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Decolorization of Cotton Pulp Black Liquor by *Pleurotus ostreatus* in a Bubble-column Reactor

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Abstract Decolorization of cotton pulp black liquor by *Pleurotus ostreatus* B1 in a bubble-column reactor (BCR) was studied. The optimal conditions for the running of BCR are 30°C, pH 6.0, aeration rate 1.2 L min^{-1}, and mycelial age 7 days. Under the optimal conditions, the BCR was run for four cycles (each cycle, 12 days) and the same mycelial pellets were reused. The ultimate decolorization and COD removal rates are 76% and 80%, respectively.

Keywords Cotton pulp black liquor · Bioreactor · *Pleurotus ostreatus* · Decolorization

Cotton linters are used in the manufacture of viscose fiber and fine paper. In chemical fiber factories, large quantities of cotton pulp black liquor are produced. Toxic and intensively colored cotton pulp black liquor causes severe environmental pollution. The primary contributor to the color and toxicity of cotton pulp black liquor is the high-molecular-weight lignin (Wang et al. 2002). As lignin is difficult to biodegrade, the conventional biological methods are not effective in treating cotton pulp black liquor. White-rot fungi have received extensive attention due to their powerful lignin-degrading enzyme systems (Pointing 2001). Many research works have been done on the treatment of lignin-related wastewater by white-rot fungi. However, most of them focused on pulp and paper mill effluents (Sahoo and Gupta 2005). Reports on the treatment of cotton pulp black liquor by white-rot fungi are very few.

Various types of reactors such as stirred-tank reactor (Gao et al. 2006), packed-bed bioreactor (Prasad et al. 2005), air-lift bioreactor (Couto et al. 2006), bubble-column reactor (Quaratino et al. 2007) etc., have been used for the production of ligninolytic enzyme by white-rot fungi. However, few studies have reported the utilization of reactors for the treatment of wastewater by white-rot fungi. Bubble-column reactors have a structure which is advantageous to gas exchange and mass transfer. Moreover, bubble-column reactors do not require a mechanical stirrer, and consequently provide a low-shear environment, which is beneficial to the production of ligninolytic enzyme by white-rot fungi (Moreira et al. 1996). *Pleurotus ostreatus* is widely recognized as one of the most efficient lignin-degrading white-rot fungi (Ha et al. 2001). This paper reports the decolorization of cotton pulp black liquor by *P. ostreatus* in a bubble-column reactor (BCR).

Materials and Methods

Pleurotus ostreatus B1 was obtained from Dalian Institute of Mushroom Study, China. The cotton pulp black liquor was collected from a chemical fiber factory, located in Yibin, China. The main characteristics of the cotton pulp black liquor were as follows: pH 9.0–10.0, COD 4,000–5,000 mg L^{-1}, colorimetric units (CU) 19,000–20,000, lignin 6,000–7,000 mg L^{-1}. Strain B1 was aseptically cultured on the potato dextrose agar plates (PDA) at 30°C for a week.

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When PDA was fully covered with mycelia, the mycelial plug (diameter 10 mm) was used as the inoculum. Four mycelial plugs were transferred to a 1,000 mL Erlenmeyer flask containing 400 mL potato extract medium. The flask was incubated on a rotary shaker (150 rpm) at 30°C. After 7 days, the pellets of strain B1 (5.0 g, dry weight) were collected and then transferred into BCR (Fig. 1). BCR was supplemented with 500 mL cotton pulp black liquor. Then the black liquor was diluted to 2,500 mL by tap water (dilution rate, 0.2). Glucose, ammonium tartrate, KH_2PO_4, $MgSO_4{\cdot}7H_2O$ and thiamine hydrochloride were added into the 2,500 mL of black liquor with final concentrations of 10, 0.2, 2, 0.5 and 0.02 g L^{-1}, respectively. The final pH was adjusted to 7.0. Temperature was maintained at 30°C by the circulation of thermo-stated water. Air was injected through a perforated pipe sparger. The aeration rate varied from 0.8–1.6 L min^{-1}. Samples were withdrawn from three different orifices along the height of BCR at an interval of 24 h. Prior to analysis, each sample was centrifuged (15°C) at 12,000 g for 10 min to remove the biomass. All the experiments were carried out at least three times. The means were statistically analyzed and the error bars depicted the 95% confidence intervals. The effects of temperature, pH, mycelial age and dilution rate on the enzyme activity and decolorization efficiency were also studied. As far as the dilution rate was concerned, the initial black liquor volume varied from 500 to 2,500 mL. Consequently, the dilution rate ranged from 0.2 to 1.0. BCR was operated under the optimum conditions. After a 12-day running, the spent black liquor in BCR was discharged empty. Then, 2,500 mL fresh amended black liquor (dilution rate, 0.2) was added into BCR for the next cycle of decolorization by the same mycelial pellets.

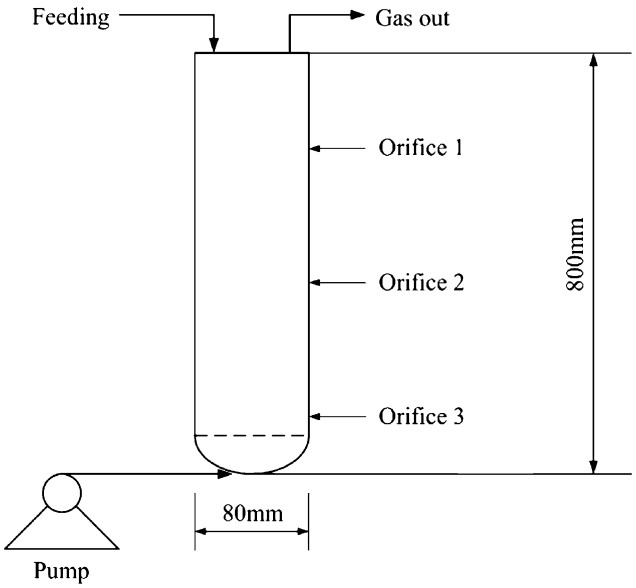

Fig. 1 The schematic diagram of BCR

The color was determined according to the CPPA standard method (CPPA 1974). The lignin content was measured using a UV–VIS spectrophotometer at 280 nm (Lundquist et al. 1977). Chemical oxygen demand (COD), ammonia concentration and mycelial dry weight were determined by standard methods (APHA 1992). Glucose concentration was determined spectrophotometrically by dinitrosalicylic acid method (Ghose 1987). Lignin peroxidase (LiP) activity was determined as described by Tien and Kirk (1984), manganese peroxidase (MnP) activity was determined as described by Glenn and Gold (1985), and laccase activity was determined as described by Niku-Paavola et al. (1990).

Results and Discussion

Figure 2 shows the effects of aeration rate on laccase activity and color removal. At an aeration rate of 1.2 L min^{-1}, the laccase activity reached 460 U L^{-1} on 10th day, 2.2 and 1.5 times of those at aeration rates of 0.8 and 1.6 L min^{-1}, respectively. Thus, aeration rate of 1.2 L min^{-1} was used for the production of laccase in the following studies. After 12 days, the color removal rate reached 72% at 1.2 L min^{-1}. It has been reported that *P. ostreatus* produces manganese peroxidase (MnP) and laccase, which catalyze the degradation of lignin (Ha et al. 2001). However, no LiP or MnP activities were detected with the strain B1. Thus, only laccase was responsible for the color removal of cotton pulp black liquor.

As shown in Fig. 3, the maximum laccase activity (460 U L^{-1}) was obtained at 30°C, resulting in the highest color removal rate (72%) and lignin removal rate (51%), respectively. The laccase activities were 139 and 167 U L^{-1} at 20°C and 40°C, respectively. Thus the color and lignin removal rates decreased significantly. These results indicate that 30°C is the optimal temperature for the decolorization of cotton pulp black liquor by the strain B1.

The highest activity of laccase (490 U L^{-1}) was observed at pH 6.0 (Fig. 4). The maximum color and lignin removal rates (76% and 55%, respectively) were also found at pH 6.0. Even at pH 8.0, the laccase still kept a high activity (294 U L^{-1}). Consequently, the black liquor was effectively decolorized by the strain B1. The pH of black liquor is generally higher (Wang et al. 2002). These results demonstrate that the strain B1 possesses potential application in cotton pulp black liquor treatment.

As can be seen from Fig. 5, when the age of mycelium was 7 days, the laccase activity was the highest (490 U L^{-1}), and the color and lignin removal rates were 76% and 55%,

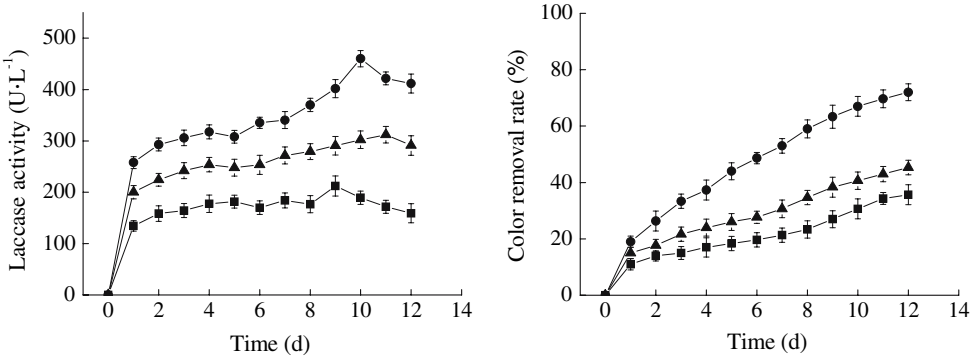

Fig. 2 The effects of aeration rate on the laccase activity (Left) and color removal rate (Right) of strain B1 in BCR (30°C, pH 7.0, mycelia age 7 days, dilution rate 0.2). ■, 0.8 L min^{-1}; ●, 1.2 L min^{-1}; ▲, 1.6 L min^{-1}

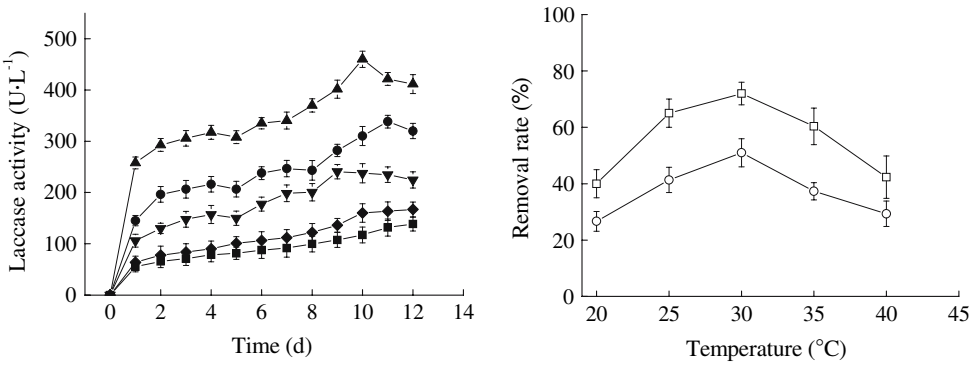

Fig. 3 The effects of temperature on laccase activity (Left) as well as color and lignin removal rates (Right) of strain B1 in BCR (pH 7.0, 1.2 L min^{-1}, mycelia age 7 days, dilution rate 0.2). ■, 20°C; ●, 25°C; ▲, 30°C; ▼, 35°C; ◆, 40°C; □, Color removal; ◯, Lignin removal

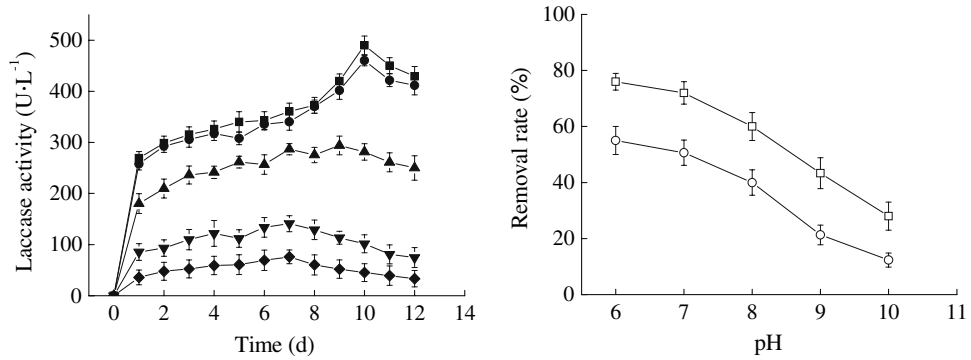

Fig. 4 The effects of pH on laccase activity (Left) as well as color and lignin removal rates (Right) of strain B1 in BCR (30°C, 1.2 L min^{-1}, mycelia age 7 days, dilution rate 0.2). ■, pH 6.0; ●, pH 7.0; ▲, pH 8.0; ▼, pH 9.0; ◆, pH 10.0; □, Color removal; ◯, Lignin removal

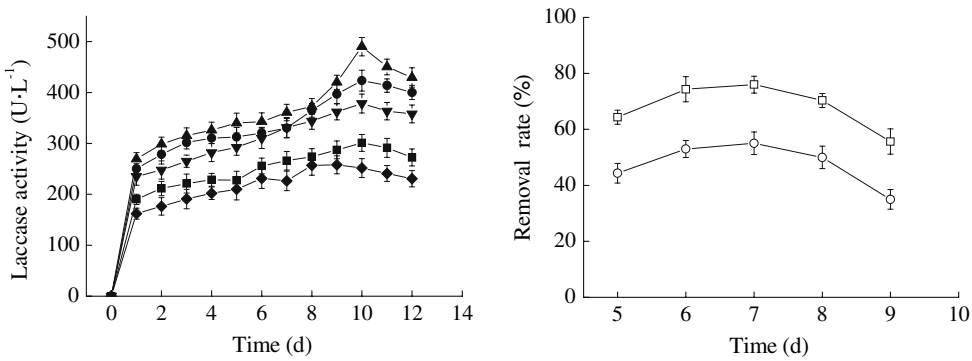

Fig. 5 The effects of mycelia age on laccase activity (Left) as well as color and lignin removal rates (Right) of strain B1 in BCR (30°C, pH 6.0, 1.2 L min^{-1}, dilution rate 0.2). ■, 5.0; ●, 6.0; ▲, 7.0; ▼, 8.0; ◆, 9.0; □, Color removal; ◯, Lignin removal

respectively. When the age of mycelium was more than 7 days, the laccase activity decreased. These results suggest that the active growth phase of the strain B1 is almost completed over 7 days.

The maximum laccase activity, color and lignin removal rates were obtained at a dilution rate of 0.2 (Fig. 6). The laccase activity decreased with the increase of the dilution rate. At a dilution rate of 1.0, the laccase activity was only

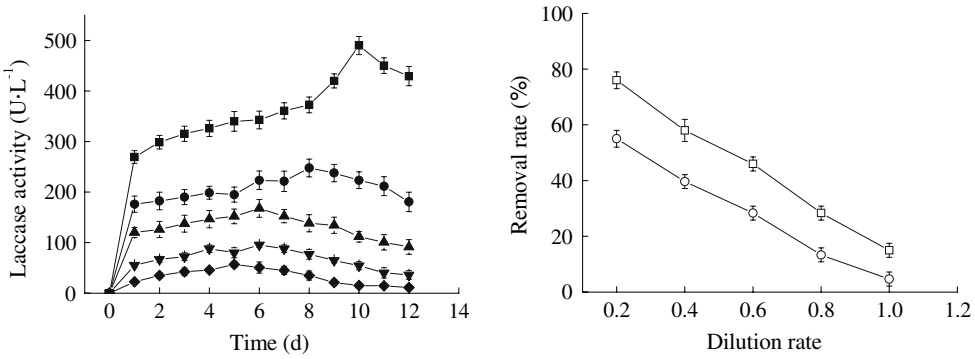

Fig. 6 The effects of dilution rate on laccase activity (Left) as well as color and lignin removal rates (Right) of strain B1 in BCR (30°C, pH 6.0, 1.2 L min^{-1}, mycelia age 7 days). ■, 0.2; ●, 0.4; ▲, 0.6; ▼, 0.8; ◆, 1.0; □, Color removal; ◯, Lignin removal

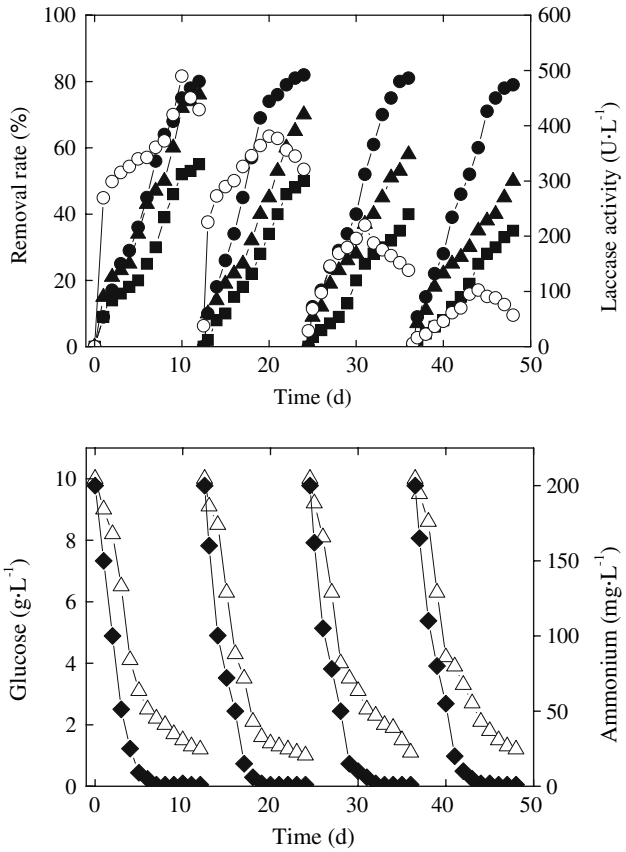

Fig. 7 Color (▲), COD (●) and lignin (■) removal, glucose (△) and ammonium (◆) consumption and laccase activity (◯) of the BCR operated in a repeated-batch mode

57 U L^{-1}. Thus, the laccase activity was inhibited when the concentration of cotton pulp black liquor increased.

Bubble-column reactor was operated in a repeated batch mode under optimum conditions. It was run for 48 days and the same mycelial pellets were reused for four cycles. Figure 7 shows the variation of laccase activity, color, lignin and COD removal rates, and consumption of glucose and ammonium. For the first two cycles, the color removal rates were 76% and 70%, respectively. For the next two cycles, the color removal rates decreased to 58% and 50%, respectively. These results demonstrate that even after four cycles, the strain B1 can still decolorize cotton pulp black liquor effectively. The reuse of mycelium may have a great economic benefit. The variation of lignin removal followed the same trend. The lignin removal rate was 55% at the first cycle and then decreased to 35% after the fourth cycle. Glucose was consumed at an average rate of 0.73 g L^{-1} day^{-1}. Most ammonium was consumed after 6 days. The COD removal rate of each cycle was about 80%. As the cotton pulp black liquor consisted of lignin and other organic compounds, the lignin removal, glucose consumption and organic compounds degradation contributed to the higher COD removal rate. Yang and Wang (1999) reported the treatment of cotton pulp black liquor by activated sludge. The COD removal rate was 58%–64%. Font et al. (2003) investigated the treatment of black liquor from a soda pulping mill by white-rot fungus *Trametes versicolor*. The color removal rate was 70%–80% and the COD removal rate was 60%. From this study, it can be concluded that BCR with *P. ostreatus* has a great potential for being applied to the treatment of cotton pulp black liquor.

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