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

initiated by the addition of H_2O_2 (0.5 mmol/L). At given time intervals, 2 mL aliquots of the reaction solution were removed and the MNPs were immediately recovered by magnetic separation. The concentration of chlorophenol in the supernatant was determined spectrophotometrically [29].

2.5. Assays of enzymatic activity

A colorimetric method was used to determine the activity of the enzyme using phenol, 4-AAP, and H_2O_2 as substrates [30]. The catalytic reaction was monitored by recording the absorbance of the red product at 510 nm. One unit of activity was defined as the amount of HRP required to hydrolyze 1×10^{-6} mol of H_2O_2 per minute under the conditions stated above.

2.6. Recycling experiments

The used immobilized enzyme was collected by centrifugation and magnetic separation, and washed with deionized water to remove the reaction solution. The recovered catalyst was reused in subsequent degradation reactions under the experimental conditions used in the original reaction.

3. Results and discussion

3.1. Characterization of Fe_3O_4/GO composites

A TEM image of the prepared Fe_3O_4/GO composite (Fig. 1(a)) clearly shows GO sheets uniformly decorated with Fe_3O_4 nanoparticles with a narrow size distribution, some of which aggregated to give larger particles. The magnetization curves of the Fe_3O_4/GO nanoparticles and Fe_3O_4 nanoparticles were measured at room temperature; the results are shown in Fig. 1(b). The hysteresis loops of the dried Fe_3O_4/GO and Fe_3O_4 nanoparticles are S-shaped curves; this shows that the samples have negligible coercivity (Hc) and remanence, and are therefore typical superparamagnetic materials. The specific saturation magnetizations (M_s) of the Fe_3O_4/GO and Fe_3O_4 nanoparticles are 26.0 and 68.8 emu/g, respectively.

XPS is an effective technique for determining surface chemical states. Fig. 2(a) shows the C 1*s* deconvolution spectra of Fe_3O_4/GO MNPs; there are four peaks, centered at 284.5, 286.5, 288.2, and 289.0 eV, and these are assigned to non-oxygenated carbon (C=C/C–C) in aromatic rings, C–O (epoxy and alkoxy), C=O, and O–C=O groups, respectively. The spectrum shows that non-oxygenated carbon (284.5 eV) and C–O (286.2 eV) are the predominant carbon forms in the GO. The formation of Fe_3O_4 nanoparticles is confirmed by the high-resolution Fe 2*p* spectrum (Fig. 2(b)), in which the observed peaks are at 724.8 and 711.2 eV, characteristic of Fe $2p_{1/2}$ and Fe $2p_{3/2}$ of Fe_3O_4 [31], rather than at 710.35 and 724.0 eV, from γ-Fe_2O_3 [32].

3.2. Effect of reaction conditions

3.2.1. pH values

The effect of pH values on the degradation of 2-chlorophenol, 4-chlorophenol, and 2,4-dichlorophenol by the immobilized enzyme was investigated. The results are shown in Fig. 3.

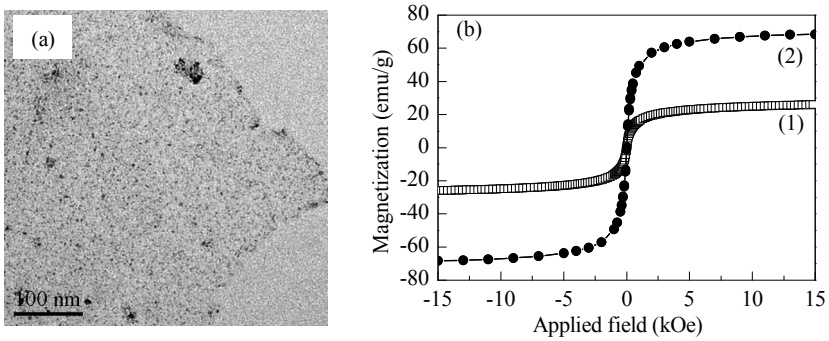


Fig. 1. (a) TEM image of Fe_3O_4/GO MNPs and (b) magnetization curves of Fe_3O_4/GO MNPs (1) and Fe_3O_4 MNPs (2) measured at 27 °C.

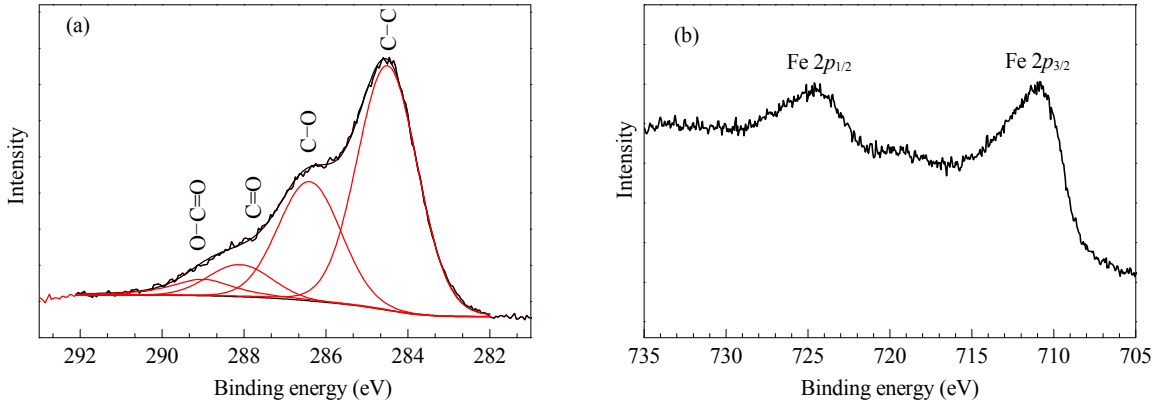


Fig. 2. XPS profiles of Fe_3O_4/GO MNPs. (a) C 1*s*; (b) Fe 2*p*.

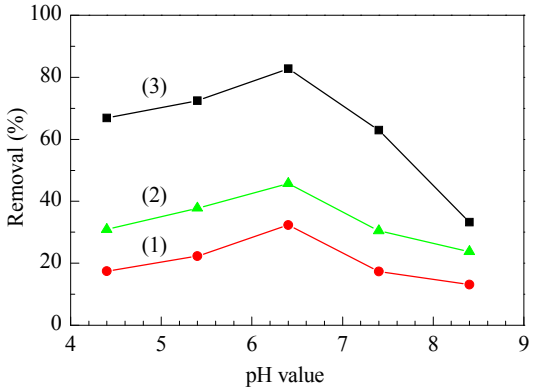


Fig. 3. Effect of pH values on chlorophenol degradation. (1) 2-chlorophenol; (2) 4-chlorophenol; (3) 2,4-dichlorophenol.

The optimum pH for the degradation of chlorophenol compounds using the immobilized HRP is observed at around pH = 6.4, with maximum removals in 2 h of 83%, 46%, and 32% for 2,4-dichlorophenol, 4-chlorophenol, and 2-chlorophenol, respectively. The catalytic activity of the immobilized enzyme may be decreased by denaturation under acidic or basic conditions.

3.2.2. *Reaction temperature*

An immobilized enzyme has an optimum temperature range in which its activity is maximum. The degradation of 2-chlorophenol, 4-chlorophenol, and 2,4-dichlorophenol using immobilized HRP was studied. Fig. 4 shows that the removals of 2,4-dichlorophenol and 2-chlorophenol initially increase with increasing temperature. A plateau appears in the temperature range 25–30 °C, and the highest removals, i.e., 83% and 32% for 2,4-dichlorophenol and 2-chlorophenol, respectively, are achieved. The degradation efficiency of 2,4-dichlorophenol and 2-chlorophenol catalyzed by the immobilized enzyme decrease at temperatures higher than 30 °C; this is ascribed to enzyme inactivation at high temperatures. For 4-chlorophenol, the maximum removal is observed at 20 °C. It is concluded that the immobilized HRP has different optimum temperatures for different substrates. The optimum temperature range of the immobilized enzyme is easily achieved, therefore this method could be used in the treatment of industrial wastewater.

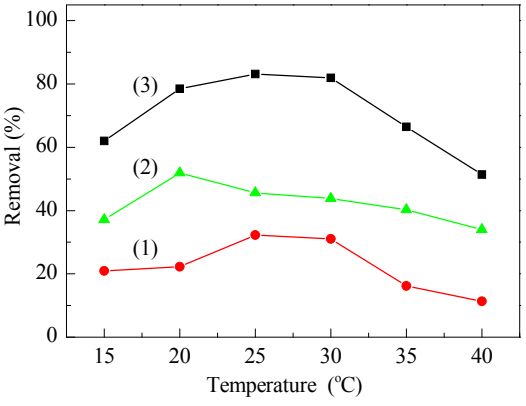


Fig. 4. Effect of temperature on chlorophenol degradation. (1) 2-chlorophenol; (2) 4-chlorophenol; (3) 2,4-dichlorophenol.

3.2.3. *H_2O_2 concentration*

The cosubstrate H_2O_2 activates the enzymatic reaction to produce peroxidase radical intermediates, which attack the chlorophenol compounds to form free radicals [33]. Optimization of the conditions for the removal of chlorophenols was performed using different concentrations of H_2O_2. Fig. 5 shows that the removals of 2-chlorophenol, 4-chlorophenol, and 2,4-dichlorophenol increase significantly with increasing H_2O_2 concentration from 0.05 to 0.5 mmol/L. When the H_2O_2 concentration is higher than 0.5 mmol/L, the chlorophenol degradation efficiency gradually decrease. It has been reported that excess H_2O_2 inhibits the peroxidase activity by inactivating the enzyme [34]; this is in agreement with the results of our study.

3.2.4. *Reaction time*

Fig. 6 shows the effect of time on the degradation of 2-chlorophenol, 4-chlorophenol, and 2,4-dichlorophenol using the immobilized enzyme. The immobilized enzyme activates H_2O_2, therefore chlorophenol removal initially increases rapidly and then reaches a plateau after 120 min. The highest percentage of removal is observed for 2,4-dichlorophenol, followed by 4-cholrophenol and 2-chlorophenol; 83% of the initial 2,4-dichlorophenol is degraded in 120 min. This observation is in agreement with the results of previous studies [35]. The percentage of removal is probably affected by the different

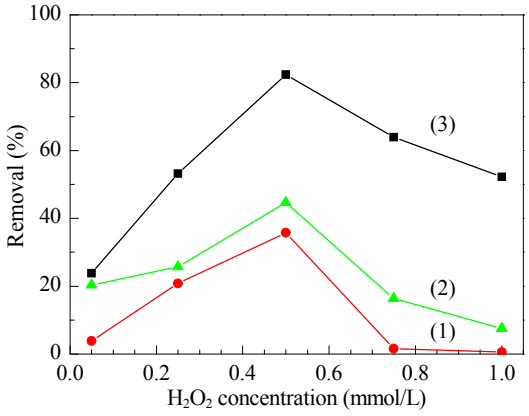


Fig. 5. Effect of H_2O_2 concentration on chlorophenol degradation. (1) 2-chlorophenol; (2) 4-chlorophenol; (3) 2,4-dichlorophenol.

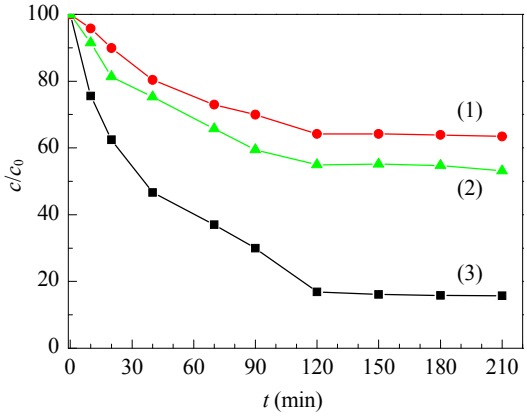


Fig. 6. Effect of time on chlorophenol degradation. (1) 2-chlorophenol; (2) 4-chlorophenol; (3) 2,4-dichlorophenol.

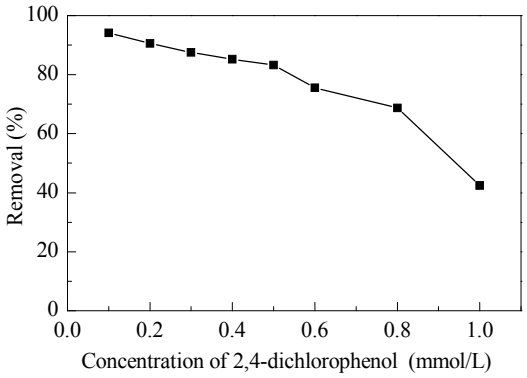


Fig. 7. Effect of initial 2,4-dichlorophenol concentration on degradation.

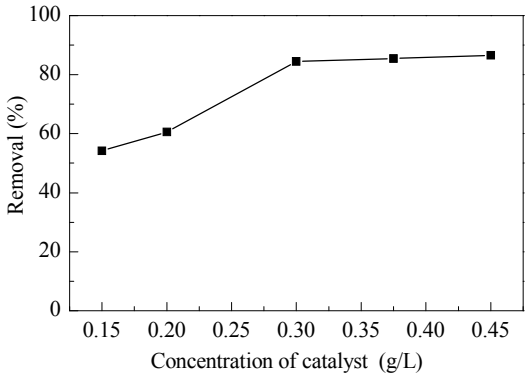


Fig. 8. Effect of catalyst concentration on 2,4-dichlorophenol degradation.

numbers and positions of the electron-withdrawing substituents [36]. Because it has a larger number of substituents on the benzene ring, 2,4-dichlorophenol can be removed via a greater variety of reaction pathways. In addition, the different phenoxy radical intermediates, which are produced by enzymatic catalysis, may have different toxicities to the enzyme.

3.2.5. 2,4-Dichlorophenol concentration

The substrate concentration in the aqueous phase significantly affects enzyme-mediated reactions. The effect of the 2,4-dichlorophenol concentration on the removal efficiency was investigated, and the results are shown in Fig. 7. The 2,4-dichlorophenol removal efficiency decreases with increasing 2,4-dichlorophenol concentration. These results enable the initial concentrations of chlorophenols in wastewaters needed to achieve optimum removal efficiency to be determined.

3.2.6. Optimum catalyst dose

The catalyst concentration is also a key factor in chlorophenol removal. The optimum dose of immobilized HRP was determined by performing experiments under specified experimental conditions; the results are shown in Fig. 8. The results clearly show that immobilized HRP can catalytically oxidize 2,4-dichlorophenol effectively. 2,4-Dichlorophenol removal initially increases with increasing catalyst concentration, and then remains almost constant above 0.30 g/L of catalyst. The highest proportion of the cost of enzymatic treatment of wastewater is derived from the enzyme, therefore the balance between the removal efficiency and enzyme cost should be optimized. The optimum catalyst dose is 0.30 g/L.

3.3. Biochemical properties of immobilized HRP

3.3.1. Optimum pH value and temperature

Fig. 9(a) shows the effect of pH values on the relative activity of free and immobilized HRP. The optimum pH values for free and immobilized HRP are both around 6.4. The relative activity of the free and immobilized enzymes decreases when the pH is too acidic or too alkaline. However, the immobilized HRP retains about 85% of its activity at pH = 9.4, whereas free HRP retains about 54% of its activity at this pH value. The pH profile of the immobilized enzyme is broader than that of free HRP, which indicates that the immobilization method preserves the enzymatic activity over a wider pH range [11]. The effects of temperature on the activity of free and immobilized HRP are shown in Fig. 9(b). The relative activity of the free enzyme initially increases with increasing temperature, and then declines with increasing temperature above 30 °C. The decrease in activity can be attributed to deactivation of the enzyme at high temperatures. The immobilized HRP also has maximum activity at 30 °C, but the performance of the enzyme at higher temperatures is improved by immobilization. The immobilized enzyme tolerates a much wider range of temperatures. These results suggest that immobilization stabilizes the enzyme and enables it to resist denaturation by various factors

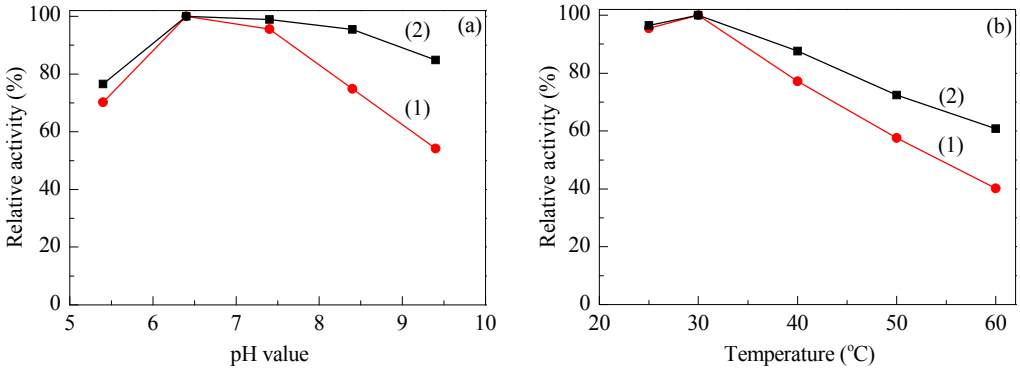


Fig. 9. Effect of (a) pH values and (b) temperature on activity of (1) free and (2) immobilized HRP.

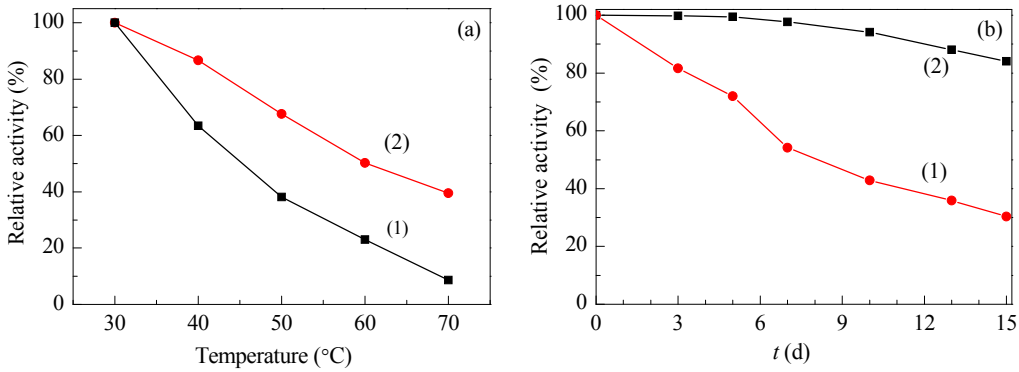


Fig. 10. (a) Thermal and (b) storage stability of (1) free and (2) immobilized HRP.

such as acids, alkalis, and heat.

3.3.2. *Thermal and storage stability*

The immobilized HRP showed good thermal stability during its use in the oxidative removal of organic pollutants. The thermal stability of the immobilized and free enzymes were determined by measuring the residual activity of immobilized HRP incubated at temperatures of 30–70 °C. Fig. 10(a) shows that after heat treatment at 70 °C, the relative activity of free HRP decreases to 9% of the initial value, but the immobilized HRP retains 40% of its initial activity. These results suggest that the immobilized enzyme is more resistant to the heat than the free HRP; this can be attributed to limitation of the conformational changes of HRP molecules in the support matrix.

The storage stability of free and immobilized HRP was also investigated. As shown in Fig. 10(b), the activity of free HRP gradually declines at room temperature. Under the same storage conditions, the activity of the immobilized HRP decreases more slowly than that of the free enzyme. The immobilized HRP retains about 84% of its relative activity during storage for 15 d, whereas the relative activity of the free HRP declines to 30% of its initial value. The immobilized HRP is therefore more stable than the free HRP. The Fe_3O_4/GO magnetic nanocomposite has a stabilizing effect, and the immobilized enzyme has a longer shelf-life than its free counterpart [11].

3.4. *Reusability of immobilized HRP*

Compared with the free enzyme, the immobilized enzyme can be easily separated from the reaction solution and reused. The recovery and reusability of HRP immobilized on the Fe_3O_4/GO magnetic nanocomposite were studied. The used immobilized enzyme was recovered by magnetic separation, washed with water to remove the reaction solution, and reused in a subsequent reaction under the same experimental conditions. The removal of 2,4-dichlorophenol using the recycled immobilized enzyme is shown in Fig. 11. The immobilized HRP retains 66% of its initial activity for the first four cycles, showing that the immobilized enzyme has moderate stability and can be recovered and reused. There are two possible reasons for the reduction in the activity. (1) Polymers produced during the enzymatic reaction may cover the immobilized enzyme and block the active sites of the enzyme, leading to decreased activity. (2) The immobilized enzyme could be inactivated by free radicals during enzymatic oxidation of chlorophenol, which would contribute to the activity reduction in the next cycle [11,34]. Use of the immobilized enzyme would greatly reduce the cost of the enzymatic system in practical application because of the ease of separation and good stability.

3.5. *Chlorophenol oxidation products*

The chlorophenol oxidation products were identified using GC/MS; the results are shown in Table 1. Several two-ring aromatic chlorinated compounds were detected. Oxidative coupling reactions of radicals with dimers could result in the generation of trimers, tetramers, pentamers, and so on. Alternatively, the addition of radicals to dimers could lead to formation of high-molecular-mass species. Both processes ultimately result in the generation of polymers, which precipitate from aqueous media [37]. A possible 2,4-dichlorophenol degradation pathway, based on the GC/MS analysis, is shown in Scheme 1. There are two main pathways for 2,4-dichlorophenol degradation: generation of 2-chloro-1,4-benzoquinone and transformation to polymer sediments. HRP is present mainly on the GO surface because the large amount of surface functional groups on GO favor HRP immobilization. Additionally, EDC is used to crosslink the HRP and GO to improve the stability of the immobilized HRP. In the catalytic procedure, HRP activates chlorophenol oxidation by H_2O_2. The immobilized HRP donates a lone

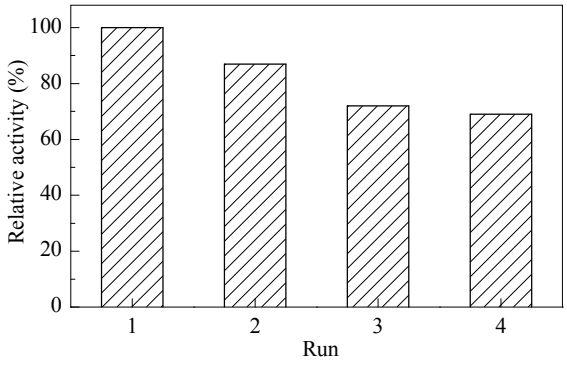
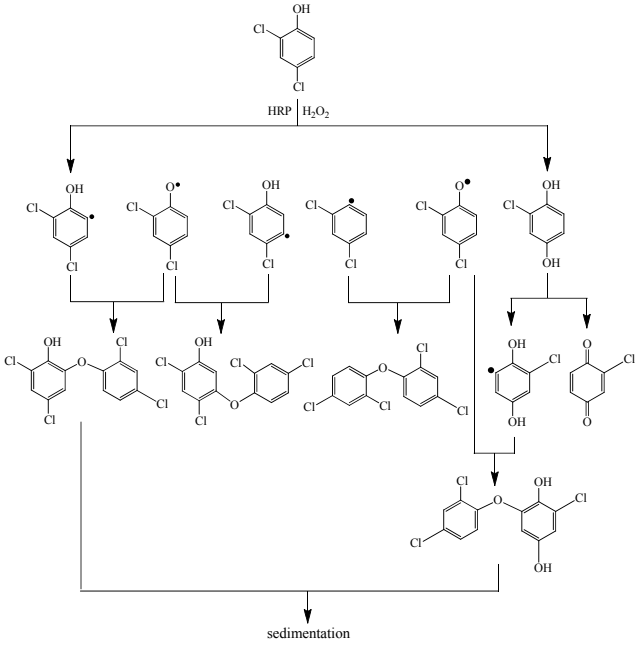


Fig. 11. Activity of reused catalyst.

Table 1
Oxidation products formed during degradation of chlorophenols.

2-Chlorophenol	4-Chlorophenol	2,4-Dichlorophenol
2-Chloro-6-(2-chloro-phenoxy)-phenol	2-(4-Chlorophenoxy)-4-chlorophenol	2,4-Dichloro-6-(2,4-dichloro-phenoxy)-phenol
2-Chloro-4-(2-chloro-phenoxy)-phenol	3-(4-Chlorophenoxy)-4-chlorophenol	2,4-Dichloro-5-(2,4-dichloro-phenoxy)-phenol
Trimer	2-(4-Chlorophenoxy)-1,4-benzenediol	2-Chloro-6-(2,4-dichloro-phenoxy)-1,4-benzenediol
	p-Benzoquinone	2-Chloro-1,4-benzoquinone
	Hydroquinone	2-Chloro-1,4-benzenediol
	Trimer	Biopolymer/trimer



Scheme 1. Possible degradation pathways of 2,4-dichlorophenol with immobilized HRP as catalyst.

pair of electrons to GO, and the electrons are transferred to H_2O_2, resulting in formation of a high-oxidation-state intermediate from HRP [38]. The intermediate catalyzes the oxidation of phenols, generating phenoxy radicals, and is itself reconverted to HRP by two one-electron reduction steps. These free radicals spontaneously form insoluble polymers, which can be removed from solution by sedimentation and filtration.

4. Conclusions

Magnetic Fe_3O_4/GO MNPs were synthesized using an ultrasound-assisted in situ precipitation method. HRP was covalently immobilized on the Fe_3O_4/GO composites using EDC as a coupling agent. The immobilized HRP was used as an enzymatic catalyst to activate H_2O_2 for degradation of 2,4-dichlorophenol, 4-chlorophenol, and 2-chlorophenol. The optimum pH for chlorophenol oxidation using the immobilized HRP was around 6.4. The temperature did not greatly influence chlorophenol removal; the optimum temperature was in the range 25–30 °C. When the molar ratio of H_2O_2/chlorophenol in the batch reaction system was 1.0, maximum removal was achieved within 2 h. The order of the chlorophenol removal efficiencies was 2-chlorophenol < 4-chlorophenol < 2,4-dichlorophenol. The immobilized enzyme had high storage stability. It was recovered by magnetic separation, and the recovered nanoparticles were reused as a catalyst for the enzymatic degradation of chlorophenols.

References

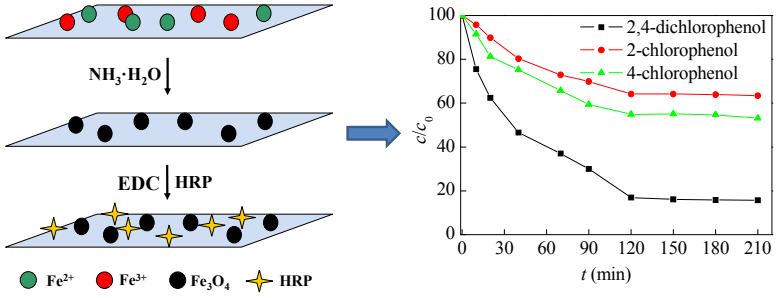
[1] Zhang J B, Xu Z Q, Chen H, Zong Y R. *Biochem Eng J*, 2009, 45: 54
[2] Elghniji K, Hentati O, Mlaik N, Mahfoudh A, Ksibi M. *J Environ Sci*, 2012, 24: 479
[3] Jia J B, Zhang S P, Wang P, Wang H J. *J Hazard Mater*, 2012, 205-206: 150
[4] Pasang T, Ranganathaiah C. *Ind Eng Chem Res*, 2013, 52: 7445
[5] Zhou L C, Meng X G, Fu J W, Yang Y C, Yang P, Mi C. *Appl Surf Sci*, 2014, 292: 735
[6] Munoz M, de Pedro Z M, Casas J A, Rodriguez J J. *Chem Eng J*, 2013, 228: 646
[7] Xu L J, Wang J L. *Appl Catal B*, 2013, 142-143: 396
[8] Jiang Y J, Tang W, Gao J, Zhou L Y, He Y. *Enzyme Microb Technol*, 2014, 55: 1
[9] Li H, Shen T T, Wang X L, Lin K F, Liu Y D, Lu S G, Gu J D, Wang P, Lu Q, Du X M. *Bioresource Technol*, 2013, 137: 286
[10] Yamada K, Shibuya T, Noda M, Uchiyama N, Kashiwada A, Matsuda K, Hirata M. *Biosci Biotechnol Biochem*, 2007, 71: 2503
[11] Bayramoğlu G, Arıca M Y. *J Hazard Mater*, 2008, 156: 148
[12] Dong S P, Mao L, Luo S Q, Zhou L, Feng Y P, Gao S X. *Environ Sci Pollut Res*, 2014, 21: 2358
[13] Magario I, García Einschlag F S, Rueda E H, Zygadlo J, Ferreira M L. *J Mol Catal A*, 2012, 352: 1
[14] Laurenti E, Ghibaudi E, Todaro G, Pia Ferrari R. *J Inorg Biochem*, 2002, 92: 75
[15] Chang Q, Zhu L H, Jiang G D, Tang H Q. *Anal Bioanal Chem*, 2009, 395: 2377
[16] Bukhari A, Idris A, Atta M, Loong T C. *Chin J Catal*, 2014, 35: 1555
[17] Chang Q, Tang H Q. *Molecules*, 2014, 19: 15768
[18] Jiang G D, Lin Z F, Chen C, Zhu L H, Chang Q, Wang N, Wei W, Tang H Q. *Carbon*, 2011, 49: 2693
[19] Zhang F, Zheng B, Zhang J L, Huang X L, Liu H, Guo S W, Zhang J Y. *J Phys Chem C*, 2010, 114: 8469
[20] Zhang J L, Zhang F, Yang H J, Huang X L, Liu H, Zhang J Y, Guo S W. *Langmuir*, 2010, 26: 6083
[21] Chang Q, Tang H Q. *Microchim Acta*, 2014, 181: 527
[22] Zuo X L, He S J, Li D, Peng C, Huang Q, Song S P, Fan C H. *Langmuir*, 2010, 26: 1936
[23] Zamani F, Izadi E. *Chin J Catal*, 2014, 35: 21
[24] Borlido L, Azevedo A M, Roque A C A, Aires-Barros M R. *Biotechnol Adv*, 2013, 31: 1374
[25] Hu Y, Meng L J, Niu L Y, Lu Q H. *Langmuir*, 2013, 29: 9156
[26] Chen M L, He Y J, Chen X W, Wang J H. *Langmuir*, 2012, 28: 16469
[27] Wu S, Wang H N, Tao S Y, Wang C, Zhang L H, Liu Z G, Meng C G. *Anal Chim Acta*, 2011, 686: 81

Graphical Abstract

Chin. J. Catal., 2015, 36: 961–968 doi: 10.1016/S1872-2067(15)60865-7

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

Qing Chang, Guodong Jiang*, Heqing Tang, Na Li, Jia Huang, Laiyan Wu
South-Central University for Nationalities; Hubei University of Technology



Horseradish peroxidase (HRP) was immobilized on superparamagnetic Fe_3O_4/graphene oxide and used for enzymatic removal of chlorophenols. The immobilized enzyme had high stability and was recovered by magnetic separation.

[28] Hummers W S Jr, Offeman R E. *J Am Chem Soc*, 1958, 80: 1339
[29] Wu Y M, Taylor K E, Biswas N, Bewtra J K. *Enzyme Microb Technol*, 1998, 22: 315
[30] Nicell J A, Wright H. *Enzyme Microb Technol*, 1997, 21: 302
[31] Chandra V, Park J, Chun Y, Lee J W, Hwang I C, Kim K S. *ACS Nano*, 2010, 4: 3979
[32] Lu J, Jiao X L, Chen D R, Li W. *J Phys Chem C*, 2009, 113: 4012
[33] Mohan S V, Prasad K K, Rao N C, Sarma P N. *Chemosphere*, 2005, 58: 1097
[34] Cheng J, Yu S M, Zuo P. *Water Res*, 2006, 40: 283
[35] Wright H, Nicell J A. *Bioresource Technol*, 1999, 70: 69
[36] Song H Y, Liu J Z, Xiong Y H, Weng L P, Ji L N. *J Mol Catal B*, 2003, 22: 37
[37] Munoz M, de Pedro Z M, Casas J A, Rodriguez J J. *J Hazard Mater*, 2011, 190: 993
[38] Zhang T, Xu X L, Jin Y N, Wu J, Xu Z K. *J Mol Catal B*, 2014, 106: 56