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# Simultaneous Laccase Production and Color Removal by Culturing Fungus *Pycnoporus* sp. SYBC-L3 in a Textile Wastewater Effluent Supplemented with a Lignocellulosic Waste *Phragmites australis*

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**Abstract** We conducted experiments to culture *Pycnoporus* sp. SYBC-L3 in a medium comprising an industrial waste (dye-containing textile effluent) and a lignocellulosic waste (*Phragmites australis*) that achieved laccase production while having the color removed from the wastewater. Our experimental results showed that the fungus grew well in liquid submerged cultivation with the diluted textile effluent as the sole culture medium, but relatively low extracellular laccase activity (1.8 U/mL) was produced. Addition of the lignocellulosic biomass enhanced laccase production and color removal. The highest laccase activity was found to be 6.5 U/mL in the presence of *Phragmites australis* stem. Under this condition, 70 % color removal occurred in the culture medium. This study provided an alternative novel scheme to remove color in textile wastewater while having an economic value added by producing laccase.

**Keywords** Textile · Effluent · *Phragmites australis* · Lignocellulosic biomass · Laccase · Decolorization

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Synthetic dyes such as triarylmethane, indigoid, azo, and anthraquinonic dyes, are extensively used in textile industry. A great amount of dye-containing textile effluents are directly discharged into wastewater every year (Abadulla et al. 2000). Discharge of untreated textile wastewater into the environment can pose a great threat to the water sources and severely influences the environmental health. It is thus of great significance to find efficient methods to remove color from textile effluents.

Some studies have shown the ability of certain enzymes such as peroxidase, laccase, lignin peroxidase and manganese peroxidase to effectively degrade organic pollutants (Colosi et al. 2006) and decolorize synthetic dyes for environmental protection (Baldrian 2004). Most previous studies focused on using such enzymes or fungal cultures that produce such enzymes for treatment of dye-containing textile wastewater (Blánquez et al. 2008). We in this study tested an alternative novel strategy in which we used dye-containing textile wastewater as a medium to culture a laccase-producing fungus *Pycnoporus* sp. SYBC-L3 to achieve simultaneous color removal from the wastewater and enzyme production as a useful product. Considering the limit of nutrients in effluent water, we also tested the addition of a lignocellulosic waste, namely *Phragmites australis* (the common reed), to serve as a supplement in the culture medium for laccase production.

Laccase is a multi-copper oxidase capable of catalyzing a wide range of phenolic compounds (Gianfreda et al. 1999). Valuable industrial and environmental applications have been identified for laccase, such as pulping and bleaching (Arias et al. 2003), delignification (Ibarra et al. 2006), dye decolorization (Baldrian 2004), pollutants removal (Attanasio et al. 2005; Lu et al. 2009), and biosensor (Vianello et al. 2006). Production of laccase at high quantity and low cost is required to realize its full

application potential. The scheme that we proposed and tested in this study is thus of both environmental and economic values by removing color from textile wastewater while producing laccase.

## Materials and Methods

The fungus *Pycnoporus* sp. SYBC-L3 (18S rRNA sequence deposited in GenBank under accession number GU182936) was a stock culture of the Laboratory of Biochemistry, School of Biotechnology, Jiangnan University, Jiangsu Province, China. And maintained on potato dextrose agar slants at 4°C with periodic transfer. The strain was first sub-cultured on potato dextrose agar plate at 30°C and then was inoculated into growth medium containing 5 % glucose, 1 % yeast extract, and 0.5 % peptone to culture a seed solution at 200 rpm and 30°C. After 48 h of incubation, 10 % of the inoculum was then transferred into a liquid culture medium which consisted of a textile effluent diluted with tap water to different levels, as mentioned in "Effect of textile effluent concentration on laccase production". The cultivation was carried out at 30°C on a rotatory shaker at 200 rpm.

The textile effluent was collected from a river near a local textile industry in Wuxi, Jiangsu Province, China. The effluent was deep red with an acidic pH of 4.2, and the composition of the effluent was unknown. *Phragmites australis* was collected from a riverside at Jiangnan University, Wuxi, Jiangsu Province, China. The collected *Phragmites australis* was air-dried in open air and smashed into powder with a disintegrator and passed through a 5 mm sieve.

Laccase activity was determined with 2, 6-Dimethoxyphenol (DMP, Fluka, Chemi new Ulm, Switzerland) as substrate and the oxidation was monitored at 469 nm ($\varepsilon = 49.5\ mM^{-1}\ cm^{-1}$) (Litthauer et al. 2007). The assay system (3 mL) contained 0.1 mL of laccase sample, 0.5 mL of 10 mM DMP and 2.4 mL sodium citrate phosphate buffer (0.1 M, pH 3.5). One unit of enzyme activity was defined as the amount of enzyme that oxidized 1 μmol of DMP per min at 35°C.

To examine the effect of textile effluent on laccase production, different levels of textile effluent (10 %, 30 %, 50 %, 80 %, 100 %) which was diluted with tap water was used as a liquid medium for laccase production, and laccase activity was determined accordingly.

To examine the effect of *Phragmites australis* on laccase production, different quantity (2 g/L, 4 g/L, 6 g/L, 10 g/L, 12 g/L) of *Phragmites australis* leaf or stem powder was supplemented into the liquid medium containing 80 % textile effluent, and laccase activity was determined accordingly.

The absorption spectrum of the textile effluent was recorded from 350 nm to 800 nm at room temperature with 6.67 % textile effluent (diluted with tap water) using a UV–vis spectrophotometer (U-3010, HITACHI, Japan). To determine the decolorization of textile effluent during the laccase production, 80 % textile effluent was used as liquid medium supplemented with 10 g/L *Phragmites australis* leaf and stem powder, respectively. The decolorization rate of textile effluent referred to Rodríguez and was calculated at day 9 with the following formula: $(A_i - A_f)/A_i \times 100\ \%$, $A_i$ means the absorbance at 516 nm before cultivation while $A_f$ means the absorbance at 516 nm after cultivation (Rodríguez 2007).

## Results and Discussion

Figure 1 shows the result for the fungus *Pycnoporus* sp. SYBC-L3 grown in textile effluent diluted with tap water at different levels. The increasing concentration of textile effluent enhanced laccase production, and the highest laccase activity of approximately 1.8 U/mL was reached with 80 % textile effluent, while 100 % textile effluent showed inhibitory effect on laccase production. We also examined cell dry weight to reflect fungal growth during cultivation, which showed a similar trend as the laccase activity during cultivation. The best fungal growth was obtained at 80 % of textile effluent and 100 % textile effluent dramatically inhibited the fungal growth and in turn greatly decreased laccase production (data not shown). Fermentation time required to reach the maximum laccase activity was different in different cultures, as seen in Fig. 1, with day 5, 7, 8, 9 and 10, respectively, for the cultures having 10 %, 30 %, 50 %, 80 % and 100 % textile effluent. The difference may be caused by difference in the induction of laccase secretion and toxicity of the culture solutions containing different textile effluent concentrations. Along with laccase activity increasing, the color of culture medium was observed slowly decreasing. The stimulation effects of dyes on laccase production during cultivation have also been reported in previous papers by D'Souza et al. (D'Souza et al. 2006; Enayatzamir et al. 2009). Enayatzamir and coworkers obtained a high decolorization effect on Reactive Black 5 (RB5) and produced laccase at the same time by adding the dye into the culture medium in a fixed-bed bioreactor.

Most earlier studies focused on decolorization of dyes or dye-containing effluents, by mixing them with to fungal culture broth. Studies using dye-containing effluent as the sole culture medium for fungal growth and simultaneous laccase production remained scarce. This study examined the feasibility of cultivating fungal in a real dye containing effluent to remove color and simultaneously produce useful

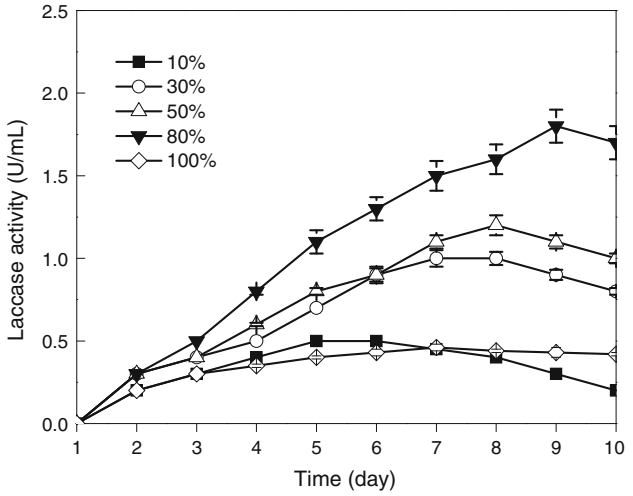


**Fig. 1** Laccase production by *Pycnoporus* sp. SYBC-L3 in the textile effluent diluted to varying concentrations

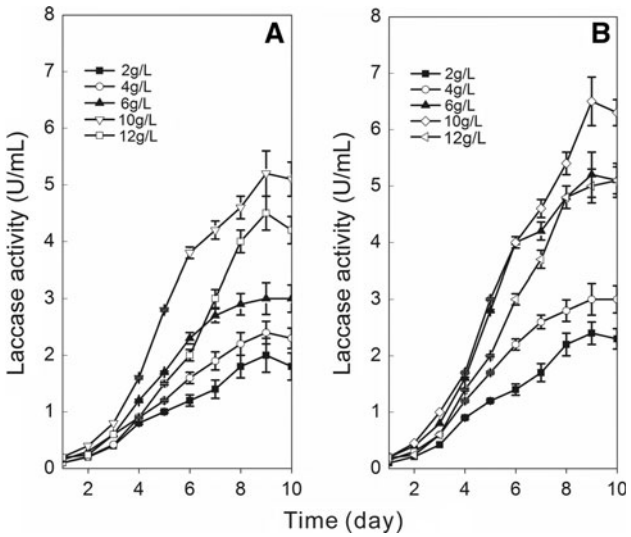


**Fig. 2** Effect of *Phragmites australis* leaf (**a**) and stem (**b**) on laccase production by *Pycnoporus* sp. SYBC-L3 in 80 % textile effluent

laccase. Compared to other laccase production studies, e.g. 6,590.26 (U/L) using optimized or partially optimized media (Arockiasamy et al. 2008), the laccase production level in this study was apparently low, which can be attributed to limit of nutrients in the textile effluent.

As shown in Fig. 1, an optimum concentration of textile effluent seemed to exist with regard to laccase production. Laccase activity increased as the textile effluent concentration increased up to 80 %, while the 100 % textile effluent showed an inhibitory effect. Nevertheless, even the highest laccase activity at 80 % textile effluent was still quite low due to the limit of nutrients in textile effluent. Therefore, we conducted additional experiments to evaluate how addition of a lignocellulosic waste *Phragmites australis* may impact on laccase production.

Figure 2 shows laccase production by the fungus *Pycnoporus* sp. SYBC-L3 grown in 80 % textile effluent supplemented with different quantity of *Phragmites australis* leaf and stem powder. Laccase production was evident in all samples, peaked on day 9, and the highest laccase activity of about 5.2 U/mL was found with the addition of 10 g/L *Phragmites australis* leaf powder (Fig. 2A). A similar trend was also observed for the cultures supplemented with *Phragmites australis* stem powder (Fig. 2b). The highest laccase activity of about 6.5 U/mL was detected on day 9 with the addition of 10 g/L *Phragmites australis* stem powder. However, the effect of *Phragmites australis* stem powder was better than leaf powder on laccase production, which may be due to nutrient differences between leaf and stem. The effect of lignocellulosic waste on laccase production were also found in other studies involving grape stalk, barley bran (Lorenzo et al. 2002) and barley stem (Moldes et al. 2004). After the addition of *Phragmites australis* stem powder into the culture medium as nutrient, laccase production (6.5 U/mL) was dramatically increased than that of cultivation without any supplement (1.8 U/mL). This laccase production level was comparable to a few previous studies using optimized culture media, e.g., 4.16 U/mL from *Cladosporium cladosporioides* (Halaburgi et al. 2011).

The UV–vis spectrum of the diluted textile effluent showed an absorbance peak at around 516 nm (Fig. 3a). The absorbance characteristic was close to that of azo dye Mordant Red 5G (at 536 nm). The absorbance value at 516 nm of the medium, which was composed of textile effluent and *Phragmites australis* leaf or stem powder, dramatically decreased after the fungal cultivation. The color of the medium changed into light (Fig. 3b-B2, B3) from bright red (Fig. 3b-B1). The levels of decolorization in the cultures with *Phragmites australis* stem powder were greater than those with *Phragmites australis* leaf powder (Fig. 4). The relative decolorization levels seemed to correspond with the laccase production levels in cultures having different quantity of supplements (Fig. 2).

Water-pollution control is one of the most challenging areas (Banat et al. 1996), and increasing efforts are directed to research and development of novel wastewater treatment methods (Singh et al. 2010). Microbial or enzyme-mediated decolorization and degradation of dyes have been shown to be a potentially viable, environmental friendly and cost-effective approach for removing dyes from the environment. Most previous studies focused on the use of crude or pure laccases for the treatment of textile effluent (Wu et al. 2002) or synthetic dyes (Li et al. 2009). Pant et al. studied solid state fermentation by fungi using distillery wastewater as a natural medium and achieved laccase production and decolorization (Pant et al. 2007).

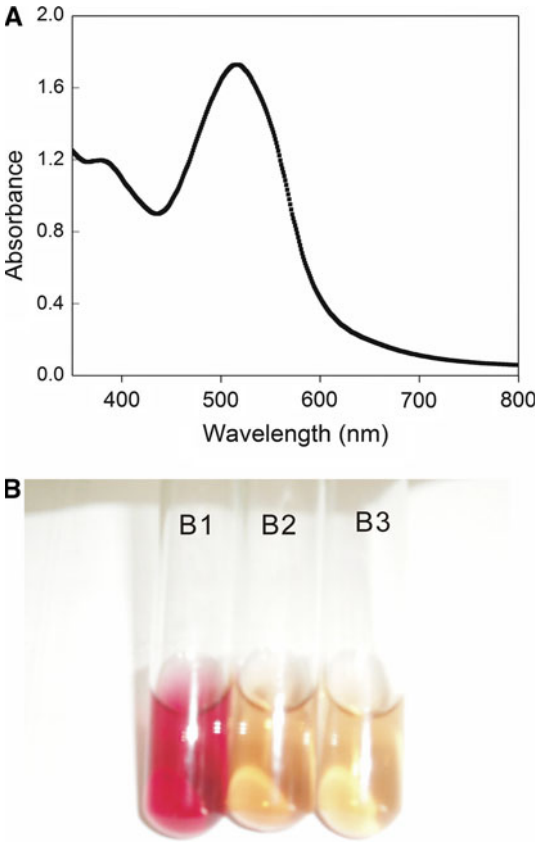


**Fig. 3** **a** The UV–vis absorption spectrum of textile effluent. **b** *Color* change of the medium before (*B1*) and after the fungal cultivation using *Phragmites australis* leafs (*B2*) or stems (*B3*) as an amendment

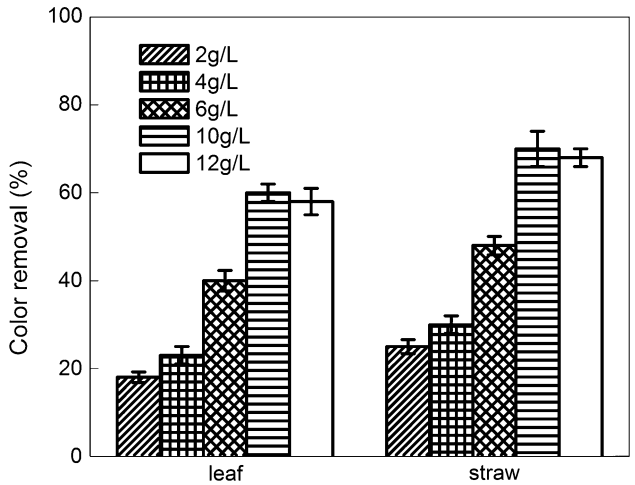


**Fig. 4** Removal of color in the textile effluent with *Phragmites australis* supplemented and *Pycnoporus* sp. SYBC-L3 cultured

However, reports on using dye-containing effluent as a natural medium for cultivation to obtain a decoloration effect have been rare. To the best of our knowledge, our study marked the first report on using a real textile effluent as the culture medium for simultaneous color removal and laccase production. Amendment of a lignocellulosic waste *Phragmites australis* enhanced the process performance by supplementing nutrients. This study also suggested the versatile potential of the fungus *Pycnoporus* sp. SYBC-L3 in various biotechnological and environmental applications.

The present study demonstrated a novel scheme by combining an industrial waste (dye-containing textile effluent) and a lignocellulosic waste (*Phragmites australis*) to serve as an effective medium for laccase production while having the color removed from the wastewater. The stem of *Phragmites australis* seemed to have a better stimulation effect on laccase production than the leaf. Along with the laccase production as high as 6.5 U/mL, about 70 % color removal was achieved in our experiments. The scheme may provide an alternative approach to color removal in textile wastewater while having an economic value added by producing laccases.

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