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Nanoparticulate Functional Materials: Green synthesis and application for the degradation of phenolic compounds

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Abstract. Zinc oxide nanoparticles was synthesized by microwave decomposition of zinc acetate precursor. Horseradish peroxidase (HRP) was immobilized on nano-ZnO. The catalytic activity, physical, and chemical properties of immobilized HRP as well as the interaction between HRP and nano-ZnO was studied. The Nano-ZnO immobilized HRP showed a better property in the thermostability, active pH stability. The Nano-ZnO is more effective than free HRP in the removal of many phenolic compounds. The results show that nano-ZnO is strong functional materials for envrionment protection.

Introduction

Nowadays, nanoparticles are incorporated in many dailyused products, such as electronic devices, sunscreens, cosmetics, paints, and water purification systems, due to their unique properties[1].

Nanoparticles have also a strong potential useability for biologicalpurposes owing to large specific surface area and functional groups. For example, biosensors[2], water-insoluble drug delivery[3].

Phenolic mixtures are common toxic chemicals present in wastewaters[4],The hazardous nature of phenolic compounds and their widespread occurrence in the environment have been well understood[5]. Numerous physical, chemical, and biological approaches have been developed for removing of phenolic compounds[6-7]. However, these methods frequently presented many disadvantages, such as low efficiency.So it is necessary to develop the new methods to degrade these phenolic mixtures.

An alternative method is to use horseradish peroxidase (HRP). This method was first proposed by Klibanov and colleagues[8-9]. Immobilized enzymes have several advantages over free enzymes, including better stability, repeated or continuous use, easy separation from the reaction mixture, possible modulation of the catalytic properties, and prevention of enzyme contamination etc[10-11]. The property and structure of the substrate play an important role to the catalytic activity and property of the immobilized enzyme.Owing to their large surface area, high surface reactivity, and strong adsorption capacity, nano materials are ideal substrates for enzyme immobilization.

The main objective of this article is to preparate ZnO nanoparticle and use it as a substrate for HRP immobilization. The physical and catalytic properties, and applications for removing phenolic compound were explored.

Experimental

Preparation of ZnO nanoparticles. ZnO nanoparticles was synthesized according to the literature[12]. Zinc acetate was dissolved in 50 mL of deionized water, and then solid NaOH was added slowly into the zinc acetate dehydrate solution under magnetic stirring at room temperature and formed a transparent $Zn(OH)_4^{2-}$solution. The white precipitate was collected by centrifugation, washed with deionized water and ethanol several times, and dried in vacuum oven at 40 ℃.

Immobilization of HRP on ZnO nanoparticle. ZnO was washed three times with potassium phosphate buffer and modified with glutaraldehyde. After repeat wash step as described above, ZnO were added to potassium phosphate buffer containing HRP. The mixtures were incubated by shaking for 2 h at 0 ℃ and centrifuged. The solid materials and the supernatants were collected respectively. The solid was then washed three times with the same buffer to remove physical adsorbed HRP. Both the resulting immobilized enzymes and the supernatants were stored at -10 ℃ prior to use.

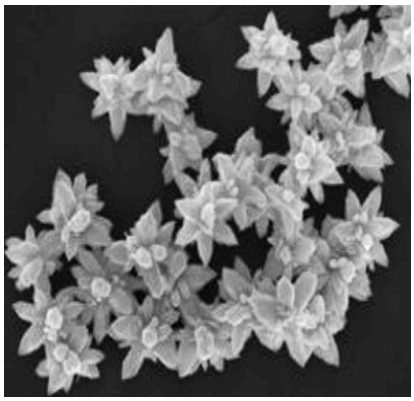
Enzyme activity measurements.A colorimetric assay was carried out according to the literature[13]. The assay uses phenol, 4-aminoantipyrine (4-AAP), and hydrogen peroxide as color-generating substrates. The absorption of the red product of the reaction (quinoneimine) was used to monitor catalytic reaction.

Phenolic compound precipitation reaction. Phenolic precipitation reactions were carried out in duplicate. The centrifuge tube contained 1mL of a mixture of 1mM aromatic compound, 1.2mM hydrogen peroxide and 0.83 U/mL HRP enzyme.Samples were centrifuged to remove precipitate at the end of reaction. The supernatant was used to determine the phenolic compound removal efficiency.

Phenolic compound assay. Residual aromatic compounds in the clear supernatants were measured by a colorimetric method using solutions of potassium ferricyanide (83.4 mM in 0.25 M sodium bicarbonate solution) and 4-aminoantipyrine (20.8 mM in 0.25 M sodium bicarbonate solution). Absorbance values were transformed to phenolic compound concentrations in the sample by using a calibration curve.

Results and discussions

Characterization of the support. The ZnO nanoparticles were characterized by scanning electron microscopy and FT-IR. Large surface area, well-distributed amino-group werer clearly observed on Fig.1(SEM images) and Fig.2(FT-IR spectroscopy). Fig.1 showes the flower-like ZnO nanoparticles formed and Fig.2 shows the FT-IR spectra of ZnO nanoparticles. So requirement as enzyme immobilization supporter is meeted.



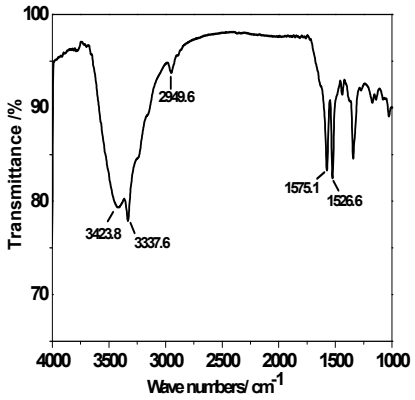


Fig. 1 SEM images of ZnO nanoparticles **Fig. 2 FT-IR spectrum of ZnO nanoparticles**.

Physical Properties

Effect of pH on the activity of free and immobilized HRP. Free and immobilized HRPactivity were examined in the pH range 4.0–10.0 at 25˚C and the results were presented in Fig.3. Immobilized HRP showed better stability at basic pH and have wider pH range than free enzymes.

Thermal stability of the free and immobilized HRP. High thermal stability of the immobilized enzyme is another merit in application. Enzyme were incubated in the absence of the substrate at various temperatures range of 25- 60 ˚C over different time and the results were presented in Fig.4.Then the activity were determined respectively. there is no much difference between the two kinds enzymes in Fig.4 when temperature was keeped under 40 ˚C. However, over 50˚C, the free

enzymes activity decreased quickly with temperature increased while immobilized enzyme retained higher activity.When temperature increased to 60 ˚C,the free and immobilized enzyme reduced their activity from a level of 100 % to 9.8 % and 45.3 % respectively. This might arise from the covalently bound enzyme being protected from conformational changes imposed by heat[14-15].

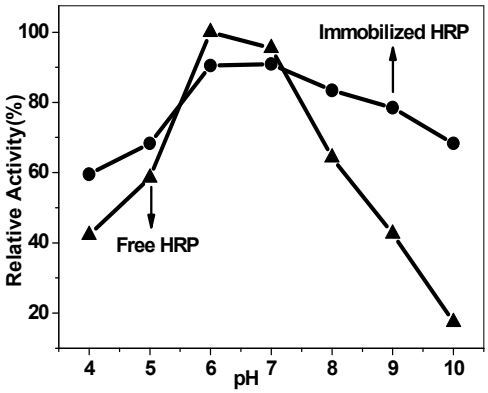


Fig.3 Effect of pH on the activity of free and immobilized HRP

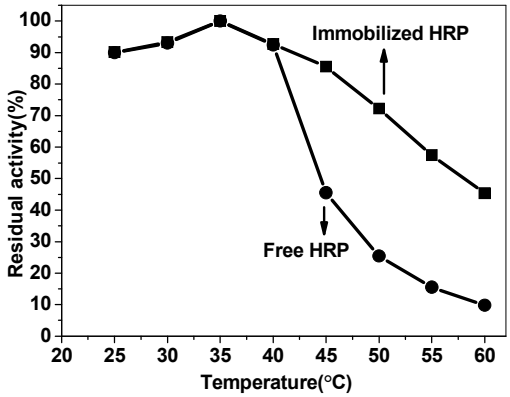


Fig. 4 Thermal stability of the free and immobilized HRP

Storage stability of the free and immobilized HRP. The free and the immobilized HRP enzyme were stored in phosphate buffer at 4˚C for a predetermined period of time. As seen in the Fig.5, under the same storage conditions, the activity of the immobilized HRP decreased slower than that of the free HRP. The experiment revealed that storage stability of the immobilized HRP was improved compared to free enzyme. The reason may be connect with the improved resistance to thermal denaturation and conformational change.

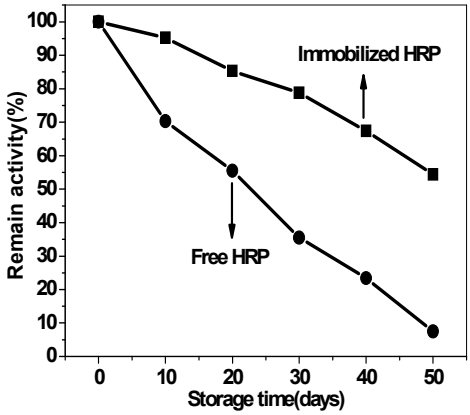


Fig.5 Comparison of the free and immobilized HRP Storage stability

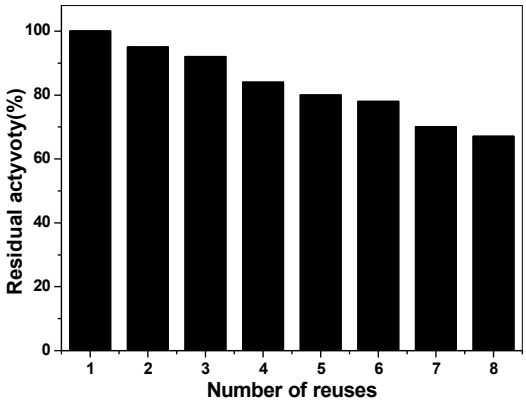


Fig.6 Activity of immobilized HRP repeated cycles

Removing of phenolic compounds The ability of the immobilized HRP to remove phenolic compounds was tested .in aqueous solution. Thel removal efficiency for the immobilized HRP and free HRP as summarized in Table 1. The immobilized HRP exhibited a slightly higher removal efficiency for phenol and *p*-Chlorophenol.

Table 1 Removal of phenolic compounds by free and immobilized HRP

(removal efficiency)/%

（substrate）	Free enzyme	immobilized enzyme
(phenol)	61.3	88.5
(*p*-Chlorophenol)	50.4	65.8

Reusability of the immobilized HRP. Reusability is a crucial feature of immobilized enzyme preparations in most practical applications. It is investigated using regular assay procedure as described in Enzyme activity measurements. After each cycle of the reaction, the reaction mixture was centrifuged to remove the supernatant and washed three times with the same buffer. Then the activity of immobilized HRP was assayed by repeating the above steps. The dependence of relatve activity on the number of reuses of immobilized HRP is illustrated in Fig.6. The relative activity of immobilized HRP decreased with the increase of the number of reuses Inactivation and enzyme bleed are the most prominent problems[16].

Conclusion

（1） HRP had been successfully immobilized on the ZnO nanoparticles via interaction between functional groups of support and the enzyme.

（2）The immobilized HRP could retain more of the activity in wider ranges of pH, better thermostability and Storage stability. It is good results that higher removal efficiency and better reusability for degradation phenol and p-Chlorophenol, which indicated that ZnO nanoparticles should be promising in wastewater treatment.

Acknowledgement

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