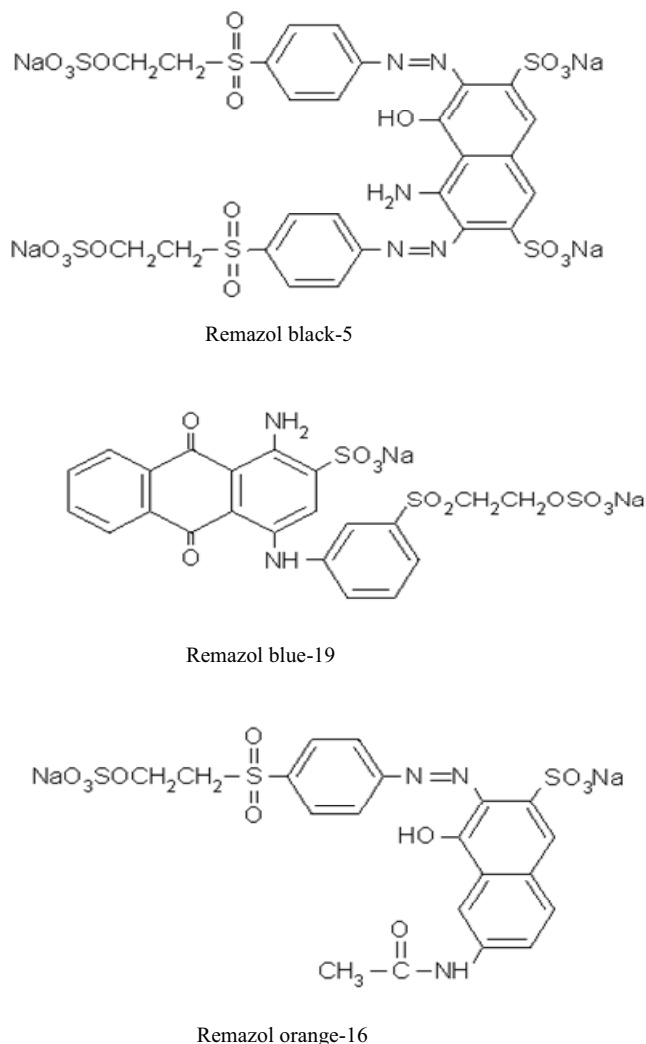


Fig. 1 Chemical structures of textile dyes used in the present study.

was collected by centrifugation at $23,000 \times g$ for 30 min at 4°C (REMI-C24 Centrifuge, Mumbai, India). The supernatant was discarded and the pellets dissolved in deionized water and combined to get a protein concentration of 2 mg/ml. The extract was dialysed against 5 l deionised water overnight at 4°C using dialysis membrane with a 10 kDa-cut off (GeNei, Bangalore, India). The dialysate (1 ml) was mixed with 1 ml of sodium acetate (pH 5.0) and 8 ml of distilled water. The dialyzed sample was subjected to gel filtration on Sephadex G-100 pre-equilibrated with 100 mM sodium acetate buffer (pH 5.0). The sample was loaded onto the column and the column was eluted with the same buffer at a rate of 20 drops per tube and 1-ml fractions were collected (Bio-rad-2110 Fraction Collector, USA). Absorbance of these fractions was recorded at 280 nm for protein content in UV spectrophotometer (Chemito, Mumbai, India). The ammonium sulphate-precipitated fraction was resolved into different protein peaks on Sephadex G-100. Aliquots from 1-ml fractions corresponding to different peaks were tested for laccase activity.

Characterization of purified laccase

SDS-PAGE was carried out according to the method of Laemmli (1970) with some modifications. A 5% stacking gel [3.4 ml H_2O , 0.85 ml 30% acrylamide, 0.32 ml 1 M Tris (pH 6.8), 0.05 ml 10% SDS and 0.05 ml APS (freshly prepared), 0.025 ml TEMED] and 12% resolving gel [3.2 ml H_2O , 4 ml 30% acrylamide, 2.5 ml 1.5 M Tris (pH 8.8), 0.1 ml 10% SDS and 0.1 ml 10% APS (freshly prepared)]. Freshly prepared running buffer [25 mM Tris, 192 mM glycine and 0.1% SDS (pH 8.3)] was used. Samples (~10-50 μg protein) were mixed with sample loading buffer (6X) final concentration (120 mM Tris (pH 6.8), 30% (v/v) glycerol, 4% SDS, 4% mercaptoethanol and 0.02% bromophenol blue) and boiled for 3

min and electrophoresed at a constant 200V for 30 min. For molecular weight calibration, a broad range molecular weight marker [consisting of myosin, rabbit muscle (205 kDa), phosphorylase B (97.4 kDa), bovine serum albumin (66 kDa), ovalbumin (43 kDa) and carbonic anhydrase (29 kDa)] was used as the protein standard and prepared according to the manufacturer's instructions (GeNei, Bangalore, India).

Coomassie staining

The gel was stained with Coomassie blue R-250 (Sigma, Hyderabad, India) [2 g Coomassie blue, 300 ml methanol, 100 ml glacial acetic acid and 400 ml water] for 2 h and destained overnight in half strength destaining solution [150 ml methanol, 50 ml glacial acetic acid and 600 ml water]. Further destaining was carried out with several changes of deionised water.

Effect of substrate concentration on enzyme activity

Pre-steady state kinetic analysis was performed using guaiacol at different concentrations ranging from 1 to 10 mM. The enzyme concentration was adjusted in such a way that the reaction was kept linear for at least 2 min. The fraction without enzyme served as the control. Three independent experiments were carried out at each concentration for substrate guaiacol and the mean value was subjected to statistical analysis. The kinetic constants K_m and V_{max} were calculated using Graph pad prism software (www.graphpad.com). Enzyme activities were calculated using an extinction coefficient of $6,740 \text{ M}^{-1} \text{ cm}^{-1}$ and expressed as katal (1 mol of substrate conversion/s).

Effect of pH on enzyme activity

The effect of pH on enzyme activity was investigated by determining the activity at various pH values. The used buffer systems were 50 mM: glycine/HCl buffer pH 2-3; sodium citrate buffer pH 4-5; sodium phosphate buffer, pH 6-7; Tris/HCl buffer pH 8 and glycine/NaOH buffer pH 9-11. The pH activity of laccase was investigated by measuring the enzyme activities after 3 h of incubation at 4°C with buffers of various pH values ranging from 2 to 9.

Effect of temperature on enzyme activity

The temperature profile of the purified enzyme was studied by measuring the activity at different temperatures viz., 4, 10, 20, 30, 40, 50, 60 and 70°C. The purified laccase (20 η kat/ml) solution was incubated in 50 mM sodium citrate buffer at different temperatures and the activity was determined with guaiacol as substrate.

Effect of inhibitory compounds on laccase activity

The effect of three different inhibitors, SDS, EDTA and arginine with different concentrations ranging from 0.5 to 2.5% were tested on laccase activity. The purified laccase (20 η kat/ml) was incubated with inhibitors for 10 min and the enzyme activity was assayed in triplicate as described above using guaiacol as substrate.

Decolorization of textile dyes with purified laccase of *S. ostrea*

Stock solutions of dyes (Remazol black-5, Remazol blue-19 and Remazol orange-16) were diluted in sodium phosphate buffer (100 mM; pH 5.9), usually to a final concentration of 200 mg/l. Purified laccase was added at activities of 20 η kat/ml. The experiments were conducted in a total volume of 10 ml (in 15 ml plastic screw cap tubes) in triplicate. The controls contained dye solutions without enzymes and *vice versa*. All the screw cap tubes were incubated at 30°C in a rotatory shaker (100 rpm) and the color was measured spectroscopically at the absorbance maximum of the dye at different time intervals.

RESULTS AND DISCUSSION

Laccase production and purification

Laccase production was observed from day 2 after inoculation, and reached a maximum on day 4 (1.006 η kat/ml) in the control. The addition of 0.02% guaiacol to the medium enhanced laccase activity on day 4 to 6.600 η kat/ml, which is approximately 6.65-fold higher than without guaiacol (control) (Fig. 2). As in the control, the highest level of laccase activity was also recorded on day 4 in guaiacol-induced medium. Similar results were obtained by Koroljova *et al.* (1998) in a comparative study on induction of laccase by a basidiomycete *Coriolus hirsutus* by different inducers. But in that study, the inducer, guaiacol, enhanced laccase production by about 2.6-fold, lower than the 6.65-fold in our study. The extracellular laccase enzyme was purified to homogeneity as evident from the appearance of single band on SDS-PAGE (Fig. 3). The extracellular laccase enzyme was purified as evident from the Coomassie staining of the protein on SDS-PAGE (Fig. 3). The presence of a single band on the gel confirmed the purification with the protocol employed in this study. The two-step protocol employed in this study for laccase isolation and purification enabled us to obtain an enzymic preparation purified 70-fold. The results are summarized in Table 1. More than 100 laccases have been purified from fungi and been more or less characterized (Baldrian 2006). Several purification steps are required to obtain a preparation free of both pigment and other contaminated proteins. Multiple steps like ultra-filtra-

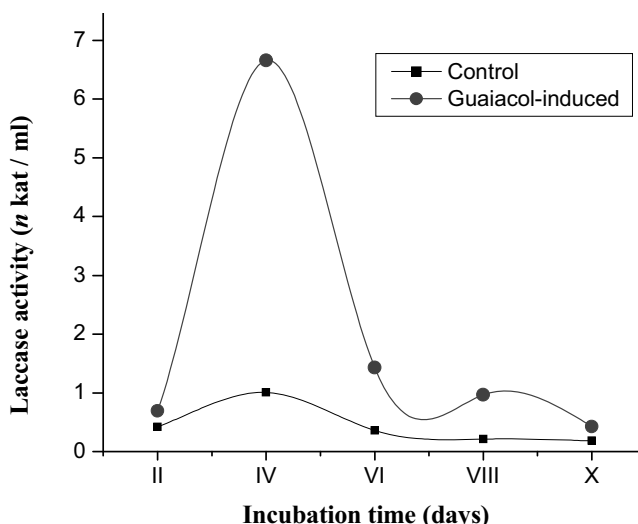


Fig. 2 Production of laccase by *Stereum ostrea* on guaiacol induced medium. The data are the mean values obtained in triplicate assays.

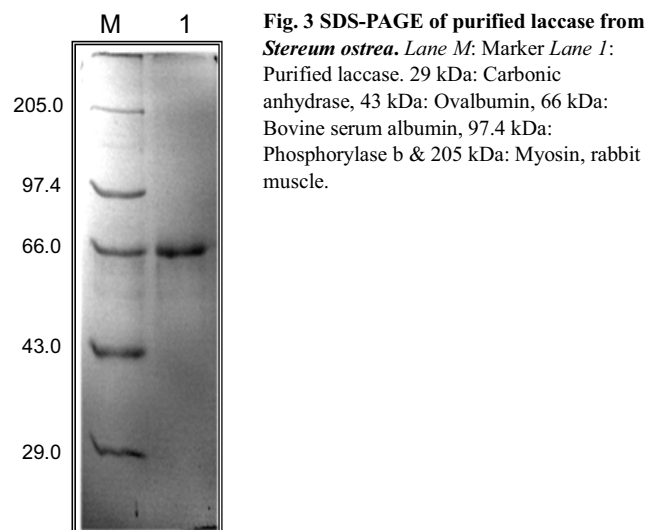


Table 1 Summary of purification of extracellular laccase from *Stereum ostrea*.

Step	Total volume (ml)	Total protein (mg)	Total activity (ηkat)	Specific activity (ηkat/mg)	Yield (%)	Purification fold
Crude	100.0	490.0	6664	13.6	100.0	1.0
(NH ₄) ₂ SO ₄ precipitation	10.0	2.0	960	480.0	14.40	35.30
Sephadex G-100	2.0	0.2	190.4	952.0	2.85	70.0

tion, precipitation using ammonium sulphate or organic solvents, ion exchange and size exclusion chromatography have been used for the purification of laccase from the culture filtrate (Koroljova *et al.* 1998; Murugesan *et al.* 2006; Park and Park 2008). In this study, laccase was purified from the culture filtrate of *S. ostrea* by a two-step protocol, using ammonium sulphate precipitation (80% w/v) and Sephadex G-100 column chromatography.

Characterization of purified laccase

Purified laccase of *S. ostrea* showed a single protein band on SDS-PAGE stained with Coomassie blue R-250. The apparent molecular mass of the purified laccase was 66.0 kDa (Fig. 3), a value consistent with most fungal laccases, in particular, other white rot fungi (60-80 kDa) (Youn *et al.* 1995; Giardina *et al.* 1999; Soden *et al.* 2002; Jordaan *et al.* 2004; Rosana *et al.* 2007). Several fungal species produce a wide variety of isoenzymes (Soden *et al.* 2002; Palmieri *et al.* 2003; Farnet *et al.* 2004). The white-rot fungus *Pleurotus ostreatus* produces at least eight different laccase isoenzymes, six of which have been isolated and characterized (Palmieri *et al.* 1993, 2003). Some fungal laccases are monomeric proteins (Koroljova *et al.* 1998; Murugesan *et al.* 2006). Typical fungal laccase is a protein of approximately 60-70 kDa with an acidic isoelectric point around 4.0 (Baldrian 2006). Like in the present study, the molecular mass of purified laccase of *Trametes versicolor* CCT 4521 showed a molecular mass of 66 kDa (Rosana *et al.* 2007). The molecular mass of *S. ostrea* laccase proved to be close to those of laccases from other fungi: *Coriolus hirsutus*, 66 ± 3 kDa (Koroljova *et al.* 1998); *Coriolus versicolor*, 64 kDa (Fahraeus and Reinhammar 1967); *Coriolus anisoporus*, 57.5 kDa (Vaitkyavichyus *et al.* 1984); *Coriolus pinstitus*, 63 kDa (Xu *et al.* 1996); *Marasmius quercophilus*, 63 kDa (Dedeyan *et al.* 2000); *Chaetomium thermophilum*, 77 kDa (Chefetz *et al.* 1998); *Coriolopsis rigida*, 55 kDa (Saparrat *et al.* 2002); *Lentinula edodes*, 53 kDa (Nagai *et al.* 2002). In contrast to these molecular masses, in a few other cases, like in *Phanerochaete flavido-alba*, laccase migrated as a single band with an apparent molecular weight 94 kDa (Perez *et al.* 1996). This molecular weight was much higher than that reported for white rot fungal laccases (Coll *et al.* 1993; Hattaka 1994; Srinivasan *et al.* 1995).

Effect of substrate concentration

Pre-steady state kinetic analysis was carried out as described in the materials and methods section using guaiacol as substrate. As the substrate concentration increased a linear relationship between laccase activity and substrate concentration was observed. The K_m and V_{max} values for guaiacol were found to be 13.25 mM and 255 η kat/mg of protein respectively. The K_m value of purified laccases from *Lentinula edodes* using guaiacol as substrate was 0.917 mM and 0.350 mM, respectively (Nagai *et al.* 2002). The K_m and V_{max} values for guaiacol from the purified laccase of *Pleurotus florida* were 28.5 mM and 0.175 μkat/mg (Das *et al.* 1997). In case of native *Melanocarpus albomyces* the K_m value with guaiacol as substrate was 910 ± 80 μM, while, with recombinant *M. albomyces*, the K_m value was 890 ± 80 μM (Kiiskinen *et al.* 2004).

Effect of pH on laccase activity

The influence of pH within a range of 2 to 9 on laccase

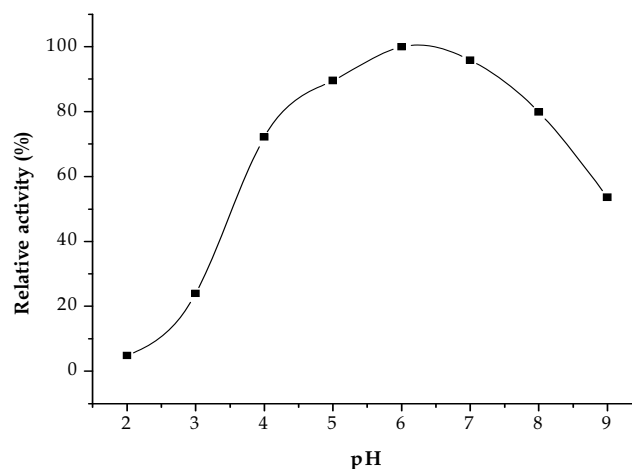


Fig. 4 Effect of pH on laccase activity. The data are the mean values obtained in triplicate assays.

activity of *S. ostrea* was studied and the results were plotted (Fig. 4). The maximum activity was observed at pH 6.0 when measured with the phenolic compound guaiacol. The pH activity profiles of purified laccases indicated pH optima around 4-6, when measured with phenolic substrates (Palmieri *et al.* 1993; Eggert *et al.* 1996; Xu 1997; Chefetz *et al.* 1998; Schneider *et al.* 1999; Garzillo *et al.* 2001). The decrease in laccase activity in neutral or alkaline pH values is affected by increasing hydroxide anion inhibition, because as a small anion, hydroxide ion is also a laccase inhibitor (Xu 1997). On the other hand, the increasing pH decreases the redox potential of the phenolic substrate, which makes the substrate more susceptible to oxidation by laccase (Xu 1997). Oxidation of non-phenolic substrates, such as ABTS, does not involve proton exchange, and therefore nearly monotonic pH activity profiles with highest activities at pH values of 2-3 are obtained (Garzillo *et al.* 2001).

Effect of temperature on laccase activity

The effect of varying temperature on purified laccase was examined (Fig. 5). The optimum temperature was 40°C above which the enzyme activity was drastically reduced (Fig. 5). Temperature stabilities of laccases vary considerably, depending on the source of the organism. In general, laccases are stable at 30-50°C and rapidly lose activity at temperatures above 50°C (Perez *et al.* 1996; Edens *et al.* 1999; Jung *et al.* 2002; Kim *et al.* 2002; Palonen *et al.* 2003; Cordi *et al.* 2007; Rosana *et al.* 2007).

Effect of inhibitory compounds on laccase activity

The effect of inhibitory compounds on laccase activity was determined (Fig. 6). The effect of the inhibitory compounds EDTA, SDS and arginine on laccase activity was determined. With SDS, the percentage inhibition increased as the concentration of SDS decreased from 5 to 0.5%. With the metal ion chelator EDTA, however, an opposite trend i.e., increase in inhibition of laccase activities with increase in EDTA concentration was recorded, suggesting that the enzyme is metal-dependent. These observations were similar to the results made by others on laccase when using the same substances (Castro-Sowinski *et al.* 2002; Jordaan *et al.* 2004; Farnet *et al.* 2004). In the case of arginine, laccase

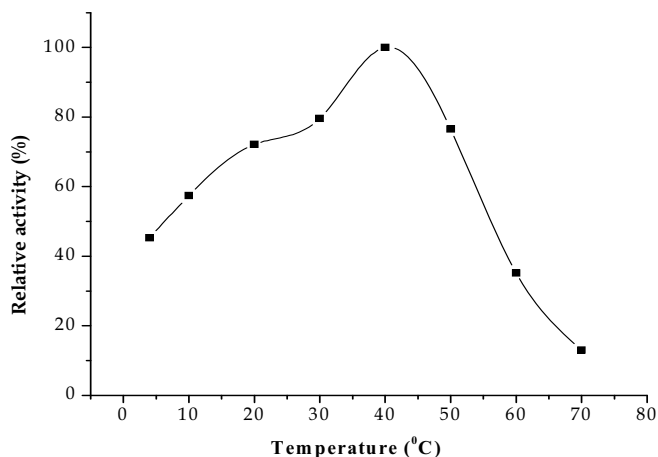


Fig. 5 Effect of temperature on laccase activity. The data are the mean values obtained in triplicate assays.

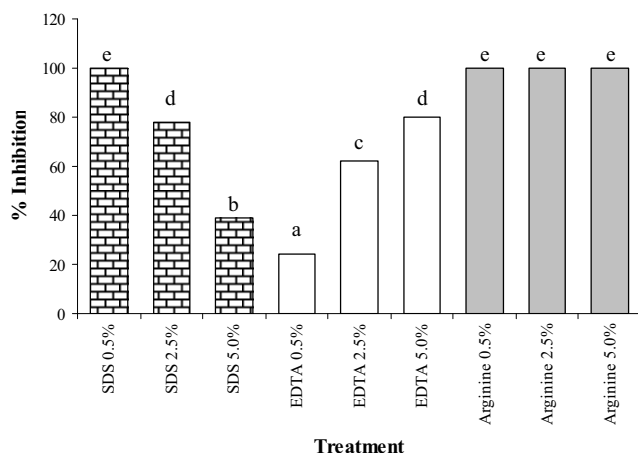


Fig. 6 Effect of inhibitors on purified laccase activity. The data are the mean values obtained in triplicate assays.

activity was completely inhibited at all tested concentrations (Fig. 6). All three inhibitors inhibited laccase activity. One interesting finding was that a lower concentration of SDS showed greater inhibition than the higher concentration. Diamantidis *et al.* (2002) reported the purification and characterization of laccase from *Azospirillum lipofereum* 4T. They described a multimeric laccase enzyme that could be activated by adding SDS.

Dye decolorization by purified laccase of *S. ostrea*

Three textile dyes, Remazol black-5, Remazol blue-19 and Remazol orange-16 were treated with purified laccase of *S. ostrea*. The range of activity on decolorization of Remazol black-5, Remazol blue-19 and Remazol orange-16 were 94, 89 and 92%, respectively (Fig. 7). More than 90% of decolorization activity was observed after 16 h incubation with laccase at 20 η kat/ml for all the textile dyes used in the present study. The extent of decolorization was higher for all the tested textile dyes. The decolorization rate increased with an increase in incubation time (Fig. 7). So the white rot fungus *S. ostrea* was effective in degrading textile dyes. It has been documented that laccases play a key role in dye decolorization. Laccases from *Phanerochaete chrysosporium* and *Pleurotus sajor-caju* are used to decolorize azo dyes (Chagas and Durrant 2001). Complete decolorization of synthetic dyes was achieved by *P. sajor-caju* cultures (Murugesan and Kalaichelvan 2003). *P. sajor-caju*, and a laccase purified from the fungus, were able to decolorize basic dyes methyl violet and emerald green (Arulmani *et al.* 2005). Partial decolorization of two azo dyes (orange G and amaranth) and complete decolorization of two triphenyl-methane dyes (bromophenol blue and malachite green) was

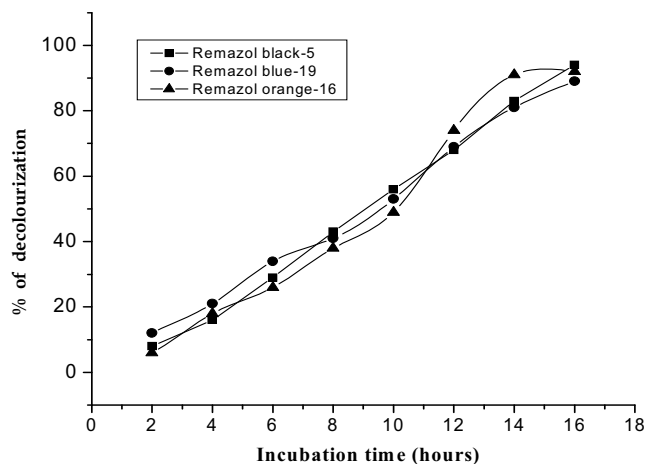


Fig. 7 Decolorization of textile dyes by purified enzyme of *Stereum ostrea*. The data are the mean values obtained in triplicate assays.

achieved by cultures of *Pycnoporus sanguineus* producing laccase as the sole phenoloxidase (Pointing and Vrijmoed 2000). Mishra *et al.* (2000) reported that many white-rot fungi are well suited for the treatment of a broad range of textile dye effluents due to the versatility of the lignin-degrading enzymes they produce. They investigated decolorization of a number of recalcitrant reactive azo and acid dyes using culture filtrate and purified laccase from the fungus *Cyathus bulleri*. Selvam *et al.* (2003), whose findings also support this study, noted that the white rot fungus *Thelephora* sp. was used for decolorization of azodyes such as orange G, Congo red and amido black 10B.

Enzyme-based decolorization is an efficient method and of current interest in industrial effluent treatments (Abadulla *et al.* 2000a; Murugesan *et al.* 2006). Laccase-mediated textile dye decolorization has been described with crude and purified forms from many fungi; however, most of the laccases required redox mediators (Abadulla *et al.* 2000b; Zille *et al.* 2003; Baldrian 2004). In the present study, purified laccase of *S. ostrea* was able to decolorize three textile dyes without any additional redox mediator. This is advantageous to facilitate the benefit of using immobilized enzymes for textile dye degradation. The purified enzyme showed maximum decolorization activity on all three textiles used in the present study. The variation of decolorization extent is possible due to the structural variation of these dyes (Nyanhongo *et al.* 2002). Thus, this study demonstrates the ability of purified laccase of *S. ostrea* in decolorizing different textile dyes and suggests that this enzyme could be used for the decolorization of industrial effluents. The use of laccases in the textile industry is growing very rapidly, since, besides decolorizing textile effluents, they are used to bleach textiles of fabrics (Zille 2003). Other studies have shown that fungal laccases are able to decolorize and detoxify industrial dyes *in vitro* (Abadulla *et al.* 2000a; Schliephake *et al.* 2000).

CONCLUSION

To the best of our knowledge, there has been no study on dye decolorization with laccase purified from *Stereum ostrea*. Therefore, an attempt was made in this study to demonstrate the decolorization potential of the purified laccase. Our results provide evidence of extracellular laccase production by *S. ostrea* and its possible role in textile dye decolorization. Laccases by *S. ostrea* cultivated in the presence of guaiacol were purified by an easy and rapid process. The properties of the purified enzyme are similar to those of analogous enzymes of other fungi and they have a high potential for industrial applications. These preliminary results open up the possibility to apply a laccase-mediator system in bioremediation studies.

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