



Enhanced phenol degradation in coking wastewater by immobilized laccase on magnetic mesoporous silica nanoparticles in a magnetically stabilized fluidized bed

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ABSTRACT

The immobilized laccase on magnetic mesoporous silica nanoparticles has been developed for efficient phenol degradation. The degradation rate of phenol by the immobilized laccase was 2-fold higher than that of the free laccase, and the immobilized laccase retained 71.3% of its initial degradation ability after 10 successive batch treatments of coking wastewater. The phenol degradation in the coking wastewater was enhanced in a continuous treatment process by the immobilized laccase in a magnetically stabilized fluidized bed (MSFB) because of good mixing and mass transfer. The degradation rate of phenol maintained more than 99% at a flow rate of less than 450 mL h⁻¹ and decreased slowly to 91.5% after 40 h of the continuous operation in the MSFB. The present work indicated that the immobilized laccase on magnetic mesoporous supports together with the MSFB provided a promising avenue for the continuous enzymatic degradation of phenolic compounds in industrial wastewater.

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1. Introduction

Phenol in industrial wastewaters has severe toxicity to human health, and a number of physical, chemical and biological methods have been developed for the removal or degradation of phenol (Ahmaruzzaman, 2008; Bayer et al., 2010; Ho et al., 2010; Wu et al., 2001). Due to the high solubility of phenol, it is still difficult to achieve satisfactory removal efficiency by current treatment techniques (Saitoh et al., 2011). Up to date, there is still a great interest to develop novel technologies for the convenient, robust and cost effective treatment of phenol and phenolic compounds.

Enzymatic wastewater treatment is a promising technology for the degradation of phenol and phenolic compounds with high degree of specificity and minimal environmental impact (Gomez et al., 2009). Laccase belongs to a group of polyphenol oxidases containing copper atoms in the catalytic center, and laccase-induced oxidation of phenol and phenolic compounds has been extensively investigated recently (Georgieva et al., 2010; Jeon et al., 2011; Justino et al., 2010; Kolb et al., 2012; Miele et al., 2010). The essential limitation of laccase used in degradation of phenol and phenolic compounds is its low stability and productivity, as well as its high production cost (Busca et al., 2008). In order to reduce these limitations in its industrial application, laccase has

been reported to be successfully immobilized on various carriers for improving biodegradation of phenol and phenolic compounds (Ispas et al., 2009; Liu et al., 2011; Lloret et al., 2011; Park et al., 2005).

To date, there is no report on enzymatic treatment of phenolic compounds in coking wastewater. The immobilized laccase on Cu²⁺-chelated magnetic mesoporous silica nanoparticles exhibited remarkably improved catalytic capacity and stability properties in previous study (Wang et al., 2010). The objective of the current study aims to investigate the degradation performance of phenol and total phenolic compounds in coking wastewater by the immobilized laccase. Scale-up biodegradation process of phenol and total phenolic compounds in coking wastewater was further evaluated in a magnetically stabilized fluidized bed (MSFB).

2. Methods

2.1. Materials

3-Chloropropyl-trimethoxysilane (CPTS), 2,2-azinobis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) and laccase from *Trametes versicolor* were purchased from Sigma-Aldrich. Polyoxypropylene diamine (H₂NCH(CH₃)CH₂[OCH₂CH(CH₃)]_nNH₂, *n* ~ 33, MW = 2000, Jeffamine D-2000) was purchased from Huntsman Corp. All other materials were of analytical grade and were obtained from Beijing Chemical Reagents Company (Beijing, China). The coking wastewater collected from the outlet of a coke plant (Jinan,

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Shandong Province, China) contains 448 mg L⁻¹ of total phenolic compounds including 295 mg L⁻¹ of phenol. Because the optimal pH value for degrading phenol with the immobilized laccase is 6.0, the pH value of the coking wastewater was adjusted to 6.0 by adding HCl before the enzymatic treatment.

2.2. Laccase immobilization on Cu²⁺-chelated magnetic mesoporous silica nanoparticles

The prepared Cu²⁺-chelated magnetic mesoporous silica nanoparticles (MMSNPs-CPTS-IDA-Cu²⁺) with an average particle size of 287 nm have a pore volume of 2.14 cm³ g⁻¹, Barrett–Joyner–Halenda (BJH) pore size of 14.5 nm and Brunauer–Emmett–Teller (BET) surface area of 579 m² g⁻¹ (Wang et al., 2010). The saturation magnetization (Ms), remnant magnetization (Mr) and coercivity (Hc) of these magnetic mesoporous silica nanoparticles are 4.874, 0.056 emu g⁻¹, and 3.462 Oe, respectively. Laccase was immobilized on MMSNPs-CPTS-IDA-Cu²⁺ via metal affinity adsorption. Hundred milligrams of these particles was mixed with 100 mL of 0.1 mg mL⁻¹ laccase solution prepared with tartaric acid buffer (pH 3.5). The mixture was subsequently incubated at 25 °C with shaking at 150 r min⁻¹ for 1 h. The laccase-adsorbed magnetic particles were separated from the enzyme solution magnetically and washed with tartaric acid buffer until that no protein was detected in the supernatant. The laccase adsorption capacity on MMSNPs-CPTS-IDA-Cu²⁺ is 98.1 mg g⁻¹-particles, and the immobilized laccase has an activity of 29.6 U mg⁻¹-particles.

2.3. Batch treatment of phenol and coking wastewater with immobilized laccase in flasks

Phenol was used to evaluate enzymatic degradation performance by the immobilized laccase in buffer solutions. The enzymatic oxidation of phenol was carried out in shake flasks on a rotary shaker (150 r min⁻¹) at 25 °C. The effects of pH, temperature, initial phenol concentration and laccase amount on catalytic activity of free and immobilized laccase were determined in these flasks. Kinetic parameters (K_m and V_{max}) of the free and immobilized laccase were determined by measuring initial rates of the reaction with phenol concentration in the range of 0.2–10 mmol L⁻¹ in buffer solutions at 25 °C.

For analyzing polymer size of the degradation products, the phenol oxidation process was carried out at the optimal conditions in 1 mmol L⁻¹ phenol solution by using the immobilized laccase. The phenol oxidation process was stopped at different reaction times by removing the immobilized laccase with the magnet for analyzing the polymer size.

Effect of the degradation products on the enzymatic phenol oxidation was tested at different concentration of the degradation products for the free and immobilized laccase. To achieve the enough the degradation products, phenol oxidation was conducted at 1 mmol L⁻¹ substrate with the immobilized laccase. After 60 min, the immobilized laccase was separated by magnet and the reaction solution was filtrated with 0.22 µm film. The resulted degradation products were washed with Millipore water for three times, collected, and suspended in the water by ultrasound for further use.

Oxidation of phenol and total phenolic compounds in coking wastewater was conducted in 250 mL shake flasks on a rotary shaker (150 r min⁻¹) at 25 °C, and samples were withdrawn for measuring both phenol and total phenolic compounds. The reusability of the immobilized laccase was assessed by repeated oxidation reaction of phenol in both buffer system and coking wastewater. At the end of each oxidation cycle, the immobilized laccase was separated by magnet and the procedure was repeated with a fresh aliquot of substrate at the same reaction condition.

2.4. Continuous treatment of coking wastewater in MSFB

The MSFB was designed for the continuous treatment of phenols in coking wastewater (Fig. 1). The MSFB consists of a glass column (D_{in} : 1.5 cm, H : 20 cm) and four copper wire coils which is connected to a power supply (0–10 A, 0.1 A sensitivity) and produces the axially uniform magnetic field (120 Gs) to prevent the escape of the magnetic particles from the bed. All experiments were carried out at 25 ± 1 °C in the MSFB which was controlled by circulating water through the water jacket. The immobilized laccase was magnetically stabilized in the MSFB, and the coking wastewater was pumped into the MSFB through the bottom inlet at a range of flow rate from 350 to 650 mL h⁻¹.

2.5. Analysis

Laccase activity was measured with ABTS in 100 mM tartaric acid buffer (pH 3.0) at 25 °C, and one unit of the laccase activity was defined as the amount of enzyme required to oxidize 1 µmol of ABTS per minute (Jung et al., 2002). Total phenolic compounds were analyzed by measurement of absorbance at a wavelength of 510 nm using UV-2100 spectrophotometer after color development by 4-aminoantipyrene method (Wolfenden and Willson, 1982).

During continuous treatment of coking wastewater in MSFB, the phenol degradation rate was calculated according the following equation.

$$\text{Phenol degradation rate (\%)} = \left(1 - \frac{\text{Phenol concentration in the effluent}}{\text{Initial phenol concentration in the coking wastewater}} \right) \times 100\% \quad (1)$$

Phenol was analyzed on an Agilent 1100 HPLC system equipped with a quaternary pump, an on-line solvent vacuum degasser, an auto sampler with 20 µL injection loop and a UV detector. An AichromBcnd-AQ-C18 column (250 mm × 4.6 mm i.d., 5 µm) was used, and an elution system consisted of 70% (v/v) methanol and 30% (v/v) water. The flow rate is 1.0 mL min⁻¹, and the analytical wavelength is 280 nm. All samples were percolated with a 0.22 µm colander before HPLC analysis (Kang et al., 2009).

Characteristics of phenol and phenolic oxide in the solution form were determined directly by Fourier transform infrared spectroscopy (FT-IR, Bruker, Vector 22, Germany) using BaF pellets. The degradation products were moderately purified from the phenol oxidation solution by a solid phase extraction cartridge (Chromond C18, 500 mg, 6 mL total volume, Macherey and Nagel). The resulted degradation products were dissolved in 1 mL Millipore water for the FT-IR observation. Polymer size of the degradation

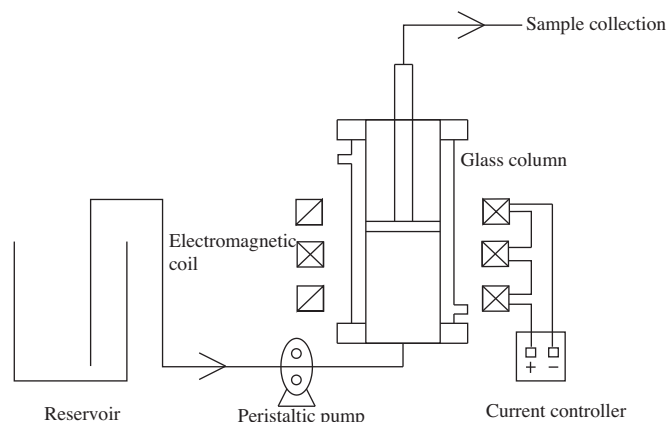


Fig. 1. Diagram of a magnetically stabilized fluidized bed.

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