Chinese Journal of Catalysis 36 (2015) 961–968

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/chnjc

Article

Enzymatic removal of chlorophenols using horseradish peroxidase immobilized on superparamagnetic Fe_3O_4/graphene oxide nanocomposite

Qing Chang [a], Guodong Jiang [b,*], Heqing Tang [a], Na Li [a], Jia Huang [a], Laiyan Wu [a]

[a] *Key Laboratory of Catalysis and Materials Science of the State Ethnic Affairs Commission and Ministry of Education, College of Chemistry and Material Science, South-Central University for Nationalities, Wuhan 430074, Hubei, China*

[b] *College of Chemistry and Chemical Engineering, Hubei University of Technology, Wuhan 430068, Hubei, China*

ARTICLE INFO

Article history:
Received 26 January 2015
Accepted 8 April 2015
Published 20 July 2015

Keywords:
Magnetic nanoparticle
Immobilized enzyme
Horseradish peroxidase
Biodegradation
Chlorophenols
Wastewater treatment

ABSTRACT

Magnetic Fe_3O_4 nanoparticles were successfully deposited on graphene oxide sheets by ultrasound-assisted coprecipitation. The nanoparticles were characterized using transmission electron microscopy, vibrating sample magnetometry, and X-ray photoelectron spectroscopy. The synthesized material was used as a support for the immobilization of horseradish peroxidase (HRP). The removals of 2-chlorophenol, 4-chlorophenol, and 2,4-dichlorophenol using the immobilized HRP were investigated. Batch degradation studies were used to determine the effects of the initial solution pH values, reaction temperature, reaction time, H_2O_2 and chlorophenol concentrations, and immobilized enzyme dosage on the removal of chlorophenols. The different numbers and positions of electron-withdrawing substituents affected the chlorophenol removal efficiency; the order of the removal efficiencies was 2-chlorophenol < 4-chlorophenol < 2,4-dichlorophenol. The oxidation products formed during chlorophenol degradation were identified using gas chromatography-mass spectrometry. The biochemical properties of the immobilized HRP were investigated; the results indicated that the storage stability and tolerance to changes in pH and temperature of the immobilized HRP were better than those of free HRP. The nanoparticles were recovered using an external magnetic field, and the immobilized HRP retained 66% of its initial activity for the first four cycles, showing that the immobilized HRP had moderate stability. These results suggest that the immobilized enzyme has potential application in wastewater treatment.

1. Introduction

Chlorophenols, which are chlorinated aromatic compounds, are widely used in the manufacture of plastics, pharmaceuticals, printing and dyeing materials, pesticides, wood preservatives, and petrochemicals [1]. They are a major group of pollutants of environmental concern because of their high toxicities. In particular, some of them have carcinogenic, teratogenic, and mutagenic effects. In 1987, the US Environmental Protection Agency categorized chlorophenols as priority pollutants and set an upper permissible limit of 0.5 mg/L in public water supplies [2,3]. Chlorophenols in aquatic environments must therefore be continuously monitored. Many techniques, including physical adsorption [4,5], catalytic oxidation [6,7], and biodeg-

* Corresponding author. Tel/Fax: +86-27-59750481; E-mail: jianggd66@gmail.com
This work was supported by the National Natural Science Foundation of China (21107143, 21207033 and 21307164) and the Fundamental Research Funds for the Central Universities, South-Central University for Nationalities (CZY15003).
DOI: 10.1016/S1872-2067(15)60856-7 | http://www.sciencedirect.com/science/journal/18722067 | Chin. J. Catal., Vol. 36, No. 7, July 2015

radation [8,9], have been used for the removal of chlorophenols. Enzymatic degradation has been extensively used because it has the merits of high specificity, selectivity, and catalytic activity, mild reaction conditions, and few byproducts [10]. The degradation of chlorophenols using horseradish peroxidase (HRP), a widely used catalyst in enzymatic reactions [11–13], proceeds through generation of phenoxy radicals, which can react with other substrate molecules to give oligomers or polymers that are much more water insoluble than the original monomers [12,14]. However, the use of free HRP is limited because it has poor stability, and it is expensive because its recovery and reuse are difficult. These drawbacks can be overcome by immobilizing the enzyme [15,16]. The immobilized enzyme should retain the same functionality and have the advantages of better storage stability, thermal stability, and ease of operation compared with those of the free enzyme in solution [17].

Graphene oxide (GO), a derivative of graphene, is a two-dimensional planar material of one-atom thickness and has a large specific surface area. It is therefore a good candidate for supporting metal oxide nanoparticles and the immobilization of a large number of enzymes [18,19]. GO contains a range of reactive oxygen functional groups on the surface, therefore it can be well dispersed in water and easily modified. The large amount of surface functional groups on GO enables rapid enzyme immobilization through electrostatic interactions [20,21]. It has been reported that GO is an ideal platform for accepting electrons from an enzyme and transferring them to a substrate [22].

The superparamagnetism of magnetic nanoparticles (MNPs) is attractive in a broad range of biomedical application because the nanoparticles can be easily separated using an external magnetic field and redispersed rapidly after removing the magnetic field [23]. Fe_3O_4 MNPs are biocompatible, are not hemolytic or genotoxic, and are superparamagnetic; they therefore have potential application in areas such as separation of biochemical products [24], magnetic resonance imaging [25], targeted drug delivery [26], and biosensing [27]. The use of solid enzyme supports and immobilized enzymes in degradation using enzymes has attracted our interest. In the present work, Fe_3O_4 nanoparticles were deposited on GO sheets using an ultrasound-assisted coprecipitation method, and HRP was then immobilized on the Fe_3O_4/GO magnetic nanocomposite. The Fe_3O_4/GO MNPs with immobilized HRP were used to remove chlorophenols in the presence of H_2O_2. The effects of the pH values, reaction temperature, reaction time, and H_2O_2, chlorophenol, and immobilized enzyme concentration on the removal of chlorophenols were investigated. The stability and reusability of the immobilized enzyme were also evaluated. The oxidation products formed during chlorophenol degradation were identified.

2. Experimental

2.1. Materials and characterization

All reagents were analytical grade and used without further purification. $KMnO_4$, $FeCl_3·6H_2O$, $FeSO_4·7H_2O$, $NH_3·H_2O$, H_2O_2 (30% *m*/*m*), 2-chlorophenol, 4-chlorophenol, 2,4-dichlorophenol, 1-ethyl-3-(3-dimethylaminopropy)carbodiimide (EDC), $NaH_2PO_4·2H_2O$, $Na_2HPO_4·12H_2O$, potassium ferricyanide, and 4-aminoantipine (4-AAP) were obtained from the Sinopharm Chemical Reagent Co., Ltd. (China). HRP (specific activity 250 units/mg, RZ ≥ 3) was purchased from the Tianyuan Biologic Engineering Corp. (China). Natural flake graphite (99%) was obtained from the Qingdao Lihaofeng Graphite Co., Ltd. (China). Doubly distilled water was used.

The product morphology was examined using transmission electron microscopy (TEM; FEI Tecnai G2 20, USA). The magnetic properties were evaluated using an ADE 4HF vibrating sample magnetometer at 27 °C (USA). X-ray photoelectron spectroscopy (XPS) was performed using a VG Multilab 2000 spectrometer (Thermo Electron Corporation, USA) with Al K_α radiation as the excitation source. Ultraviolet-visible spectroscopy was performed using an Evolution 201 spectrophotometer (Thermo Scientific, USA). The chlorophenol oxidation intermediates were analyzed using a gas chromatography-mass spectrometry (GC/MS) system (Thermo Scientific, USA) equipped with a TR-5MS column (Thermo Scientific, USA).

2.2. Synthesis of nanostructured Fe_3O_4/GO nanoparticles

GO was obtained using the method described by Hummers and Offeman [28]. The GO was added to distilled water and ultrasonically exfoliated in a bath sonicator for 30 min to obtain a GO dispersion. Fe_3O_4/GO MNPs were synthesized using an ultrasound-assisted coprecipitation method [21]. Briefly, $FeSO_4·7H_2O$ (6.0×10^{-4} mol) and $FeCl_3·6H_2O$ (9.0×10^{-4} mol) dissolved in distilled water (20 mL) were mixed with the GO dispersion. The mixture was added dropwise to ammonia water (20 mL, 12.0 mol/L) at 60 °C and reacted for 1 h under ultrasound irradiation in an ultrasound cleaning bath (KQ-200KDE, Kunshan Ultrasound Instrument Co., Ltd.). The product was washed with ethanol and distilled water until neutral and then dried to obtain GO/Fe_3O_4 MNPs.

2.3. Enzyme immobilization

GO/Fe_3O_4 MNPs (0.3 g) and HRP (3 mg) were dispersed in phosphate buffer (pH = 6, 20.0 mL), followed by addition of EDC (1.5 mL, 25 mg/mL). The mixture was incubated in a shaker at 200 r/min for 12 h at 25 °C. The nanoparticles were collected by magnetic separation and washed separately with phosphate buffer and water to remove non-specifically unbound enzyme. The immobilized enzyme was dispersed in water and stored at 4 °C.

2.4. Catalytic experiments

Immobilized enzyme solution (1.2 mL) was added to an aqueous solution of chlorophenol (40 mL, 0.5 mmol/L); the pH was adjusted to 6.4 using sodium phosphate buffer. The mixture was vibrated at a speed of 200 r/min at 25 °C. After 30 min to achieve adsorption-desorption equilibrium, the reaction was