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Simple fabrication of polymer-based *Trametes versicolor* laccase for decolorization of malachite green

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ABSTRACT

A highly efficient and stable biocatalyst (denoted D201_Lac) was fabricated by encapsulating *Trametes versicolor* laccase within a macroporous and strongly basic exchange resin D201 through a simple adsorption process. Transmission electron micrographs and Fourier transform infrared spectra of the resultant D201_Lac proved that nanosized laccase clusters were embedded into the inner nano-pores/channels of D201. As compared to the free laccase, D201_Lac showed enhanced resistance in the pH range of 3–7 or at temperature of 30–60 °C. Besides, negligible laccase was leached out from the host polymer D201 in solution of pH 3–7 and NaCl concentration up to 0.5 M, which might be attributed to the electrostatic attraction and the possible twining between long-chain laccase and the cross-linking host resin. Continuous seven-cycle batch decoloration of malachite green demonstrates that decoloration efficiency of D201_Lac kept constant for more than 320-h operation.

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1. Introduction

Laccase (benzenediol:oxygen oxidoreducatase, EC 1.10.3.2), which is widespread in fungi, is a group of polyphenol oxidases containing copper atoms. Laccase from fungi is proved effective in oxidation of various phenolic compounds with the comitant reduction of oxygen to water (Karam and Nicell, 1997). The substrate range of fungal laccase can be extended by inclusion of a mediator, such as 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonate) (ABTS), 1-hydroxybenzotriazole (Hobt), etc. (Srebotnik and Hammel, 2000; Cabana et al., 2009). The potential applications of fungal laccase in biodegradation of pollutants (Rodriguez Couto and Toca Herrera, 2006) have been well recognized. It is worth noting that laccase is often used to decolorize dye effluents and has excellent performance (Camarero et al., 2005; Chhabra et al., 2009; Khlifi et al., 2010). However, the use of laccase in large-scale industrial applications is still restricted by its relatively poor stability against environmental factors such as pH and temperature. Also, laccase is well soluble in water. Its recovery and reuse is still a challenge because laccase is currently of high cost. Previous studies (Peralta-Zamora et al., 2003; Cabana et al., 2009; Arica et al., 2009; Rekuc et al., 2010) proved that laccase immobilization is favorable to enhance its resistance against severe environmental factors (such as extreme pH or temperature) and to achieve a simple separation from the reaction systems, which is particularly significant for its recovery and reuse and for development of continuous bioprocessess for practical use.

In review of the methods for enzyme immobilization onto carriers, adsorption is obviously a basic and simple one. Various enzymes have been immobilized onto carriers by adsorption (Lei et al., 2002, 2006; Bayramoglu et al., 2011). However, leaching is a troublesome problem in the application of the resultant composite catalysts with the above-mentioned materials as carriers, especially when used in a wide pH range or at high ionic strengths. For example, Qiu et al. (2009) have ever immobilized laccase onto nanoporous gold by means of physical adsorption. As the obtained enzyme composite was introduced into a phosphate–citric acid buffer solution (50 mM, pH 4.4) for 1 h at 4 °C, a considerable amount laccase was leached out from the composite. Wang and Caruso (2004) reported that substantial amounts (up to 87%) of catalase adsorbed onto mesoporous silicas (2 nm in diameter) would be desorbed when merely immersed in 50 mM phosphate buffered saline for 48 h. To improve the stability of the enzyme composites based on adsorption technique, several strategies, either strengthening the interactions between carriers and enzymes or designing special structures to restrict enzymes within nanopores/nanochannels, have been proposed in recent years, including modifying carriers with amine or carboxylate groups, cross-linking enzymes inside nanochannels/nanopores, partially closing pore openings by silylation of the pre-loaded enzymes, and deposition of layers to cover the pore openings (Lee et al., 2009; Hartmann and Jung, 2010). As expected, they are efficient in stabilizing the enzyme within the frameworks of carriers. However, the excessive reactions used in these strategies are very likely

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