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Degradation of phenol with Horseradish Peroxidase immobilized on ZnO nanocrystals under combined irradiation of microwaves and ultrasound

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ABSTRACT

Zinc oxide (ZnO) nanocrystals with different morphologies were synthesized by varying the hydrothermal reaction conditions. Horseradish peroxidase (HRP) was successfully immobilized on ZnO nanocrystals with different morphologies. The activities of different types of the immobilized enzyme (nanodiscs, nanoflowers, and nanorods) were found to be 42.3, 26.1, and 14.1%, respectively. All three types of the immobilized enzyme showed higher phenol removal efficiency (for nanodiscs: 86.09%; for nanoflowers: 79.46%; for nanorods: 77.03%) compared to the corresponding value for the free enzyme (61.52%). The removal of phenolic compounds from aqueous solution using ZnO-immobilized HRP was explored with additional five phenolic compounds used as model pollutants. In comparison with the soluble HRP, the ZnO-immobilized HRP exhibited higher removal efficiencies for these phenolic compounds. The results indicate that the technology combined with the irradiation of microwaves and ultrasound can further promote the oxidation of primary intermediates in the conversion experiment, while the immobilization showed an obvious protective effect on HRP against the inactivation/inhibition effects.

Keywords: Phenol degradation; Immobilized enzyme; Microwaves, ultrasound

1. Introduction

Phenolic compounds are produced due to ever increasing industrial activities and are common toxic chemicals present in wastewater. The hazardous nature of phenolic compounds and their widespread occurrence in the environment have been well documented in the literature [1,2]. Therefore, it is of prime importance to develop methods which could effectively treat wastewater containing phenolic compounds. In this regard, a number of physical, chemical, and biological approaches have been developed [3–5]. Horseradish peroxidase (HRP, E.C.1.11.1.7) is one of the peroxidases, which can effectively convert phenolic compounds present in aqueous solutions to polymeric phenolic aggregates. It has extensively been used to remove phenolic compounds from the

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wastewater. However, due to its inactivation by various side reactions during the treatment process, HRP exhibits a short catalytic lifetime [6,7]. Enzyme immobilization is an effective technique used for increasing its stability during the degradation of the phenolic compounds. The immobilization process results in a decreased enzymatic activity, which limits the catalytic effect, especially in the concentrated solutions of phenolic compounds. Therefore, the development of a more economical and efficient method to degrade phenolic compounds is a key research goal in this area.

During the past two decades, chemical effects of the simultaneous irradiation of microwaves and ultrasound on the degradation process have been investigated in detail [8]. This novel irradiation process can accelerate the degradation rate of the phenolic compounds [9]. Cravotto et al. [10] examined the degradation of 2,4-dibromophenol using Fenton's reagent under the combined irradiation of microwaves and ultrasound. Their results showed that phenols present in wastewater in a concentration of 100 mg/l were completely degraded in six hours. Results from other studies revealed that when microwaves were used in the treatment process, a considerable improvement in the oxidative decomposition efficiency for different chemical contaminants in aqueous solutions was observed [11–15]. Microwaves' irradiation stimulates the generation of free radicals from oxidants, which results in improved degradation rate and reaction time. Due to this reason, it has been used both as a stand-alone degradation technology and in combination with other degradation technologies. Ultrasound can produce large amounts of hydroxyl radicals particularly in combination with Fenton and photo-Fenton processes. These hydroxyl radicals participate in the whole reaction as interim reaction products [16–18]. The result induced by this procedure is similar to the chemical effects stimulated by microwaves' irradiation. Moreover, cavitation effect of liquid induced by ultrasound is supportive to the degradation process. A previous study [19] has reported the degradation of 2,4-dinitrophenol (DNP) with a combination of hydrodynamic cavitation and advanced oxidation processes. The results showed that DNP with a concentration of 20 ppm was completely degraded within 60 min. However, when only hydrodynamic cavitation was used, the degradation yield decreased to about 12.4%. Due to its high adsorption capacity, activated carbon was used to degrade phenolic compounds with and without microwaves' irradiation [20–22]. Hunag et al. [23] used modified activated carbon to degrade 4-chlorophenol in the presence of hydrogen peroxide. Functional group present on the surface of the activated carbon, which was introduced by a preliminary chemical pretreatment, played a crucial role in the degradation process. In comparison with single granular activated carbon, relatively higher removal efficiency was achieved, which suggested that modification with chemical methods may be an attractive alternative for removing the organic pollutants in wastewater treatment process. In order to reduce the processing cost, regeneration of activated carbon saturated with phenol solution has also been studied [24]. Spent carbon can be regenerated with a combination of thermal and ozone treatments, though 15 wt% of it may be lost during the regeneration process. Although the regeneration of spent carbon signifies progress in the field of wastewater treatment, yet there are still some issues requiring research attention in this field. One of these issues is that, when phenols are present in high concentration, the removal efficiency achieved in the absence of a catalyst is not satisfactory.

In one of our previous studies, graphene oxide (GO)-immobilized HRP was used to remove the phenolic compounds [25,26]. In comparison with the process in which free HRP was used, the results for GO-immobilized HRP showed higher removal efficiency. GO shows good dispersion in phosphate buffer, and therefore, it is very difficult to centrifuge immobilized enzyme from the reaction system. It will lead to a significant loss of enzyme, and therefore, to improve the separation process, multiple centrifugation cycles would be needed. Hence, the degradation process could only be applied to micro-systems such as a system consisted of 1 mL volume. In the present work, we have synthesized ZnO nanoparticles with three different morphologies and have used them as HRP immobilization matrix. ZnO nanoparticles can easily be separated and hence provide the ease to process larger volumes of the phenolic solution. The catalytic activity and the amount of saturated immobilized HRP on three different morphological ZnO nanoparticles were studied. In addition, the application of the immobilized HRP for the removal of phenol under combined irradiation of microwaves and ultrasound was explored and compared with that of the soluble HRP.

2. Experimental

2.1. Chemicals and materials

HRP was purchased from Shanghai YuanJu Bio-Tech Co., Ltd., China, and was stored at −20°C before use. All other chemicals including phenol, 4-methoxyphenol, $Zn(Ac)_2{\cdot}2H_2O$, $(CH_3)_4NOH$, 3-aminopropyltriethoxysilane (APTES), and tetraethyl

orthosilicate (TEOS) were of analytical grade and were purchased from Sigma–Aldrich Co., Ltd., China. All chemicals were used as purchased without any further purification.

3. Methods

3.1. Synthesis of ZnO nanoparticles

About 5 mmol of zinc acetate was dissolved in 25 mL of methanol. Then, 15 ml of aqueous $(CH_3)_4NOH$ was slowly added to zinc acetate solution. The mixture was stirred for 15 min using a magnetic stirrer at room temperature. Then, the mixed solution was heated in an oven at 75°C for 24 h. The heated solution was then naturally cooled to room temperature. After cooling, the solid product was separated from the reaction solution by centrifugation. It was washed three times using ethanol and water alternately before being suspended in a 100 ml mixture of CH_3CH_2OH and H_2O. 500 µl of APTES/TEOS with a volumetric ratio of 1:4, respectively, was added to the mixture solution. The resulting mixture was sonicated for 1 h. The final product was washed three times and was then dried at room temperature.

3.2. Immobilization of HRP

About 5 mg ZnO nanoparticles were washed three times with 0.1 M potassium phosphate buffer (having a pH of 7) and were modified with 5 ml glutaraldehyde by shaking the mixture for 2 h at 0°C. The solid product was centrifuged and washed three times with the same buffer (0.1 M potassium phosphate having a pH of 7). ZnO nanoparticles were added to 2 mL of 0.1 M potassium phosphate buffer (having a pH of 7) solutions, each containing a different concentration of HRP. The mixtures were stirred and kept incubated for 2 h at 0°C. Each mixture was then centrifuged to separate the immobilized and free enzyme. Solid ZnO nanoparticles and the supernatants were collected. The solid was washed three times with 0.1 M potassium phosphate having a pH of 7. Both the resulting immobilized enzymes and the supernatants were stored at −10°C prior to use. The supernatant was used to measure the concentration of the residual enzyme to determine the enzyme loading on ZnO.

3.3. Determination of enzyme concentration

Enzyme concentration was determined by Lowry's procedure as modified by Peterson [27]. The amount of coupled enzyme is defined as the difference between the initial amount of the added enzyme and the amount in the supernatant after immobilization.

3.4. Enzyme activity assay

A colorimetric assay was used to evaluate the catalytic activity of the immobilized HRP [28]. The immobilized HRP was added to the assay solution which contained 1 mL of 60 mM phenol, 14.38 mM 4-aminoantipine (4-AAP), and 1.21 mM hydrogen peroxide in 1 mL of 0.1 M potassium phosphate buffer (having a pH of 7). The catalytic reaction was monitored by analyzing the absorption of the red-colored product of the reaction (quinoneimine with an extinction coefficient of 7,210 M^{-1} cm^{-1}) at 510 nm [29]. Unit HRP activity (U) was defined as the amount of HRP required to hydrolyze 1 µmol of hydrogen peroxide converted per minute under the conditions stated above.

3.5. Removal of the phenolic compounds

The experimental rig consisted of microwaves (MW) and ultrasound (US) generators, a circulating pump and a condenser. These units were set up in series as shown in Fig. 1. 10U of immobilized horseradish peroxidase was added to the solution which contained 60 mM phenol and 1.21 mM hydrogen peroxide in a 50 mL volume of 0.1 M potassium phosphate (having a pH of 7). MW generator was switched on with the power set to 600 W to increase the temperature from room temperature to 60°C. The 850 kHz US generator was operated at 40 W. When the

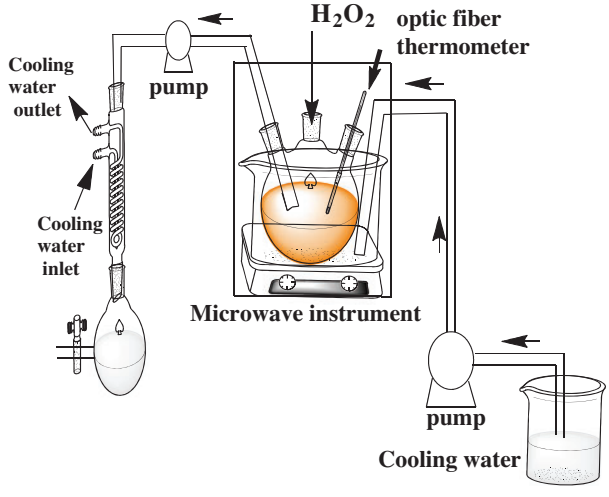

Fig. 1. Schematic diagram of experimental facility.

temperature reached the desired value, the power of MW generator was reduced to 50 W and cooling water was used to maintain the temperature at this value. After the completion of reaction, 1 mL aliquots were periodically extracted from the reaction system and were analyzed for the quantitative evaluation of the residual concentration of the phenolic compounds in the supernatants using UV–vis spectrophotometer and HPLC. For colorimetric assay, 50 μL of clear supernatants of the reactions was mixed with 100 μL of potassium ferricyanide (83.4 mM), 100μL of 4-AAP (20.8 mM), and 750 μL of 0.1 M phosphate buffer. After complete development of the color in the reaction mixture, the absorbance was measured at 505 nm. Absorbance values were transformed to phenolic compound concentrations using a calibration curve which was plotted according to the standard phenol concentration with the initial catalytic reaction rates. For samples measured by the HPLC, the phenolic compounds were eluted isocratically with a mixture of 70% methanol and 30% mobile phase of phosphate buffer solution (having a pH of 2) having a flow rate of 0.8 mL min^{-1}.

4. Results and discussion

4.1. Characterization of the support

Fig. 2 shows the scanning electron microscope (SEM) nanoparticles having different morphologies. The concentration of methanol in the solution affected the morphology of ZnO. ZnO nanorods were obtained when the volume ratio was 50% (see Fig. 2(a)). ZnO nanodiscs were obtained when the volume ratio was 25% (see Fig. 2(b)). Flower-like ZnO nanoparticles were obtained when the volume ratio was 10% (see Fig. 2(c)). Fig. 3 shows the FT-IR spectra of ZnO nanoparticles. The peak at 465.3 cm^{-1} represents the Zn-O stretching vibration. The peak at 1050.0 cm^{-1} can be attributed to the stretching vibration of Si-O. The peak at 2939.2 cm^{-1} is due to the C–H stretching vibration. The two peaks at 3425.8 and 3457.7 cm^{-1} were due to $-NH_2$. The peak at 1323.7 cm^{-1} corresponds to the stretching vibration of C–N, indicating that there are $-NH_2$ groups on the surface of nanoparticles. As shown in Fig. 4, the protein was connected to ZnO nanoparticles by glutaraldehyde, which played the role of a cross-linking agent in the reaction.

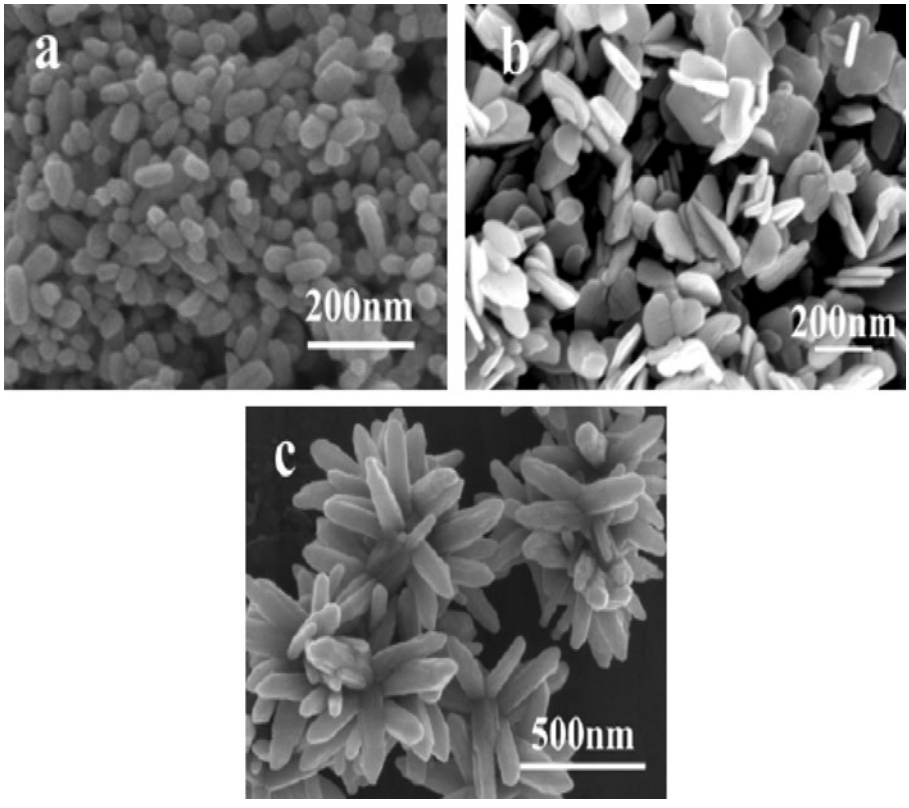

Fig. 2. SEM images of ZnO (a) nanorods, (b) nanodiscs, and (c) nanoflowers.

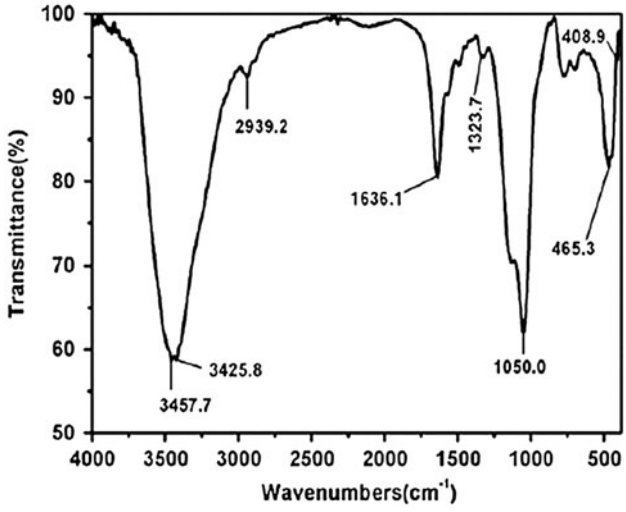

Fig. 3. FT-IR spectrum of ZnO nanoparticles.

4.2. HRP immobilization

The UV spectrum of the supernatant separated from the immobilized system has been shown in Fig. 5(a). In comparison with the blank, the absorption value for the supernatant, immobilized with different morphological ZnO, decreased significantly at 403 and 207 nm. The maximum absorption of the HRP solution at 270 and 403 nm, which can be attributed to the amino acids and iron porphyrins, shows linear dependence on the protein concentration. These results suggest that part of the protein is adsorbed on the matrix (ZnO). A colorimetric assay (as mentioned above) to determine the activity of the enzyme immobilized on the ZnO (see Fig. 5(b)) shows that the HRP has been adsorbed on the ZnO, which can also be proven by the maximum absorption peak appearing at 510 nm. A lower absorption of the supernatant at 270 and 403 nm shows the completion of a more thorough immobilization process associated with the increasing absorption of ZnO at 510 nm. However, from the absorption values of the blue and orange curves in Fig. 4(b), it can be inferred qualitatively that activity of the immobilized enzyme was significantly reduced compared to the same amount of the free enzyme. A number of previous studies have reported similar results. One reason for such results may be the change in the active site of the enzyme, which was influenced by the cross-linking agent and the enzyme support in the immobilization process. The catalytic ability of the immobilized enzyme on different morphologies of ZnO was examined under excess catalytic substrates. As seen in Fig. 5(c), the initial reaction rates of

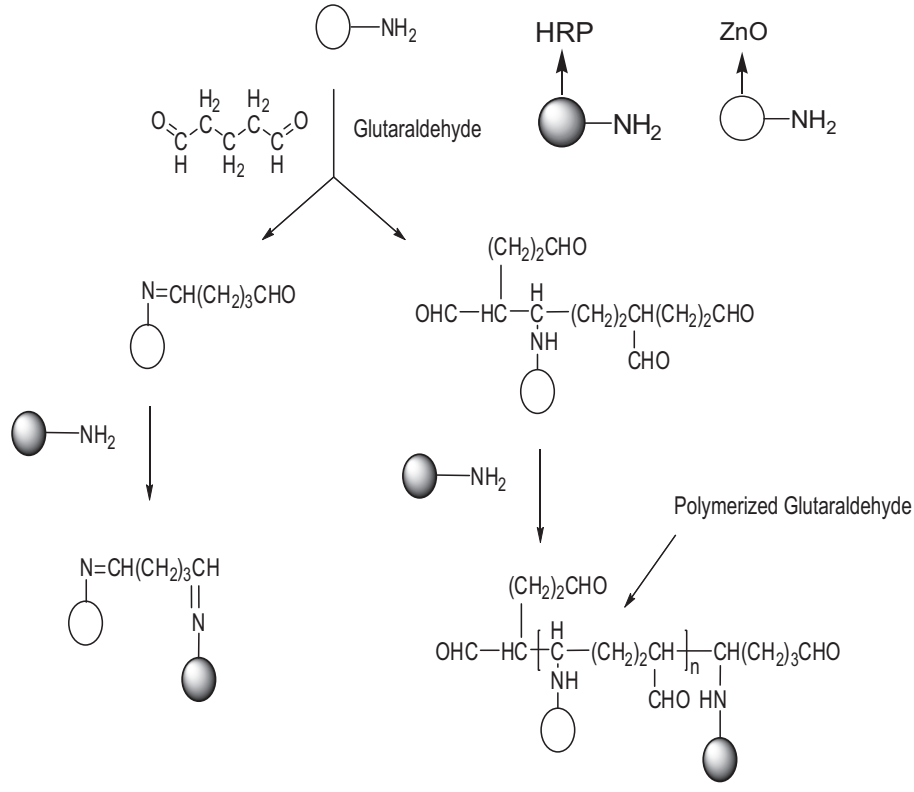

Fig. 4. Schematic of immobilization procedure.

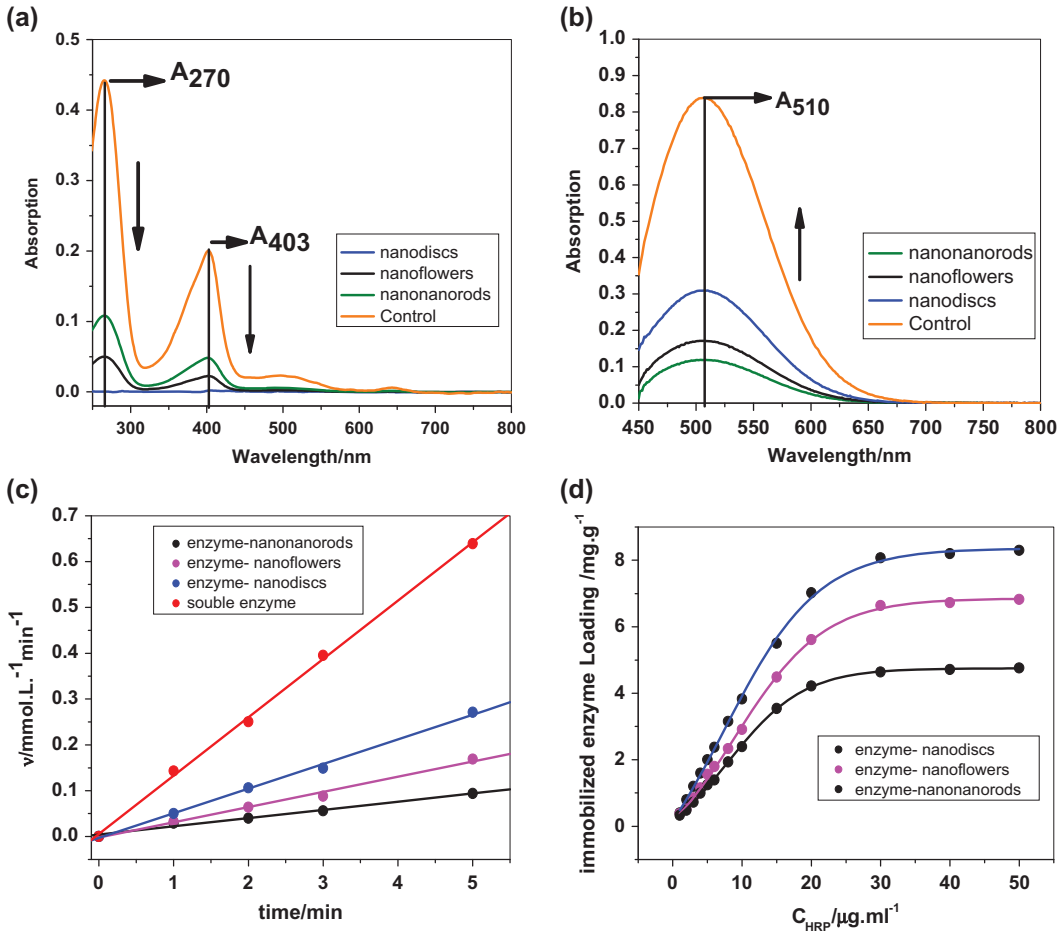

Fig. 5. (a) UV analysis graph data of supernatant, (b) UV analysis graph data of Immobilized enzyme, (c) Initial reaction rates of ZnO-bound HRP vs. time. The activities were measured with HRP(5 μg mL^{-1}) phenol (60 mM), 4-AAP (14.4 mM), and H_2O_2 (1.2 mM), (d): Effect of the total concentration of HRP on the HRP loading on ZnO. The concentration of ZnO was kept constant (5 mg). HRP were added to phosphate buffer (0.1 M, pH 7) containing ZnO, and the immobilization was performed at 0℃ for 2 h.

enzyme-catalyzed reactions follow the order of: free enzyme > enzyme-nanodiscs > enzyme-nanoflowers > enzyme-nanorods. The activities of the enzyme immobilized by different morphologies declined to 42.3% (for nanodiscs), 26.1% (for nanoflowers), 14.1% (for nanorods) compared to that of the soluble HRP. This may be due to the affinity of substrate of different morphological units of ZnO to the HRP, which could affect the catalytic efficiency of the enzyme. Thus, we suggest that the morphology of ZnO should be considered as an important factor for the enzyme immobilization process. Fig. 5(d) shows the highest loadings of HRP on the ZnO nanorods, nanodiscs, and nanoflowers, respectively. The maximum loading of HRP on ZnO nanodiscs was 8.27 $mg.g^{-1}$ (which is the highest among the three morphologies), 6.77 and 4.73 mg g^{-1}, respectively. The results also show that the amount of immobilized enzyme at saturation was influenced by the shape of immobilization matrix.

4.3. Removal of the phenolic compounds

Degradation experiments were conducted for aqueous solutions containing different concentrations of phenol. For comparison, different types of immobilized and free enzymes with equal catalytic activity were used to decompose phenol. Fig. 6(a) shows that similar phenol conversions (between 92 and 100%) were achieved with free and immobilized HRP, although the time consumed to achieve the maximum conversions was different in each case. It is clear that removal efficiency of phenol treated with free HRP increased rapidly during the first 5 min and remained practically unchanged during the next 35 min. More

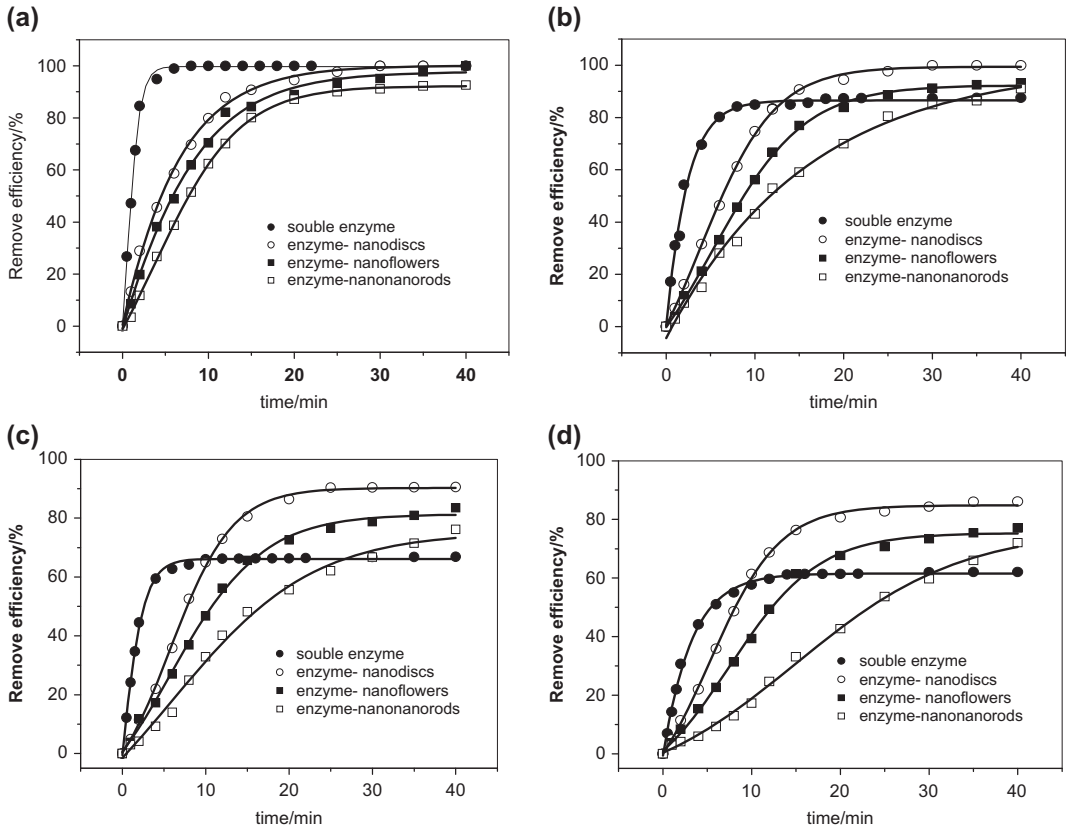

Fig. 6. Kinetics curves of phenol degradation with different phenol concentration. (a) 10 mM, (b) 20 mM, (c) 30 mM, and (d) 40 mM Starting phenolic compounds 10 mM, HRP 10U, H_2O_2 1.2 mM. MW power = 550 W US power = 40 W T = 60℃, pH 7.0.

time was taken by the enzymes of different morphologies for treating the solution containing same concentration of phenol. The reason may be connected to the difference in reaction rates for the homogeneous and heterogeneous reaction systems catalyzed by free and immobilized HRP.

However, Figs. 6(b)–(d) illustrate that higher conversions were achieved with immobilized HRP than the corresponding value with soluble HRP. From Fig. 5(b), it can be observed that 100% conversion could not be achieved except in the case of immobilized HRP on nanodiscs. The removal efficiency dropped to varying degrees for the rest of the three systems. The values for the removal efficiency for immobilized HRP on nanoflowers, nanorods, and free HRP were 93.3, 91.6, and 86.5%, respectively. When the phenol concentration was increased to 30 mM, lower efficiencies were observed for all cases. These results were similar to the result of Fig. 6(d), which shows that for higher phenol concentration, the difference in conversions becomes more obvious. It has been reported by many researchers that HRP may become inactive during the enzymatic reaction due to the interactions between the phenoxy radicals and/or the polymers produced and the enzymes' active sites. These results, together with those mentioned in the HRP immobilization section, led us to affirm that, in the catalytic system having higher concentration of the phenol, the enzyme achieved the inactive state more quickly, especially the free enzyme. The HPLC results for the degradation of phenol are shown in Fig. 7. It is obvious that the peak area of phenol decreased, while a new absorption peak also appeared in the spectra. These results suggest that the phenol was decomposed into other substances. In comparison with Fig. 7(b), the declining tendency of the phenol's absorption peak area was more obvious in Fig. 7(c). This suggested that the degradation efficiency using immobilized enzyme was higher than that obtained using the free enzyme. According to the values obtained from standard curve, the value for the degradation of phenol catalyzed by immobilized enzyme was 81.19%. However, the value dropped to only 60.1% when the degradation was catalyzed by free enzyme. This was

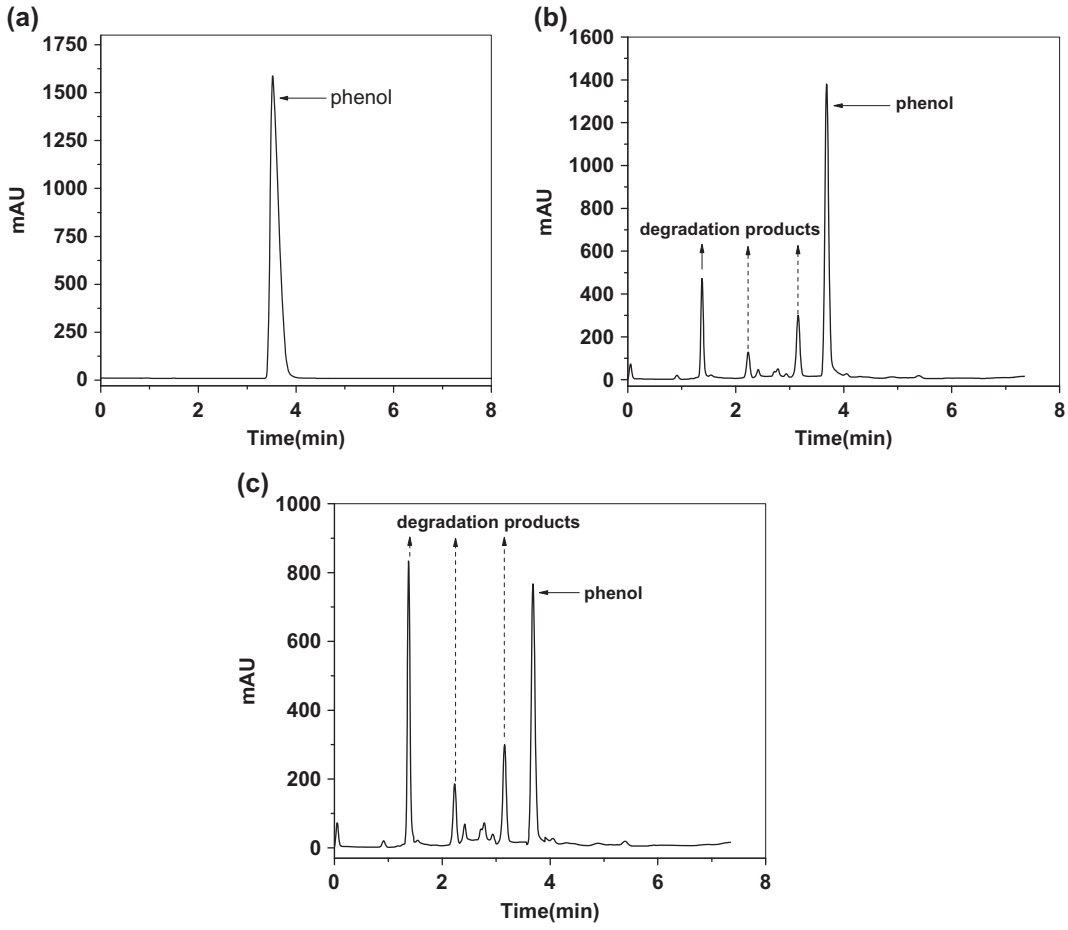

Fig. 7. HPLC data of phenol degradation with free and immobilized enzyme (a); standard sample (b); degradation with free enzyme (c); degradation with immobilized enzyme (nanodiscs). The experimental conditions are consistent with the conditions of Fig. 6(d).

Table 1
Removal of phenolic compounds by free and immobilized HRP

	Removal efficiency	
Substrate	Free HRP (%)	Immobilized HRP (%)
Phenol	61.4	86.1
2,4-dimethoxyphenol	22.3	43.6
p-chlorophenol	20.4	25.9
4-methoxyphenol	88.2	88.2
3-aminophenol	85.5	92.2

consistent with the results obtained in Fig. 6. Since, in addition to phenol, wastewater also contains some other toxic derivatives of phenolic compounds; therefore, some more common phenolic compounds were added to wastewater samples to study their catalytic degradation. The results obtained have been presented in Table 1. Table 1 shows that the immobilized enzyme exhibited higher catalytic ability during the degradation process and therefore resulted in a higher degradation efficiency. The observation is consistent with the results obtained using only phenol as the targeted model pollutant for the degradation experiment, though the observation is not very obvious for some phenolic compounds (such as 4-methoxyphenol and 3-aminophenol). Considering its high efficiency and better thermal stability,

Table 2
Comparison among phenol degradation efficiency reported in this work and in literature

Kind of process	Experiment conditions	Degradation yield (%)	Refs.
MW-UIT- Immobilized HRP. On ZnO nanocrystals	C_{phenol} = 10 mM $C_{hydrogen\ peroxide}$ = 1.2 mM volume = 50 mL irradiation time 5 min MW power = 550 W US power = 40 W reaction time = 20 min T = 60℃, pH 7.0	100	Present Study
MW/ClO_2-CUO_x/Al_2O_3	C_{phenol} = 1.06 mM C_{ClO2} = 80 mg/L $Ccuo_X$ = 50 g/L T = 50℃, pH 9.0 volume = 100 mL contact time 5 min	92.24	[30]
Fenton-MW-H_2O_2	C_{phenol} = 120 mg/L $C_{hydrogen\ peroxide}$ = 10 mg/L $C_{Fe^{2+}}$ = 11.112 mg/L Room temperature pH 3.0 volume = 8 mL reaction time 40 min	100	[31]
UV-MW-H_2O_2	C_{phenol} = 1 mM $C_{hydrogen\ peroxide}$ = 1 M reaction time 30 min pH not specified T = 20˚volume = 50 mL	100	[32]
MW-UlT-H_2O_2	C_{phenol} = 1 mM 2 mL(30%) H_2O_2. Room temperature pH 6.0 volume = 1,250 mL reaction time = 120 min MW power = 600 W US power = 40 W	76	[33]
Immobilized HRP on glass bead	$C_{chlorophenol}$ = 2 mM $C_{hydrogen\ peroxide}$ = 2 mM C_{enzyme} = 20 mg/L volume = 50 mL reaction time = 30 min pH 7.0	100	[34]
Immobilized HRP on graphene oxide free HRP	C_{phenol} = 60 mM, $C_{hydrogen\ peroxide}$ = 1.2 mM volume = 1 mL reaction time = 30 min pH 7.0	64 57.1	[26]

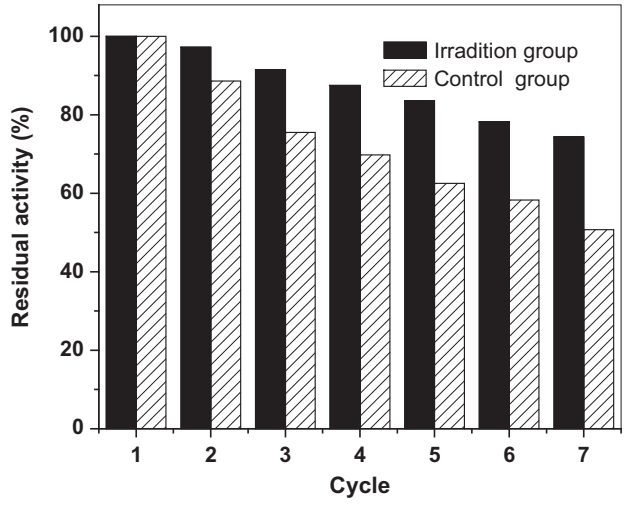

Fig. 8. Reusability of immobilized HRP with Microwave-ultrasound induced.

immobilized enzyme appears to be a more suitable choice for the degradation of phenol in wastewater.

Meanwhile, the nonthermal effects of the microwaves affirmed the important role these play in the oxidation of phenol and its intermediates and which could protect the active center of the enzyme from being destroyed. Ultrasound technique, which is regarded as an activating agent for both H_2O_2 and OH radicals produced from the aqueous solution, was used to degrade phenol in aqueous solution in the presence of hydrogen peroxide. An increase in the phenol concentration from 10 mM to 40 mM means that more intermediates and OH radicals are generated during the oxidation process. So both the free and the immobilized enzyme would become easily inactive due to interactions with the intermediates. It was observed that the decline in the activity of the immobilized enzyme was slower compared to that of the free enzyme. The removal efficiency obtained using three different types of the immobilized enzyme (flower-like, nanodiscs, and nanorods) decreased by 13.91, 22.97, and 20.54%, respectively. In case of the free HRP, the corresponding drop in removal efficiency was 34.48%. The results indicate that the method of combined irradiation of microwaves and ultrasound can promote the oxidation of primary intermediates in the conversion experiment, while the immobilization shows an obvious protective effect on HRP against the inactivation/inhibition effects, which have been mentioned in the Introduction section.

A comparison of the results obtained in this study with the already reported values in literature is shown in Table 2. Although to the existence of some differences in the experimental conditions does not allow a direct comparison, yet the results obtained from the current work seem to be the most effective. For the use of ClO_2 in the degradation process, the limited range of pH and lack of suitable catalysts should primarily be considered. Although microwaves can promote the degradation reaction rate due to nonthermal effects, yet it requires more time especially when

ferrous ions and hydrogen peroxide were used for the oxidation process. It is difficult to meet the practical requirements for the treatment of high concentration phenolic wastewater. Degradation technology combined with irradiation of microwaves and ultrasound is an attractive option due to enhanced/synergistic effects of microwaves on the sono-degradation of phenol without a catalyst. Slower kinetics and higher energy consumption are two of the major disadvantages of the combined technology. Immobilized enzyme on glass bead shows higher removal efficiency compared to the preceding results (as listed in Table 2). However, further application of the combined technology is restricted due to high enzyme concentration required in the reaction system, which will result in a high cost. Compared to the results obtained in one of our previous studies, results from current investigation show better degradation efficiency and a shorter reaction time. These results are also consistent with the results shown in Fig. 4. Thus, we conclude that the oxidation process using the technology of immobilized HRP combined with microwaves and ultrasound irradiation would provide a novel treatment method for the phenolic wastewater and has better prospects to be used in practical applications.

4.4. Reusability of the immobilized HRP

One attractive advantage of using the immobilized enzyme is that it can easily be separated from the reaction system and reused. The ability to regenerate the immobilized enzyme greatly decreases the cost for practical applications. After the first cycle of the catalytic reaction with nanodiscs immobilized enzyme, the reaction mixture was centrifuged to remove the supernatant. The immobilized enzyme was washed and the activity was assayed again with the methods stated above in enzyme activity assay section. The graph showing the relationship between the catalytic cycles and the enzyme's activities of the immobilized HRP has been plotted in Fig. 8. After 7 cycles, the immobilized enzyme, which was continuously irradiated by microwaves and ultrasound during the catalysis process, showed better reusability than the enzyme not undergoing microwaves and ultrasound irradiation. The value of activity for the enzyme undergoing irradiation fell to 75%, while the corresponding value for the enzyme not undergoing irradiation was found to be 45%. The reusability of the immobilized enzyme depended not only on the solid support type but also on the extent to which enzyme was inactivated (due to interactions between the active sites and inert intermediate products). The active sites might get covered and the extent of reaction in the next cycle would be restrained. As stated above, it was very conducive to oxidize primary intermediates and decompose excess phenoxy radicals if microwaves and ultrasound irradiation was used. Additionally, repeated washings and reaction cycles may disturb the interactions between ZnO and HRP, thus leading to gradual bleeding of enzyme from the ZnO support, which may also contribute to the reduction of enzyme's activity.

5. Conclusions

HRP was immobilized on different morphologies of ZnO nanocrystal surfaces using glutaraldehyde as a cross-linker. The maximum loadings of HRP on ZnO nanodiscs, nanoflowers, and nanorods were 8.27, 6.77, and 4.73 mg/g^{-1}, respectively, indicating that the saturation amount of the immobilized enzyme was influenced by the shape of immobilization matrix. The catalytic ability of the immobilized enzyme was examined under excess catalytic substrates. The initial reaction rates of enzyme-catalyzed reactions were found to be in the following order: free enzyme > enzyme-nanodiscs > enzyme-nanoflowers > enzyme-nanorods. In comparison with the activity of the soluble enzyme, the activity of different types of the immobilized enzyme (nanodiscs, nanoflowers, and nanorods) declined to 42.3% (for nanodiscs), 26.1% (for nanoflowers), and 14.1% (for nanorods), respectively. The results from the degradation experiments showed that higher conversions were achieved with immobilized enzyme than those with the soluble enzyme. The removal efficiency obtained using free enzyme dropped by 34.48% for an increase in the phenol concentration from 10 to 40 mM. In case of the different types immobilized enzyme (nanodiscs, nanoflowers and nanorods), the removal efficiency decreased by 13.91% (for nanodiscs), 22.97% (for nanoflowers), and 20.54% (for nanorods), respectively. It was also found that for higher concentrations of phenol, the difference in conversions was more obvious. Based upon these results, it is concluded that the degradation process using the technology of immobilized HRP combined with microwaves and ultrasound irradiation has better prospects for its future use in practical applications.

Acknowledgments

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