J. Microbiol. Biotechnol. (2009), **19**(11), 1421–1430
doi: 10.4014/jmb.0901.0033
First published online 16 July 2009

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Bioprocess of Triphenylmethane Dyes Decolorization by *Pleurotus ostreatus* BP Under Solid-State Cultivation

Yan, Keliang[1], Hongxun Wang[2], Xiaoyu Zhang[1]*, and Hongbo Yu[1]

[1]*College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan 430074, P. R. China*
[2]*College of Food Science and Technology, Wuhan Polytechnic University, Wuhan 430023, P. R. China*

Received: January 17, 2009 / Revised: May 12, 2009 / Accepted: June 15, 2009

With an aim to evaluate dye decolorization by white rot fungus on natural living conditions, reproducing by solid-state fermentation, the process of triphenylmethane dyes decolorization using the white rot fungus *P. ostreatus* BP, cultivated on rice straw solid-state medium, has been demonstrated. Three typical dyes, including malachite green, bromophenol blue, and crystal violet, were almost completely decolorized by the fungus after 9 days of incubation. During the process of dye decolorization, the activities of enzyme secreted by the fungus, and the contents of soluble components, such as phenolic compounds, protein, and sugar, changed regularly. The fungus could produce ligninolytic, cellulolytic, and hemicellulolytic enzymes and laccase was the most dominant enzyme in solid-state medium. Laccase, laccase isoenzyme, and the laccase mediator could explain the decolorization of malachite green, bromophenol blue, and crystal violet by the fungus in solid-state medium, respectively. It is worth noting that the presence of the water-soluble phenolic compounds could stimulate the growth of fungus, enhance the production of laccase, and accelerate dye decolorization.

Keywords: White rot fungus, solid-state fermentation, laccase, dye decolorization

Triphenylmethane dyes are used extensively in some industries including textile, food, medicine, and cosmetic [4]. Generally, all dyes used in the textile industry are designed to resist fading upon exposure to sweat, light, water, and microbial attack, so these dyes are recalcitrant molecules difficult to degrade biologically [25]. Conventional wastewater treatment processes, which are suitable for some non-xenobiotic compounds, do not work well with these xenobiotic compounds. Moreover, a great number of industrial effluent discharges in combination with increasingly stringent legislation make the search for more color removal processes an important priority [4]. Currently, the synthetic dyes used are recalcitrant to remove by physicochemical means, including adsorption, precipitation, oxidation, filtration, and photodegradation. However, the biological degradation was regarded as an economical and effective way of dealing with textile dyeing waste as it has inexpensive, eco-friendly, and less sludge-producing properties [22, 26]. For bioremediation of synthetic colorants, several microorganisms including bacteria and fungi can be employed. By far, the single class of microorganisms most efficient in breaking down synthetic dyes is the white rot fungi, which are organisms able to degrade a wide range of recalcitrant organic compounds, including various dyes [31].

White rot fungi are well known for their outstanding degradation ability because they could produce extracellular oxidative enzymes, which could act on different substrates. This ability has opened new prospects for the development of biotechnological processes aimed at the degradation of xenobiotic compounds, effluent decolorization, and biobleaching of Kraft pulp [5, 13]. The white rot fungus *Pleurotus ostreatus*, which can produce some oxidative enzymes, such as laccases, manganese peroxidases, veratryl alcohol oxidase, and peroxidase enzyme, has been studied intensely and was able to decolorize textile dyes in submerged cultures [21, 24]. However, most studies on dye decolorization by the fungus have been carried out using liquid culture condition, which does not reflect the natural living conditions of the fungus. Solid-state fermentation (SSF) is generally defined as a culture for the production of ligninolytic enzymes because it mimics the natural environment of the white rot fungi [10, 14]. The main advantages offered by this type of cultivation over liquid cultures are that they can reproduce the natural living conditions of the white rot fungi, provide nutrients, and reduce production costs, since they are usually carried out using agricultural by-products, like straw and

*Corresponding author
Phone: +86-27-87792108; Fax: +86-27-87792128;
E-mail: unclelia@gmail.com or zhangxiaoyu@mail.hust.edu.cn

bran [16]. Reports on dye decolorization by fungus on natural substrate using solid-state fermentation were scanty.

The aim of the present work was to study the process of dye decolorization by the white rot fungus *P. ostreatus* BP in a rice straw medium using solid-state fermentation. Furthermore, the roles of water-soluble phenolic compounds in dye decolorization were also investigated.

Materials and Methods

Microorganism and Preparation of Inoculum

The microorganism used in this study was a pure strain, *P. ostreatus* BP, which was obtained from the culture collection of College of Science and Technology, Huazhong Agricultural University, Wuhan, People's Republic of China. Stock cultures were maintained on PDA (potato 200 g, glucose 10 g, agar 3 g, distilled water 1 l) at 4°C. Conical flasks containing 100 ml of PDB (potato 200 g, glucose 10 g, distilled water 1 l) were each inoculated with five 5-mm plugs of active mycelium cut from PDA plate cultures, and incubated at 28°C. Prior to sterilization by autoclaving, the medium was adjusted to pH 6.5 with 20 mM acetate buffer. After 6 days, the contents of each culture flask were homogenized in a sterilized blender for 1 min, providing inoculum for the experiment.

Dye Decolorization on SSF

The ability of cultures to decolorize triphenylmethane dyes was tested, and Table 1 lists the dyes used in this study and the wavelength of maximum absorbance. Each dye, at a final concentration of 80 mg/l, was membrane-filtered through a 0.45-μm cellulose nitrate filter and mixed with the rice straw to prepare rice straw solid medium (RSSM), comprising 5 g of rice straw and 20 ml of dye at 80 mg/l. The sterilized solid media were inoculated with 5 ml of inoculum in 50-ml sterile Erlenmeyer flasks, and then made to media uniformity and static cultured at 28°C. Samples were taken at 72 h intervals. At periodic intervals, 50 ml of cold water was added to the media and the mixtures were shaken for 1 h at 4°C. The mixtures were filtered and centrifuged; the clear supernatants were used for analyzing the content of protein, total sugar, reducing sugar, glucose, phenolic compounds, enzyme activity, and pH. The residual dyes on cultures were extracted with water, followed by methanol, and dye disappearance was determined spectrophotometrically. To calculate the residual dye in the cultures, the total dye extracted with water and methanol in the abiotic control was considered as 100%.

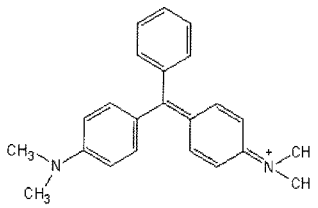
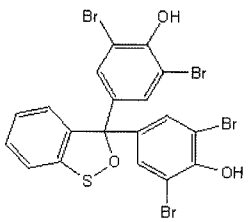
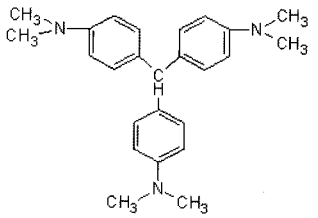
Separation of Laccase and Low-Molecular-Weight Fraction

Because the laccase activity could reach its peak on day 3, the cultured sample on day 3 was submerged, filtered, and centrifuged, and the obtained clear supernatant was used as the crude laccase. All purification steps were conducted at a temperature not exceeding 4°C. The purification of laccase from the crude laccase was carried out as described in Sual *et al.* [30]. Two laccase, Lac I and Lac II, were obtained.

The enzyme used for BB decolorization, after fractionating by ammonium sulfate at 65% saturation and dialyzing by a membrane with molecular mass cut-off of 10,000 Da, was separated with a DEAE–Sepharose column with a linear salt gradient (0.5 M NaCl) at 0.5 ml/min. The eluted fractions were assayed for BB decolorization, oxidation activity of 2,2'-azinobis (3-ethylbenzthiazoline-5-sulfonate), (ABTS) and dimethoxyphenol (DMP), and the A_{280} was monitored.

Some samples of the obtained clear supernatant were ultrafiltrated through a Millipore Ultrafree-15 Membrane (cut-off 5 kDa) and the low-molecular-weight fractions (<5 kDa) were referred to as LMWF.

Table 1. Dyes used for decolorization experiments and the wavelength of maximum absorbance.

Commercial name	Abbreviation	Chemical structure	Molecular wt.	λ_{max} (nm)	Type
Malachite green	MG		364.92	610	Triphenylmethane
Bromophenol blue	BB		669.97	592	Triphenylmethane
Crystal violet	CV		407.99	584	Triphenylmethane

Dye Decolorization by Laccase and LMWF

The color removal capabilities of laccase alone or the synergistic action of laccase and LMWF were assessed using the reaction mixture, containing 2 ml of dye (80 mg/l), 1 ml of laccase, and LMWF with different volumes (0 μl, 10 μl, 20 μl, 50 μl, and 100 μl) in 50 mM sodium acetate buffer (pH 6.0), in a tube with total volume of 3.1 ml, and it was incubated at 28°C in the dark. After 24 h incubation, dye decolorization was measured by monitoring the decrease in absorbance maximum of each dye in a UV–Vis spectrophotometer (UV-2000, U.S.A.) and expressed in terms of percentage.

Effect of Water-Soluble Phenolic Compounds on Dye Decolorization

A mixture of 100 g of rice straw plus 1,000 ml of water was autoclaved for 15 min at 120°C to extract soluble phenolic compounds. Then, the mixture was filtered and centrifuged, and the obtained clear supernatant, membrane-filtered through a 0.25-μm cellulose nitrate filter, was used as source of water-soluble phenolic compounds [16].

Two media were prepared to determine the roles of phenolic compounds in dye decolorization. One was the control medium (CM), which comprised (l^{-1}) glucose 20 g, ammonium tartrate 0.4 g, KH_2PO_4 1 g, K_2HPO_4 0.46 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, and dye 80 mg. The other was of phenolic compound medium (PCM) containing (l^{-1}) glucose 20 g, ammonium tartrate 0.4 g, KH_2PO_4 1 g, K_2HPO_4 0.46 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, phenolic compound extracted 0.08 g, and dye 80 mg. The sterile Erlenmeyer flasks with 100 ml of each medium were inoculated with 10 ml of inoculum and incubated at 150 rpm, 28°C. Un-inoculated control flasks were also included. At regular intervals, flasks were selected randomly and used for analyses. The sample was filtered and the filtrate was used to determine the decolorization rate, residual glucose, phenolic compound, Lac, MnP, and pH. The fungal biomass was washed with warm distilled water, dried at 80°C, and gravimetrically determined.

Decolorization and Enzyme Assays

The decolorization of different dyes was determined by the UV–Vis spectrophotometer UV-2000 at a settled wavelength, where the un-inoculated dye-free medium was used as blank. The decolorization efficiency was expressed as $\eta\% = (A_0 - A_1 - A_2)/A_0 \times 100$ (η is decolorization rate; A_0 is absorbance of initial dye; A_1 is absorbance of residual dye, which is a water extraction from the solid media or filtrate in liquid medium; A_2 is absorbance of adsorbed dye, which is an ethanol extraction from the solid media or the mycelium in liquid medium).

Laccase (Lac), Mn-peroxidase (MnP), and lignin peroxidase (LiP) activities were determined spectrophotometrically by oxidation with 2, 2'-azino-*bis*-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), phenol red, and Azure B, respectively [2, 33]. The activities of total cellulase (filter paper activity, FPA), endo-1,4-β-glucanase (EG), cellobiohydrolase (CBH), and xylanase (XYL) were determined by measuring the reducing sugars released from Whatman No 1 filter paper, carboxymethyl cellulose, and cellulose crystalline, respectively, as substrates, in 50 mM sodium acetate buffer, pH 4.8. Liberated reducing sugars were quantified by the Somogyi–Nelson method, using either glucose or xylose as standards [19]. The activity of 1,4-β-glucosidase (BG) and endo-1,4-β-mannanase (MAN) was estimated using *p*-nitrophenyl-L-D-glucoside (pNPG) and azo-dyed carbohydrate substrates as described previously [6]. One unit of activity was defined as the amount of enzyme releasing 1 μmole of product per minute (IU). Enzymatic activities in the extracts recovered from the liquid cultures were reported in U/ml. In terms of production, the activity was defined as unit per gram dry residue (substrate plus mycelium) (U/g).

Analysis of Water-soluble Phenolic Compounds by HPLC and LC/MS

The total phenol content was determined colorimetrically at 765 nm, using the Folin–Ciocalteau reagent [7]. The qualitative analyses of water-soluble phenolic compounds in culture fluids were estimated using HPLC and LC/MS. The HPLC analyses were carried out on a Shimadzu LC-10A Liquid Chromatograph equipped with a Phenomenex Luna column (150 mm×4.6 mm) and UV–Vis diode array detector. The column was maintained at 35°C and eluted at 0.7 ml/min with the following program: first 2 min with 30% acetonitrile; within the next 16 min, a linear gradient from 0.1% acetic acid to 30% acetonitrile; then, 2 min with 30% acetonitrile. Eluted substances were detected at 280 nm. Estimation of the water-soluble phenolic compounds in culture filtrate was obtained from the HPLC chromatograms by integrating the peaks of the substances eluted between 0 and 20 min [18]. The flow generated by chromatographic separation was directly injected into the electrospray ion source. The positiveion mode for MS analysis was selected. Preliminary analyses were carried out using full-scan and data-dependent MS scanning from *m/z* 250 to 2,000. Identities of the compounds were obtained by matching their molecular ions (*m/z*) obtained by LC–MS/MS with both the retention time and literature data [29].

Other Chemical Analyses

The moisture content of the substrate was determined gravimetrically, after drying at 60°C to constant weight. Glucose oxidase, phenol-sulfuric acid, and dinitrosalicylic acid (DNS) were used to detect the content of residual glucose, total sugar, and reducing sugars, respectively [11]. The analysis of protein concentration followed the method of Zohar *et al.* [34].

RESULTS AND DISCUSSION

Dye Decolorization by Strain BP on SSF

The decolorization of three triphenylmethane dyes including MG, CV, and BB by *P. ostreatus* BP is demonstrated in Fig. 1. The same trend in decolorization profiles for the three dyes, which increased rapidly first and kept stability afterwards, was observed. After 9 days of incubation in RSSM, the decolorization rate of MG, CV, and BB could reach up to 96.06%, 96.08%, and 97.98%, respectively. Furthermore, methanol extracts and visual analysis of the mixtures (data not shown) indicated that less than 5% of the dyes were adsorbed by the mixture of fungi plus rice straw, and the dyes were adsorbed in the rice straw but not in the mycelial mass.

Enzyme Secreted by Strain BP on SSF

The activity of a wide set of ligninolytic and cellulolytic enzymes (Lac, MnP, LiP, FPA, EG, CBH, and BG) as well

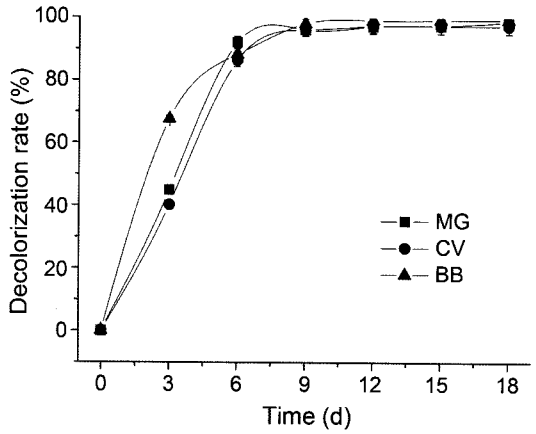


Fig. 1. Time course for dye decolorization by strain BP.
Profiles corresponding to decolorization rate in RSSM with MG (■), CV (●), and BB (▲).

as the hemicellulolytic activities (XYL, MAN) were tested in the decolorization experiment on the fungus culturing in RSSM.

During the whole decolorization process, the ligninolytic enzymes were the major enzyme and Lac was the most dominant ligninolytic enzyme produced by this fungus with low activities of MnP and no LiP (data not shown) in RSSM. Compared with the other two RSSM with MG and CV, a higher Lac activity, which was 139.6 U/g, was found on 6-day culture in RSSM with BB. However, MnP activity, reaching its peak on day 3, was higher in RSSM with CV, compared with that in the other two media (Fig. 2A). During the first 9 days of incubation, the Lac activity keeps higher levels (Fig. 2A) and the decolorization rate increased gradually (Fig. 1) in RSSM, which suggested that Lac may play important roles in dye decolorization. The fungus also could produce cellulolytic and hemicellulolytic enzymes (Fig. 2); however, the activities of these were very low compared with that of ligninolytic enzymes. These results suggested that the three dyes, decolorization might be attributed to the production of laccase.

Synergistic Actions of Laccase and LMWF for Dye Decolorization

The decolorizations of three dyes by purified laccase alone or the synergistic actions of laccase and LMWF are shown in Fig. 3. CV was slightly degraded when it was treated by

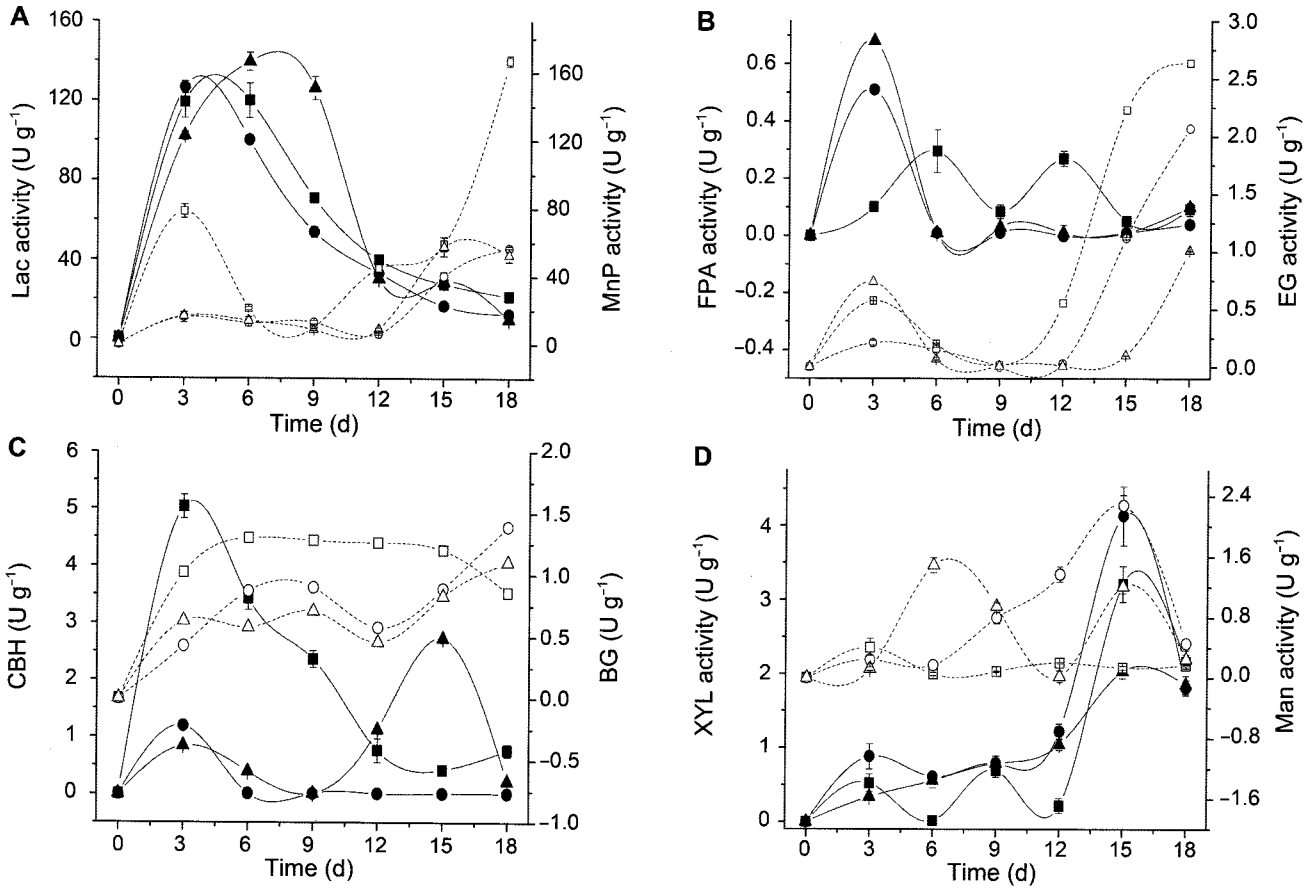


Fig. 2. Enzyme activities of crude culture filtrate from SSF culture of *Pleurotus ostreatus* BP.
The results depicted are the averages of three independent experiments and error bars indicate standard deviations. **A.** Lac and MnP activity. **B.** FPA and EG activity. **C.** CBH and BG activity. **D.** XYL and MAN activity. Profiles with solid line corresponding to left axis are for RSSM with CV (■), BB (▲), and MG (●). Profiles with dashed line corresponding to right axis are for RSSM with CV (□), BB (△), and MG (○).

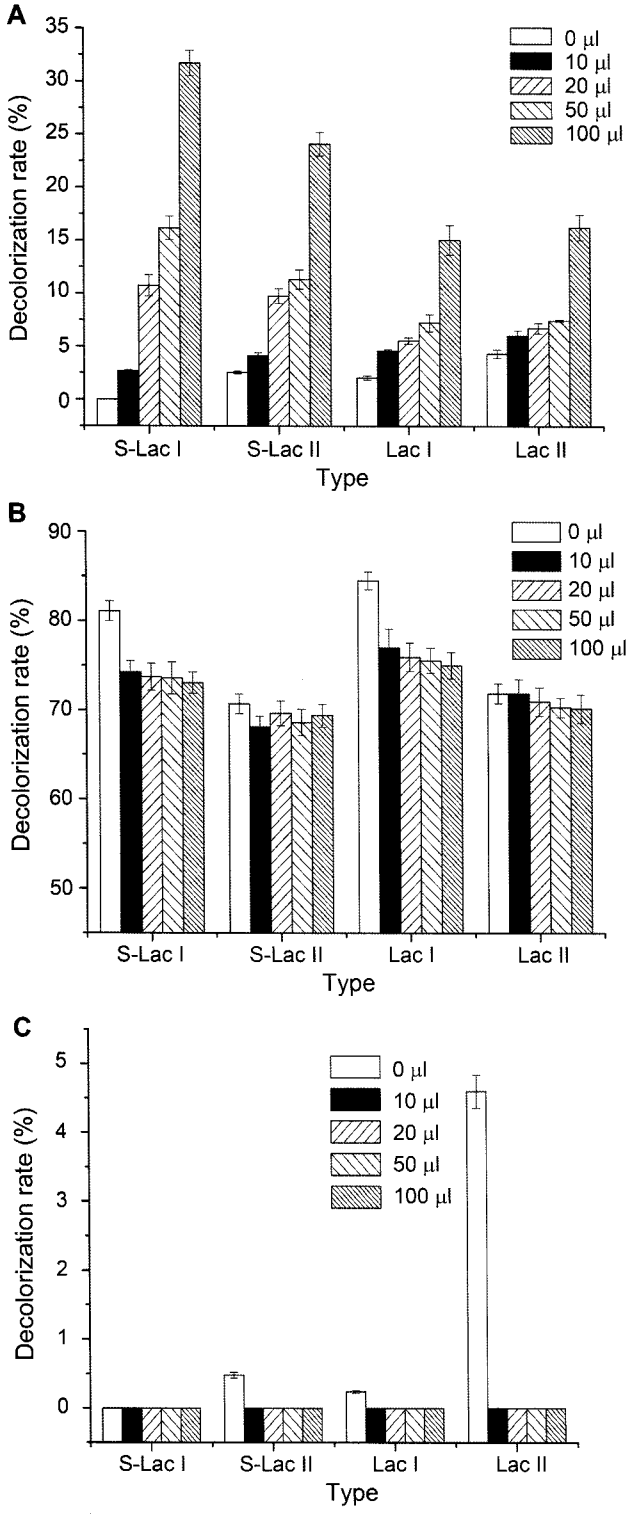


Fig. 3. Synergistic actions of laccase and LMWF for dye decolorization.

The results depicted are the averages of three independent experiments and error bars indicate standard deviations. (**A**) CV; (**B**) MG; (**C**) BB. S-Lac I and S-Lac II indicate the semi-purified Lac I and Lac II through DEAE-cellulose, respectively; Lac I and Lac II show the purified laccase through G-100. The data 0 μl, 10 μl, 20 μl, 50 μl, and 100 μl are the addition amounts of LMWF.

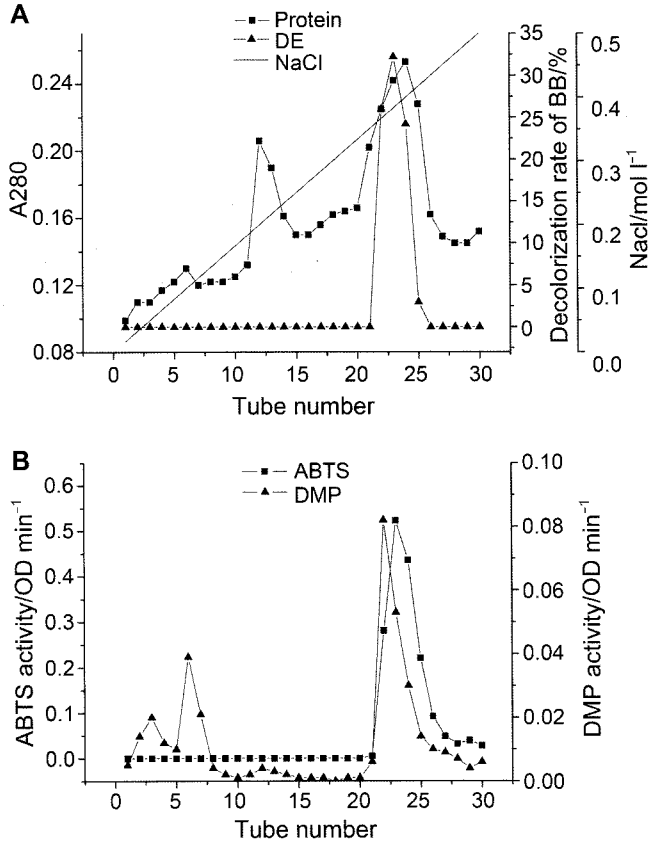


Fig. 4. Eluted curves of enzyme used for BB decolorization on DEAE–Sepharose column.

A. Decolorization curves of BB and absorbance at 280 nm. **B**. Oxidation activities of ABTS and DMP.

laccase alone (Fig. 3A), whereas the addition of LMWF enhanced CV degradation and the decolorization rate of CV increased with the addition amount of LMWF, which indicated that CV decolorization might attribute to the synergistic actions of laccase and LMWF. Additionally, significant MG degradation was observed regardless of whether it was treated by laccase alone or by synergistic actions of laccase and LMWF (Fig. 3B). In the presence of laccase alone, over 70% decolorization of MG was reached. However, the addition of LMWF resulted in a decline of MG decolorization, suggesting that the laccase alone contributes to MG decolorization, which was inhibited by LMWF. Furthermore, the laccase and LMWF could not explain the decolorization of BB and the highest decolorization rate only could reach 4.67% (Fig. 3C). To further research BB decolorization, the enzyme used to degrade BB was separated (Fig. 4), and the results showed that the enzyme that could decolorize BB has the properties of oxidizing ABTS and DMP, so the separated enzyme should be laccase isoenzyme, which showed that BB decolorization was due to the laccase isoenzyme.

The major enzymes of white rot fungi include lignin peroxidase, manganese peroxidase, and laccase. Some

white rot fungi produce all these enzymes, whereas others produce only one or two of them. *Pleurotus ostreatus* produces laccases, manganese peroxidases, veratryl alcohol oxidase, and even lignin peroxidases, and laccase is the main enzyme secreted by the fungus used in this study. *P. ostreatus* laccases have been extensively studied, which could decolorize different dye types including azo and anthraquinone dyes [12, 27] However, the reports on the triphenylmethane dyes by *P. ostreatus* laccase were few. In our study, the laccase, laccase isoenzyme, and the synergetic actions of laccase and LMWF are responsible for the decolorization of MG, BB, and CV, respectively. Chung and Aust [9] had reported the theory for an enzyme mediator, and there was a good capability of biodegradation by a laccase mediator compared with laccase alone. These conclusions suggested that CV decolorization might be attributed to the LMWF being used as the mediator of laccase, and the laccase-mediator system enhanced the CV decolorization.

Characteristics of Soluble Components on SSF

Changes of soluble components, shown in Fig. 5, such as pH, protein, total sugar, reducing sugar, glucose, and phenolic compound, were determined during the whole culture period, and a similar pattern was observed from the profiles of components in RSSM.

The system presented an acidic pH, varying from 5.6 to 6.5, and the moisture content, reducing by 46%, gradually declined in RSSM. During 9 days of incubation, there was an obvious decrease in the content of protein, reducing sugar, total sugar, and phenolic compounds. The content of total sugar and phenolic compounds declined from 6.0 to

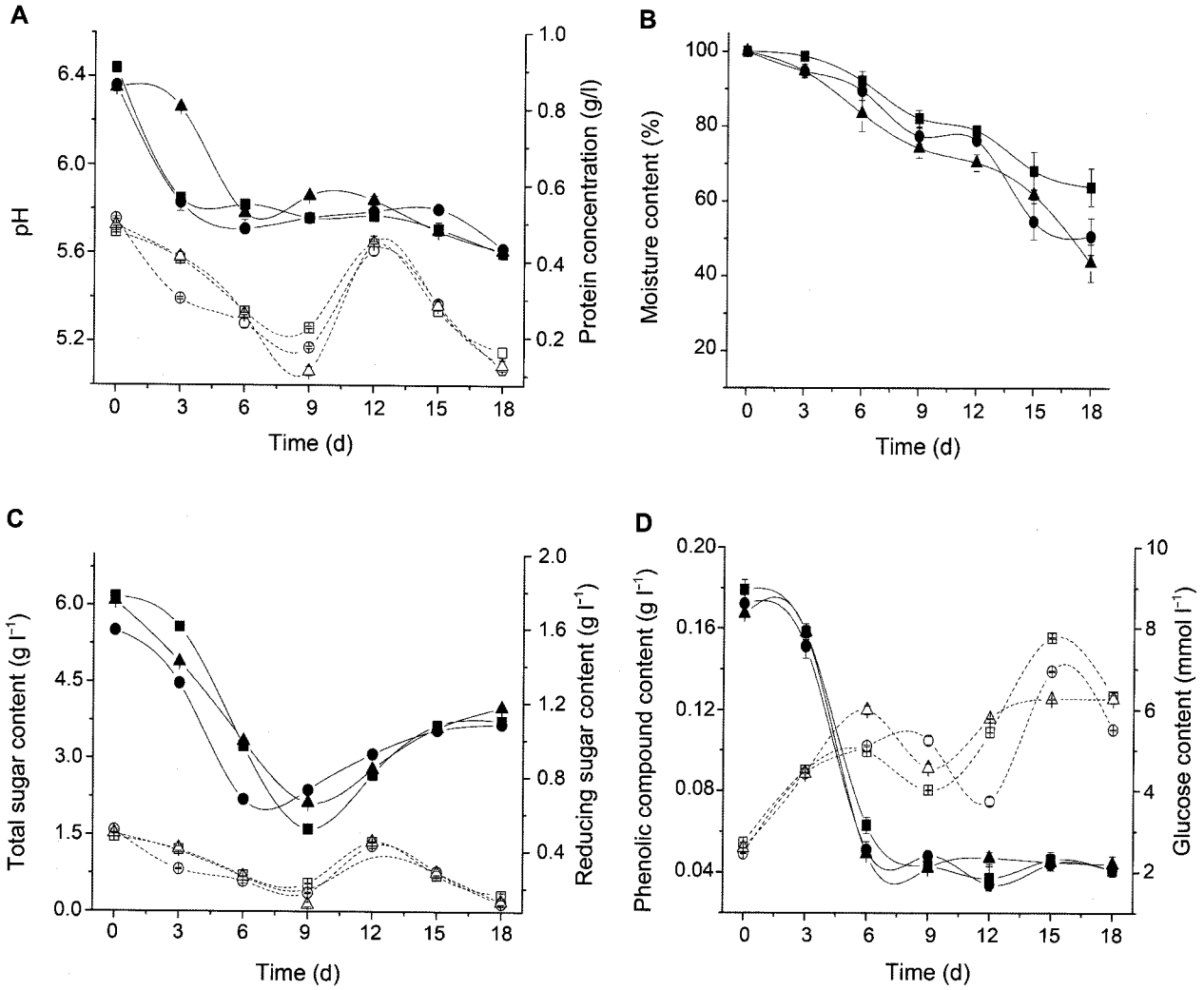


Fig. 5. Soluble components changes of crude culture filtrate from SSF culture of *Pleurotus ostreatus* BP.
The results depicted are the averages of three independent experiments and error bars indicate standard deviations. **A**. pH and protein concentration. **B**. Moisture content. **C**. Total sugar and reducing sugar content. **D**. Content of phenolic compound and glucose. Profiles with solid line corresponding to left axis are for RSSM with MG (■), BB (▲), and CV (●). Profiles with dashed line corresponding to right axis are for RSSM with MG (□), BB (△), and CV (○).

1.62–2.12 g/l and from 0.167–0.179 to 0.043–0.048 g/l, respectively. Furthermore, protein and reducing sugar content increased from 9 to 12 day culture and decreased, which lasted until the end of the experiment. The total sugar content increased and the phenolic compounds kept their stability after 9 days of incubation. Although the profiles of glucose level were irregular with the lengthening of incubation time, the general trend of glucose content presented an increase situation, reaching 5.51–6.33 mmol/l from 2.58 mmol/l.

During the first 9 days of incubation, significant decolorization of the three dyes by white rot fungus (*P. ostreatus* BP) in RSSM were obvious (Fig. 1), and the content of protein, total sugar, reducing sugar, and phenolic compounds decreased remarkably (Fig. 5), which suggested that soluble components played some roles in dye decolorization by the fungus in RSSM. Combined with the growth state of the fungus in RSSM, the conclusion could be speculated that protein and sugar might be used as carbon and nitrogen sources to support the growth of fungus. However, the glucose content increased despite that the total sugar content declined. Baldrian and Gabriel. [6] had reported that *Pleurotus ostreatus* could degrade lignocelluloses efficiently, which might lead it to degrade cellulose in RSSM by secreting enzymes to produce some glucose, resulting in the increasing of glucose content. In addition, because the laccases are multi-copper enzymes that catalyze the oxidation of a variety of phenolic compounds, with the concomitant reduction of O_2 to H_2O [15], the laccase production on SSF led to the decrease of phenolic compounds. Furthermore, it is noteworthy that some phenolic compounds were not degraded and kept their stability to the end of incubation (Fig. 5D).

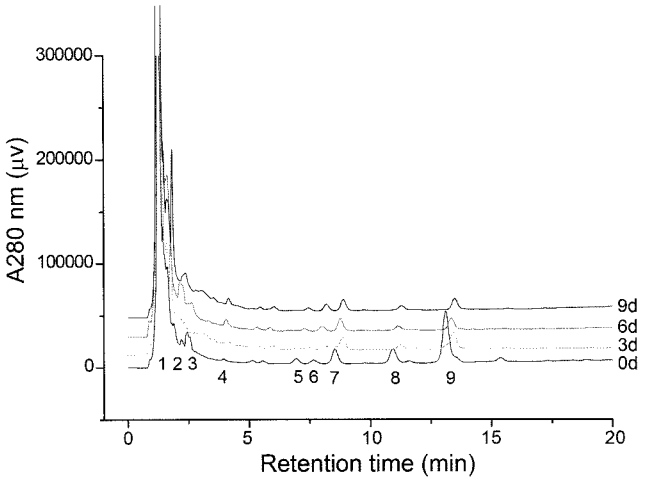


Fig. 6. Chromatograms obtained during HPLC analyses of water-soluble phenolic compounds in RSSM with MG.
In the medium, four superimposed chromatograms are presented: a chromatogram obtained during analyses of the un-inoculated medium (0d) and the other three chromatograms of the culture fluid obtained on days 3 (light gray), 6 (gray), and 9 (bold line). Peaks from 1 to 9 show the typical peaks of phenolic compounds.

Changes of Water-Soluble Phenolic Compounds

Recently, Andrea *et al.* [1] reported on phenolic compounds as likely natural mediators of laccase to oxidize non-phenolic substrates. In order to understand the changes and roles of phenolic compounds in RSSM, a qualitative analysis of water-soluble phenolic compounds was carried out with HPLC and LC–MS/MS. In all cases, the peaks corresponding to initial phenolic compounds are shown in Fig. 6, and 9 typical peaks appeared on the initial control. During the fungal growth in RSSM, the intensity of peaks 8 and 9 decreased apparently and peak 4 increased, while other peaks kept stability. Furthermore, several extracted water-soluble phenolic compounds with low molecular weight, such as ethyl vanillin, 2-ethyl-3-methyl-4-methyl phenol, α-naphthol, and 2-cyclopentane-hydroquinone (Table 2), were identified by their typical MS/MS ion *m/z* of 137, 118, 132, and 162 respectively [20, 32]. However, other peaks, except for peaks 1, 3, 4, and 7, could not be identified.

In the ligninolytic system that involved various dyes degraded by white rot fungi, the native or synthetic mediators with low molecular weight can accelerate the dye degradation [26]. Some mediators, such as phenolic compounds, ABTS, HBT, and 3-HAA, could enhance the dye degradation by laccase [1, 17]. In our study, we found four phenolic compounds with low molecular weight in the degradation system (Table 2) and laccase was the main enzyme secreted by the fungus in RSSM, suggesting that these compounds might be used as mediators of laccase. Moreover, Camarero *et al.* [8] reported that ethyl vanillin could be used as the mediators of laccase to degrade Reactive Black 5, and the compound was also found in our degradation system, so some phenolic compounds in the

Table 2. Identification of some phenolic compounds from culture fluid by LC–MS/MS.

No.	R_t (min)	MW	MS/MS ions (relative intensity)	Compounds	Ref/Std
1	2.0	148	118 (100), 148 (79.2), 84 (31.3), 144 (31.3), 138 (16.3), 130 (13.9)	2-Ethyl-3-methyl-4-methyl phenol	Ref
3	3.3	166	137 (100), 160 (47.4), 109 (15.1), 81(13.9), 53 (8.1)	Ethyl vanillin	Ref
4	4.3	145	143 (100), 132 (66.7), 86 (65.3), 118 (25.9), 114 (12.9)	α-Naphthol	Ref
7	8.8	175	162 (100), 175 (77.6), 116 (32.6), 144 (29.6), 69 (24.5)	2-Cyclopentane-hydroquinone	Ref

system might also be used as mediators of laccase for dye decolorization in RSSM.

Effects of Phenolic Compounds on Dye Decolorization

The synthetic medium was used as control medium (CM) to understand the roles of extracted water-soluble phenolic compounds in dye decolorization, the effects of phenolic compounds on dyes (MG, CV, and BB) decolorization are shown in Table 3. The added phenolic compounds could increase the biomass and Lac activity obviously in PCM compared with that in CM. After 6 days of incubation, the biomass in PCM with MG, BB, and CV could reach 5.5, 5.76, and 5.78 g/l respectively, which was higher than the corresponding biomasses of 0.11, 1.88, and 0.16 g/l in CM. However, the dye adsorbed on mycelium in PCM was less than that in CM, although the corresponding biomass had increased, which suggested that biodegradation was the major mechanism in the decolorization of dyes by the fungus in PCM. Furthermore, a significant increase in decolorization rate of the three dyes in PCM was observed compared with that in CM, and 95.19%, 96.98%, and 95.27% decolorization could be obtained in PCM with MG, BB, and CV, respectively, after 6-day incubation. In addition, phenolic compounds were consumed apparently in PCM.

From the above analysis, the addition of the extracted phenolic compounds could accelerate the degradation of the triphenylmethane dyes, increase the growth of the fungus, and enhance Lac and MnP activity. Some water-extracted plant straw had been found to stimulate fungal growth in synthetic media, and the growth-enhancing component was characterized as a flavonoid-type compound. Furthermore, the growth of fungus could be increased by adding aromatic compounds such as toluidine, vanillic acid, *p*-hydroxybenzoic acid, and aniline to the fungal media [3]. The extracted phenolic compounds from rice straw might include some water-extracted components and aromatic compounds in our study, so the fungal growth could be stimulated by the phenolic compounds extracted. Additionally, the use of inducers to enhance laccase production has been widely practiced in fungi, especially in the white rot fungus [23]. Laccase production can be induced by some compounds including aromatic or phenolic compounds such as ABTS, ferulic acid, 2,5-xylidine, guaiacol or veratryl alcohol, and some water-extracted plant straw [28], which indicated the laccase activity enhanced remarkably in our study might be attributed to the existence of phenolic compound or some water-extracted components. Fig. 3 showed laccase and its isoenzyme are responsible for MG and BB decolorization, so the enhancement of laccase activity would result in a higher decolorization rate of MG or BB (Table 3). However, it is strange that the decolorization rate of CV could reach up to 53.30% without laccase activity in CM, and the fact that the laccase–LMWF could explain the CV decolorization by the fungus had been obtained, so LMWF might play main role in CV decolorization.

In this paper, the degradation process for three triphenylmethane dyes by *P. ostreatus* BP in a solid-state system was studied. Although the three dyes used in this study

Table 3. Dye decolorization by *Pleurotus ostreatus* IMER3 in CM and PCM.

		Time (d)	Adsorption (OD)	Phenolic content (g/l)	Biomass (g/l)	Lac activity (U/ml)	MnP activity (U/ml)	Declorization rate (%)
MG	CM	0	1.26±0.05	0	0.16±0.01	0	0	0
		3	1.73±0.28	0	0.11±0.01	9.67±1.25	0	80.56±1.26
		6	0.51±0.12	0.01±0.002	0.11±0.01	9.97±0.25	0	82.30±0.59
	PCM	0	0.34±0.03	0.09±0.003	0.15±0.01	0	0	0
		3	0.15±0.02	0.03±0.001	4.90±0.01	196±5.22	0	80.18±2.02
		6	0.07±0.02	0.02±0.001	5.50±0.17	580.3±3.22	25±1.47	95.19±0.91
BB	CM	0	0.61±0.26	0	0.27±0.01	0	0	0
		3	1.56±0.17	0	0.88±0.01	35.7±1	0	9.30±0.92
		6	1.12±0.18	0.01±0.001	1.88±0.07	96.5±0.7	0	42.41±0.82
	PCM	0	0.70±0.25	0.08±0.007	0.26±0.01	0	0	0
		3	0.24±0.01	0.03±0.007	5.15±0.01	64.3±3.2	4±0.35	42.41±0.82
		6	0.17±0.05	0.02±0	5.76±0.06	127.3±3.41	22.3±3.2	96.98±0.32
CV	CM	0	1.70±0.21	0	0.16±0.03	0	0	0
		3	1.99±0.02	0	0.17±0.01	0	0	53.20±0.02
		6	1.83±0.01	0.03±0.003	0.16±0.01	0	0	53.30±2.06
	PCM	0	1.12±0.41	0.08±0.003	0.14±0.01	0	0	0
		3	0.34±0.11	0.03±0.002	3.73±0.11	316±5.33	6±0.26	61.42±0.79
		6	0.13±0.01	0.01±0.002	5.78±0.05	72.5±2.48	25±1	95.27±0.35

belong to the same group of dyes and they were degraded by the fungus effectively, the decolorization mechanisms were different in RSSM. Laccase, laccase isoenzyme, and laccase–LMWF could explain the decolorization of MG, BB, and CV, respectively. It is interesting that LMWF might lead to the CV decolorization, and the color removal mechanisms of CV by LMWF need further research. The water-soluble phenolic compound extracted from rice straw, which could stimulate the growth of fungus and enhance the production of laccase, might also contribute to dye decolorization. Solid-state cultures from *P. ostreatus* or addition of some phenolic compounds may be an attractive option for treatment of industrial effluents contaminated with dyes.

Acknowledgments

We are very grateful to the Department of Science and Technology in China for financial support of 863 Advance Technologies Research Program Fund (No. 2007AA021301). Thanks are also due to the Testing Center of the Huazhong University of Science and Technology for valuable help.

REFERENCES

1. Andrea, C., G. Carlo, and G. Patrizia. 2008. Phenolic compounds as likely natural mediators of laccase: A mechanistic assessment. *J. Mol. Cataly. B Enzym.* **51:** 118–120.
2. Archibald, F. S. 1992. A new assay for lignin-type peroxidases employing the dye Azure B. *Appl. Environ. Microbiol.* **58:** 3110–3116.
3. Ardon, O., Z. Kerem, and Y. Hadar. 1996. Enhancement of lactase activity in liquid cultures of the ligninolytic fungus *Pleurotus ostreatus* by cotton stalk extract. *J. Biotechnol.* **51:** 201–207.
4. Azmi, W., R. K. Sani, and U. C. Banerjee. 1998. Biodegradation of triphenylmethane dyes. *Enzyme Microb. Tech.* **22:** 185–191.
5. Bajpai, P. 2004. Biological bleaching of chemical pulps. *Crit. Rev. Biotechnol.* **24:** 1–58.
6. Baldrian, P. and J. Gabriel. 2003. Lignocellulose degradation by *Pleurotus ostreatus* in the presence of cadmium. *FEMS Microbiol. Lett.* **220:** 235–240.
7. Box, J. D. 1983. Investigation of the Folin Ciocalteu's reagent for determination of polyphenolic substances in natural waters. *Water Res.* **17:** 501–525.
8. Camarero, S., D. Ibarra, M. J. Martnez, and A. T. Martnez. 2005. Lignin-derived compounds as efficient laccase mediators for decolorization of different types of recalcitrant dyes. *Appl. Environ. Microbiol.* **71:** 1775–1784.
9. Chung, N. and S. D. Aust. 1995. Veratryl alcohol-mediated indirect oxidation of phenol by lignin peroxidase. *Arch. Biochem. Biophys.* **316:** 733–737.
10. Couto, S. R. and M. A. Sanromán. 2005. Application of solid-state fermentation to ligninolytic enzyme production. *Biochem. Eng. J.* **22:** 211–219.
11. Cuesta, G., N. Suarez, M. I. Bessio, F. Ferreira, and H. Massaldi. 2003. Quantitative determination of pneumococcal capsular polysaccharide serotype 14 using a modification of phenol-sulfuric acid method. *J. Microbiol. Meth.* **52:** 69–73.
12. Erkurt, E. A., A. U. Nyayar, and H. Kumbur. 2007. Decolorization of synthetic dyes by white rot fungi, involving laccase enzyme in the process. *Process Biochem.* **42:** 1429–1435.
13. Fu, Y. Z. and T. Viraraghavan. 2001. Fungal decolorization of dye wasters: A review. *Bioresource Technol.* **79:** 251–262.
14. Gao, J. M., H. B. Weng, D. H. Zhu, M. X. Yuan, F. X. Guan, and Y. Xi. 2008. Production and characterization of cellulolytic enzymes from the thermoacidophilic fungal *Aspergillus terreus* M11 under solid-state cultivation of corn stover. *Bioresource Technol.* **99:** 7623–7629.
15. Gianfreda, L., F. Xu, and J. M. Bollag. 1999. Laccases. A useful group of oxidoreductive enzymes. *Biorem. J.* **3:** 1–25.
16. Giovana, K. T., Z. Adriana, G. M. S. Cristina, and M. P. Rosane. 2004. Decolourisation of industrial dyes by solid-state cultures of *Pleurotus pulmonarius*. *Process Biochem.* **39:** 855–859.
17. Johannes, C., A. Majcherczyk, and A. Huettermann. 1998. Oxidation of acenaphthene and acenaphthylene by laccase of *Trametes versicolor* in a laccase-mediator system. *J. Biotechnol.* **61:** 151–156.
18. Kapich, A. N., B. A. Prior, A. Botha, S. Galkin, T. Lundell, and A. Hatakka. 2004. Effect of lignocellulose-containing substrates on production of ligninolytic peroxidases in submerged cultures of *Phanerochaete chrysosporium* ME-446. *Enzyme Microb. Tech.* **34:** 187–195.
19. Laura, L., H. Claudia, and L. P. Victor. 2008. Optimization of lignocellulolytic enzyme production by the white-rot fungus *Trametes trogii* in solid-state fermentation using response surface methodology. *Biochem. Eng. J.* **39:** 207–214.
20. Lowri, S. D., A. P. Gracia, and W. D. Gregory. 2008. Comparison of headspace-SPME-GC-MS and LC-MS for the detection and quantification of coumarin, vanillin, and ethyl vanillin in vanilla extract products. *Food Chem.* **107:** 1701–1709.
21. Membrillo, I., C. Sánchez, M. Meneses, E. Favela, and O. Loera. 2008. Effect of substrate particle size and additional nitrogen source on production of lignocellulolytic enzymes by *Pleurotus ostreatus* strains. *Bioresource Technol.* **99:** 7842–7847.
22. Moreira, M. T., G. Feijoo, and J. M. Lema. 2000. Evaluation of different fungal strains in the decolourisation of synthetic dyes. *Biotechnol. Lett.* **22:** 1499–1503.
23. Niladevi, K. N. and P. Prema. 2008. Effect of inducers and process parameters on laccase production by *Streptomyces psammoticus* and its application in dye decolourization. *Bioresource Technol.* **99:** 4583–4589.
24. Nilsson, I., A. Möller, B. Mattiasson, M. S. T. Rubindamayugi, and U. Welander. 2006. Decolorization of synthetic and real textile wastewater by the use of white-rot fungi. *Enzyme Microb. Tech.* **38:** 94–100.
25. Nyanhongo, G. S., J. Gomes, G. M. Gübitz, R. Zvauya, J. Read, and W. Steiner. 2002. Decolorization of textile dyes by laccases from a newly isolated strain of *Trametes modesta*. *Water Res.* **36:** 1449–1456.
26. Robinson, T., G. McMullan, R. Marchant, and P. Nigam. 2001. Remediation of dyes in textile effluent: A critical review on current treatment technologies with a proposed alternative. *Bioresource Technol.* **77:** 247–255.

27. Robinson, T., B. Chandran, and P. Nigam. 2005. Studies on the production of enzymes by white-rot fungi for the decolourisation of textile dyes. *Enzyme Microb. Tech.* **36:** 17–24.
28. Ryan, D., W. Leukes, and S. Burton. 2007. Improving the bioremediation of phenolic wastewaters by *Trametes versicolor*. *Bioresource Technol.* **98:** 579–587.
29. Seeram, N. P., R. Lee, S. H. Scheuller, and D. Heber. 2006. Identification of phenolic compounds in strawberries by liquid chromatography electrospray ionization mass spectroscopy. *Food Chem.* **97:** 1–11.
30. Sual, T. B., S. Carmen, L. Octavio, D. R. Geroffrey, and D. G Gerardo. 2008. Laccase of *Pleurotus ostreatus* observed at different phases of its growth in submerged fermentation: Production of a novel laccase isoform. *Mycolog. Res.* **112:** 1080–1084.
31. Wesenberg, D., I. Kyriakides, and S. N. Agathos. 2003. White-rot fungi and their enzymes for the treatment of industrial dye effluents. *Biotechnol. Adv.* **22:** 161–187.
32. Xua, F. X., D. E. Kochb, I. C. Kongc, R. P. Hunterb, and A. Bhandaria. 2005. Peroxidase-mediated oxidative coupling of 1-naphthol: Characterization of polymerization products. *Water Res.* **39:** 2358–2368.
33. Zhou, J. Y., F. Q. Zhang, and M. Kuwahara. 1994. Study on the Mn-peroxidase and laccase from fungus II: The Mn-peroxidase produced by a basidiomycete, *Pycnoporus sanguineus* K-2352. *Microbiology* **2:** 152–156.
34. Zohar, K., F. Dana, and H. Yitzhak. 1992. Lignocellulose degradation during solid-state fermentation: *Pleurotus ostreatus* versus *Phanerochaete chrysosporium*. *Appl. Environ. Microbiol.* **58:** 1121–1127.