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Immobilization of horseradish peroxidase enzymes on hydrous-titanium and its application for phenol removal

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In this work, hydrous-titanium was utilized to immobilize horseradish peroxidase (HRP) in order to improve its stability and adaptability under different water qualities by biomimetic titanification process. The catalytic performance of immobilized HRP for phenol removal from aqueous solution was evaluated. The physicochemical properties of immobilized HRP (IM-HRP) were characterized by means of scanning electron microscopy (SEM), transmission electron microscopy (TEM), and Fourier transform infrared spectroscopy (FT-IR). The effects of reaction conditions (H_2O_2 and IM-HRP dosage, initial phenol concentrations) on phenol removal were investigated. Enzymatic activity analysis indicated that immobilization process has no adverse effects on catalytic performance of HRP, and it was observed a slightly increase in enzymatic catalytic kinetic (k_m) of IM-HRP when compared to its free enzymes. It was found that in a synthetic condition of 37±3 °C, the IM-HRP exhibited a perfect phenol removal over 90% after reaction 15 min. In addition, the enzyme activity of IM-HRP was very stable over a wide reaction conditions, e.g. temperature from 20 to 90°C and pH from 3.0 to 11.0. Therefore, HRP enzymes loaded on hydrous-titanium was very effective to improve stability of enzymes and can alleviate the adverse effects of environmental and varying water conditions on contaminants removal.

1. Introduction

Phenolic compounds were considered as environment threats and they were usually discharged from many industrial processes, such as coal conversion, dyes production, plastic processing and so on [1]. Most of phenolic compounds in water were toxic and may pose a great threat to environment and human health. Many conventional methods had been developed and implied to remove/degrade phenolic compounds in environment, such as adsorption by carbon or membrane, chemical oxidation, biological degradation, solvent extraction and coagulation process [2, 3]. With the advent of green technology, the use of enzymes as biocatalyst attracted much attention and has become increasingly popular. Enzyme-catalytic degradation technologies provided a novel solution for pollutants abatement in environment [4 - 6]. As an effective biocatalyst, enzyme has many advantages of substrate specificity, high catalytic efficiency and the mild reaction conditions, and it could be utilized and degraded by bacteria. HRP (horseradish peroxidase) was the peroxidase extracted from plants and it was a glycosilated containing one heme ion Fe-protoporphrin (IX) prosthetic group and two Ca (II) ions, in which the combination of the two types of metal ions offered the active center [7, 8]. It has been widely used in organic synthesis, polymeric synthesis and treatment of the wastewater containing the phenolic compounds [6, 9]. It was reported that its catalytic mechanism for degradation of phenolic compounds was mainly attributed to formation of phenoxy radicals in the presence of hydrogen peroxide [10].

However, the free HRP enzymes were subject to reaction conditions and solution chemistry solutions and showed poor stability in wastewater treatment. Especially, their structure might change and lose catalytic function under adverse conditons. Hence, the full-scale applications of enzyme-related environmental technologies were constrained. In order to solve this problem[11, 12], one of the common and promising methods is the immobilizing technology loading the enzymes on the suitable carriers [13 - 16], to achieve the following advantages: easy enzymes recycling from reaction mixture, abatement of secondary pollution, improved stability and broadened adaptability of enzymes [17, 18]. For example, HRP enzymes were encapsulated into silica glass by sol-gel process to the analysis of phenolic compounds [19]. Or similarly, HRP enzymes were immobilized on magnetic core-shell particles which was the silica coated Fe_3O_4 particles modified by NH_2 for 2.4-dichlorophenol removal from aqueous solutions [20]. The results showed the immobilized HRP was more stable than free ones. Grapheme oxide/clays and the soil organic matter were used as the matrix for immobilizing the HRP enzymes, enzymes could take place readily without using any additional surface modification or cross-linking reagents [21, 22]. In

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comparison of these materials, titanium-based materials were also attracted in a number of fields [23, 24]. For instance, the titanium sol-gel process could be controlled at near neutral-pH, ambient temperature of mild within a short process period [21-24]. Jiang et al. suggested that HRP enzymes were encapsulated in phospholipid-templated titanium of Ti-BALDH for titanium source, it is very efficient to remove phenolic compounds and dye [25]. Although immobilized enzymes has many advantage over free enzyme, it was noted that the structure of the enzymes could also be denatured and reduced the enzymes activity under the long aging time of immobilization process. Therefore, its synthesis process should be carefully controlled.

In this paper, HRP enzymes were immobilized on hydrous-titanium coatings through a biomimetic titanification process. Then, the immobilized enzymes were used for phenolic compounds removal from aqueous solutions. In order to generate the 3-D open mesoporosity structure for the biocatalyst process and eliminate the special response of the enzyme to hydrous titanium forming process, the lecithins were selected as the template which could protect enzymes from the severe conditions. Firstly, the IM-HRP particles were characterized by FT-IR, TEM and SEM. The effects of pH and temperature on activity of free/immobilized HRP enzymes residual activity were evaluated. In addition, the effects of reactions conditions (time, H_2O_2, IM-HRP dosage and initial concentration) on phenol removal in the presence of IM-HRP and free HRP were compared.

2. Materials and methods

2.1 Materials

Horseradise peroxidase (HRP, RZ = 3.0, enzymetic activity ≥ 250 U/mg) was purchased from Shanghai roche pharmaceutical Ltd (Shanghai, China). Soybean lecithin (10 g/L at 25 °C), solid phenol (analytically pure; content ≥ 99.0%), 4-Aminoantipyrine (chemical pure; content ≥ 98.5%), potassium ferricyanide (analytically pure; density ≥ 99.5%), TritonX-100(chemical pure; density ≥ 0.980 at 20 °C), α-lactose monohydrate (analytically pure), anhydrous ethanol (analytically pure; content ≥ 99.7%), and tetrabutyl titanate (chemical pure; content ≥ 98.0%) were purchased from national medicine group Shanghai chemical reagent co. (Shanghai, China).

2.2 Immobilization of HRP on hydrous-titanium

In the process of preparing hydrous titanium particles as the enzyme carriers, the lecithins were dissolved in absolute ethyl alcohol and formed mixed-micelle as the biomimetic template. The lecithins could protect the enzymes from unfriendly environment [25] during hydrous-tianium particles forming progress, and the lactose acted as the structure preserving lyoprotectants which could prohibit enzyme denaturation [26].

The immobilized HRP on hydrous titanium progress was described as follows: A first solution of HRP (10 mg) in 2 ml phosphate pH 7.0 containing 0.05 g lactose was prepared at 25 °C. It was slowly added under the mechanical stirring to the second solution, prepared by dissolving 0.3 g lecithins with absolute ethyl alcohol (5 ml) under ultrasonical treated for 15 min, and then the mixture solution was stirring vigorously for 0.5 h to form soft HRP enzymes phospholipid microspheres as stock solution A.

Then, the above mixture solution A was slowly added to 3 ml of tetrabutyl titanate (1.76 mol/L, pH 7.0) solutions, also prepared at 25 °C, composed of tetrabutyl titanate and absolute ethyl alcohol as well as be under the mechanical stirring for 1 h previously. Until titania particles formed and aged for 15 min, the final particles were washed with TritonX-100 (7.5%) and ultra-pure water by twice to remove lecithins and then dried at vacuum (37 °C).

2.3 Characterization of IM-HRP

Transmission electron microscopy (TEM) and fourier transform infrared spectroscopy (FT-IR): The IM-HRP samples were ultrasonically treated with absolute ethyl alcohol for 0.5 h and dried on copper grid at room temperature for determination. TEM morphology of IM-HRP samples were carried out using an Transmission electron microscopy (TEM, H-7500, Hitachi, Japan). It was performed using an acceleration voltage of 80 KV and the magnification of 1.5×10^5. FT-IR sprectra of IM-HRP samples were measured by using a Nexus 670 normal position analysis FT-IR spectrophotometer (Thermo Fisher, USA). The FT-IR spectrum was recorded in a range from 4000 to 400 cm^{-1}.

Scanning electron microscopy: The IM-HRP samples were dried and coated with gold prior to analysis. A scanning electron microscopy (SEM, JSM35-CF) was implied to observe the surface of the IM-HRP samples under an acceleration voltage of 5.0 KV.

2.4 Measurement of enzyme activity

The enzyme activity of HRP was measured according to the method described by Worthington et al.[25]. One unit enzyme activity (U) was defined as: the amount of enzyme required to resolve 1 μmol hydrogen peroxide in the condition of pH 7.0 each minute at 25 °C, in which the relative enzyme activity could be described using the following equation:

$$\mathrm{Re}latie-activity(100\%)=\frac{enzyme-activity}{\max imum-activity}\times100\%$$

The maximum enzyme activity was regarded as the 100% of free/immobilized HRP enzymes activity to be studied respectively.

The required reagents to the process of determining enzyme activity were: 1.4 ml mixture solution of phenol (0.1723 mol/L) and 4-Aminoantypyrine (4-APP, 2.460×10^{-3} mol/L), 1.5 mL H_2O_2 (2.12 μmol/L) and 0.1 mL 0.05-0.25 U/mL enzyme solution [1]. The generating rate of the red none-precipitating products was proportional to the enzyme activity, in which the 4-APP was acted as a hydrogen donor to the reaction of enzyme catalyze the phenolic substances in the presence H_2O_2. The relative concentration of red none-

precipitating products was determined with absorbance at the wavelength of 510 nm at 25 °C. Coloration reaction rate was measured using a Purkinje TU-1901 UV-visible spectrophotometer (wavelength range from 190 to 900 nm with a 1 nm resolution).

2.5 Phenol removal test

In order to determine the reaction time required for the enzymatic capacity with the efficient phenol removal, comparative experiments were carried out for free/immobilized HRP to remove phenol from aqueous solutions. All of the experiments were performed in 100 ml conical flasks under vigorous agitation. Effects of the temperature tolerances and pH on the residual activity of free/immobilized HRP were tested by incubating enzymes in buffer solutions at pH 7.0 at the temperature from 20 to 90 °C and in buffer solutions at 25 °C within the pH range from 3.0 to 11.0 for 2 h respectively. What's more, influence of reaction conditions such as catalysis reaction time, H_2O_2 amount, immobilized HRP dosage and initial phenol concentration on phenol removal efficiency was investigated.

The concentrations of phenol in the assay solutions were measured by the method reported by previous reports [22, 26]. In brief, phenol concentrations could be quantified by measuring absorbance at 510 nm of red compounds generated in the presence of 0.20 ml of potassium ferricyanide (83.4 mmol/L $K_3Fe(CN)_6$ in 0.25 mol/L $NaHCO_3$) and 0.2 ml 4-APP (20.8 mmol/L 4-anminoantypyrine in 0.25 mol/L $NaHCO_3$).

3. Results and discussion

3.1 Immobilization of HRP on hydrous-titanium process

On account of the poor stability of free HRP enzymes and the second pollution of the treated water since the free HRP enzymes cannot be separated after treatment, we presented a method of HRP enzymes immobilization described as Scheme 1. With the protection by lecithins microspheres of HRP enzymes, hydrous-titanium formed around the lecithins microspheres as the template from tetrabutyl titanate, and the lecithins were removed by TritonX-100. The results showed the stability of IM-HRP enzymes were enhanced in harsh conditions and could be easy to be separated.

3.2 Characterization of IM-HRP

3.2.1TEM and SEM characterization: TEM analysis was used to determine the changes in morphological properties immobilized HRP enzymes. Fig. 1 (A) illustrated the TEM of the IM-HRP enzymes, it can be clearly observed that enzymes were wrapped by a layer of hydrated titanium coatings. In addition, Fig. 1 (B) also showed the SEM images of the encapsulated HRP, which presented the images of samples were reunited with the rules of large microspheres. As illustrated in Fig. (C), samples were aggregates of microspheres together. Since it had a large specific surface area, and it was advantageous to biocatalyst reaction process. This result was in agreement with the biomimetic silica particles properties reported by

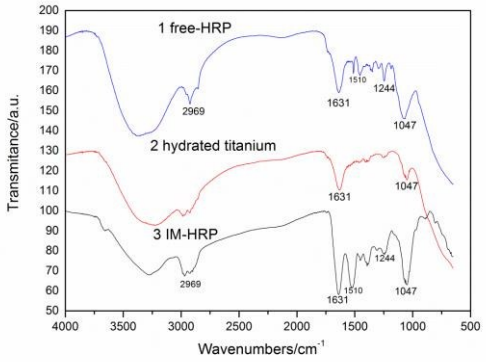
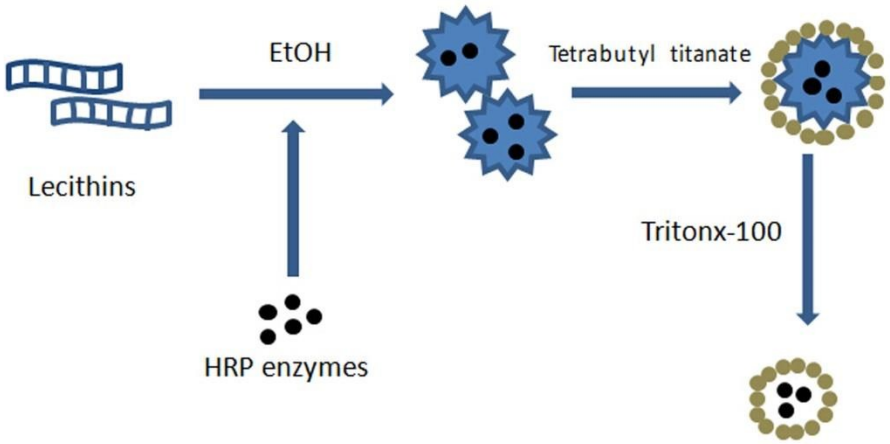


Fig. 2 - FT-IR spectra of free HRP, hydrated titanium, IM-HRP



Scheme 1 - Process of HRP enzymes immobilization

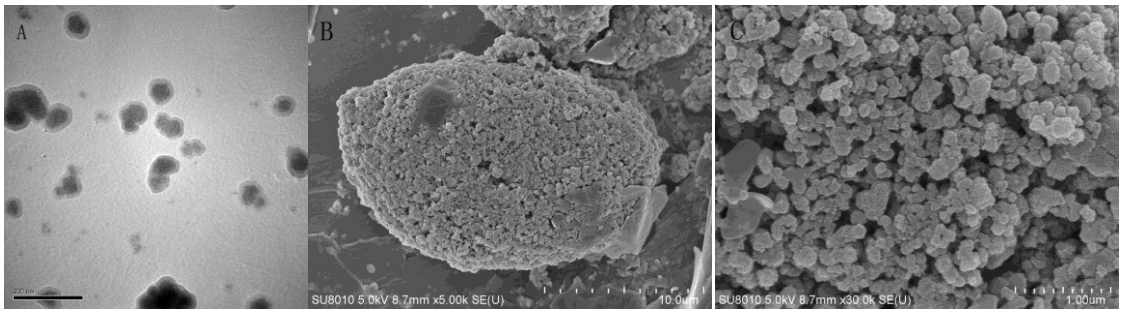


Fig. 1 - (A) TEM of the immobilized HRP (acceleration voltage of 80 KV, magnification of 1.5×10^5); (B) SEM of whole reunited immobilized-HRP; (C) SEM of the surface of IM-HRP

Luckarift.[26]. Besides, the well encapsulated HRP images can be clearly observed in their TEM images there was wrapped in a layer of hydrated titanium around the enzyme, which looked like the TiO_2-shell coating on Fe_3O_4-core nanoparticles according to the report of A.Hasanpour et al.[27]

3.2.2 FT-IR characterization:

FT-IR spectra of free-HRP/hydrous-titania/immobilized-HRP bead surfaces were presented in Fig. 2. For the free-HRP, the characteristic transmittance at 1510 cm^{-1} and 1244 cm^{-1} were related to the stretching brand of -CONH- (amide Ⅰ) and (amide Ⅱ) vibrations [9]. The sharp transmittance of the bands at 1631 cm^{-1} and 1047 cm^{-1} were associated with the stretching vibrations bands of Ti-H bonds which were features of hydrated TiO_2 particles surfaces. And the wavenumbers of 2969 cm^{-1} represented to the stretching bands of -NH_2 groups. On the other hand, after HRP enzymes were immobilized by hydrated TiO_2 coatings, the immobilized HRP particles still exhibited three characteristic stretching bands at 1510 cm^{-1}, 1631 cm^{-1} and 2969 cm^{-1} presented of -CONH- , Ti-H and –NH_2 vibrations. These results demonstrated that the enzymes were immobilized on hydrated titanium. It's assumed to be that free HRP enzymes were successfully immobilized on hydrated titanium coatings based on the results of TEM, SEM and FT-IR analysis.

3.3 Effects of reaction conditions on enzymes residual activity in different enzyme-catalyzed systems

3.3.1 Effect of the temperature on residual activity of enzymes

Temperature was believed to be one of the most important influencing factors to enzymes activity. Enzymes structure might be destroyed and consequently resulted in function loss at certain temperatures. Therefore, it was necessary to determine the effects of temperature on the activity of free/immobilized HRP enzyme. In this work, effects of temperature on the free/immobilized HRP enzymes residual activity were also investigated. The results can be found in Fig. 3, the optimal temperature of free/immobilized HRP enzymes was about 37 °C. In order to enable the free/immobilized HRP enzymes to function efficiently under the catalysis reaction process, the temperature of the catalysis reaction should be controlled by 30 - 40 °C. The results showed free-HRP enzymes almost lost activity at above temperature range 70 °C.

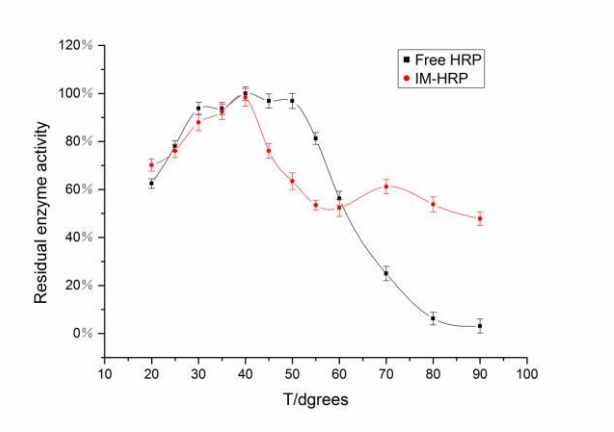


Fig. 3 - Residual enzyme activity of initial enzyme with different temperature; 5 mg of IM-HRP, the content of HRP on carrier was 3.11 mg/g; 0.1 ml free HRP (20 mg/L)

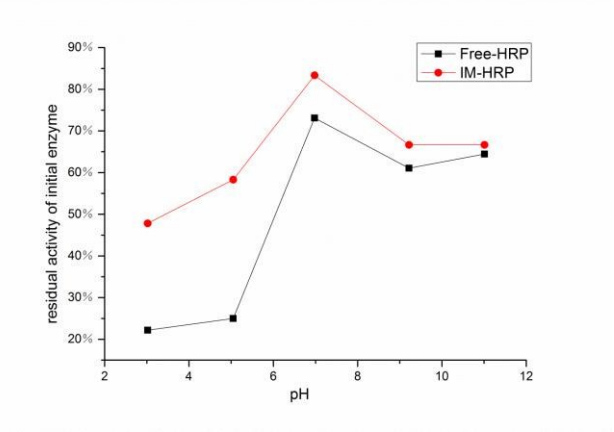


Fig. 4 - Residual enzyme activity of initial enzyme of storage 4 h in different pH under the condition 25 °C

However, IM-HRP enzymes exhibited significantly higher stability in the temperature examined compared to the free

HRP enzymes. IM-HRP enzymes still was up to 50% of residual enzyme activity under optimal conditions at temperature from 60 to 90 °C. It could be indicated that the IM-HRP enzymes were more resistant to the high temperature conditions than free HRP enzyme. This phenomenon might be due to the improved stability of HRP enzymes resulting from immobilizing the free HRP enzymes on hydrated titanium coatings.

3.3.2 Effect of pH on the residual activity of enzymes

As depicted in Fig. 4, optimal pH for both free and immobilized HRP enzymes was about 7.0. At this time, residual enzyme activity of IM-HRP and free-HRP accounted for 83.3% and 73.1% of initial value respectively. Moreover, the residual activity of immobilized HRP enzymes was more stable in the harsh pH conditions (acid and alkaline) in comparison to that of the free HRP enzymes. These results revealed that the IM-HRP enzymes were more resistant to the pH changes. It was very likely that the stability against to pH changes of HRP enzymes was improved by loading on the surface of hydrate titanium.

3.4 Catalytic reaction kinetics of free-HRP and IM-HRP

In order to understand the enzymatic capacity of free/immobilized HRP enzymes, the enzymes constant k_m (Michaelis constant) were obtained by using phenol compounds as the substrate. For enzyme catalyzed process, the reaction rate (v) and the substrate concentration of phenol ([S]) compounds conformed the Michaelis-Menten equation. The k_m could be obtained by following equation:

$$\frac{1}{v} = \frac{K_m}{v_{max}} \bullet \frac{1}{[S]} + \frac{1}{v_{max}}$$

The k_m of free/immobilized HRP enzymes were determined by 1/[S] to 1/v.

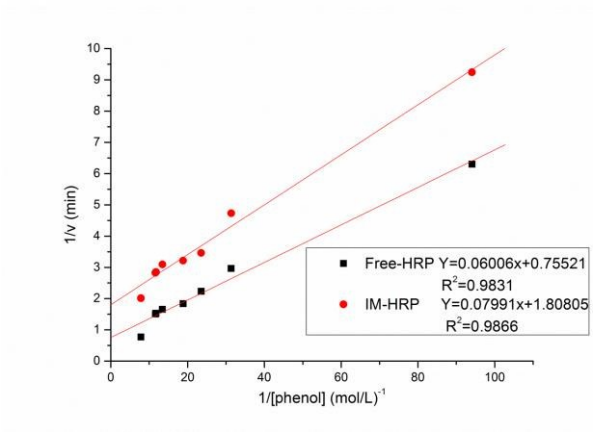


Fig. 5 - Kinetic of free and immobilized HRP

As showed in Fig. 5, experimental data were conformed well to Michaelis - Menten equation (R^2 > 0.98). The constant k_m of IM-HRP for phenol degradation was slighter higher than that of free-HRP enzymes in the present of H_2O_2. It was demonstrated that the catalytic performance was comparable to that of the free-HRP enzymes. Moreover, the increase in k_m value of IM-HRP could be explained by the reduction of enzyme activity for binding substrate after HRP was immobilized [29], or lower rate of IM-HRP enzymes binding substrate in catalysis progress compared with free-HRP.

3.5 Effects of reaction conditions on phenol removal using different enzyme-catalyzed systems

3.5.1 H_2O_2 dose

In this paper, experiments were carried out by choosing different H_2O_2 (6 g/L, 30%) dosage range 0.2 – 1.5 ml and initial phenol substrate concentrations range 10 – 100 mg/L to determine the phenol removal efficiency under catalysis with free/immobilized HRP enzymes. As depicted in Fig. 6, the phenol removal efficiency of IM-HRP and free-HRP reached the maximum at 94% and 91% respectively when H_2O_2 (6 g/L, 30%) dosage was 1.0 ml. It was clear that the phenol removal percentage of IM-HRP enzymes was higher than that of free-HRP. It was very likely to be the result of the phenol adsorption on the surface of the IM-HRP particles which were very porous. Addition of excessive H_2O_2 could also lead to a decrease in substrate removal. Thus, high H_2O_2 concentration inhibited the activity of enzymes. Some studies also suggested that that overdose of H_2O_2 could prohibit the enzyme catalysis performance [28, 29].

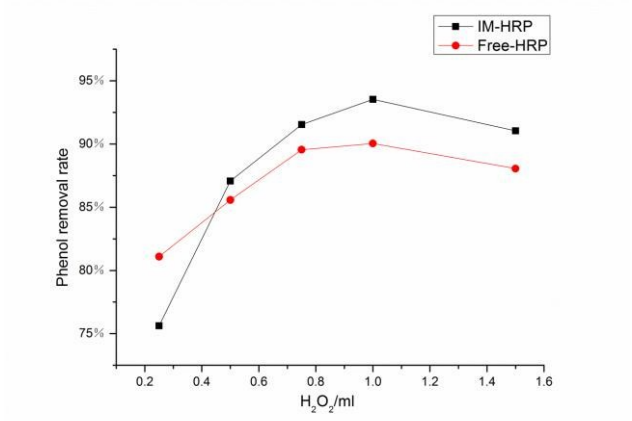


Fig. 6 - Phenol removal efficiency of H_2O_2 (6g/L, 30%) dosage; phenol concentration was 20 mg/L; reaction time was 25 min; temperature of 37 °C; IM-HRP dosage was 8 mg

3.5.2 Initial phenol concentration

The amount of initial substrate concentrations were also profiled in Fig. 7, the phenol removal percentage was sharply decreased by increasing initial phenol concentrations from 10 to 100 mg/L. The efficiency of phenol removal with initial

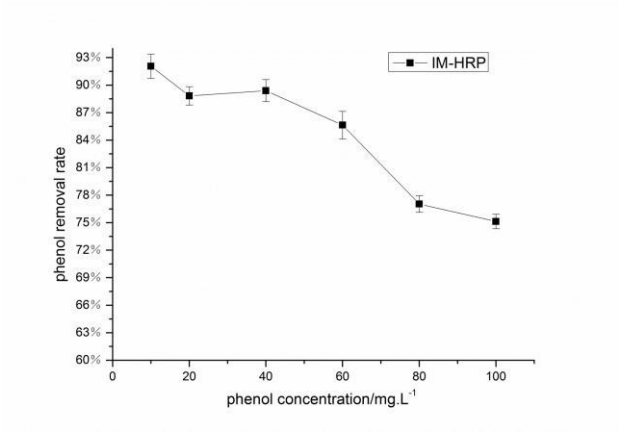


Fig. 7 - Phenol removal efficiency of initial phenol concentration under the condition 37 °C (1.0 ml of H_2O_2 (6 g/L, 30%); reaction time was 25 min. IM-HRP dosage was 8 mg)

phenol concentration was 10 mg/L on the H_2O_2 concentration of 0.212 mol/L, the phenol removal efficiency was reached by 92.05%. The results demonstrated that degradation of phenol was highly dependent on the initial phenol concentration, and the phenomenon was due to the higher initial phenol concentration leading to low catalytic reaction rate and resulting in poor phenol removal. Therefore, more catalysts and H_2O_2 needed to be added to reach a higher organic removal.

3.5.3 IM-HRP dose

The optimal phenol removal capacity of IM-HRP required to

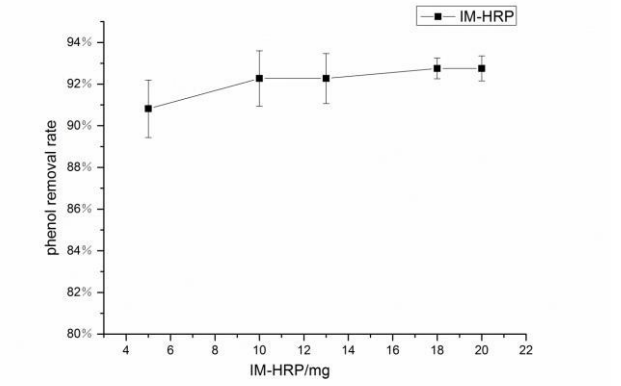


Fig. 8 - Phenol removal rate among the dosage of IM-HRP (phenol concentration was 16 mg/L; 1.0 ml H_2O_2 (6 g/L, 30%); reaction time of 25 min under the condition of 37 °C)

the catalyst reaction was showed in Fig. 8. As depicted, the maximum efficiency on phenol removal from aqueous solutions was achieved with 18 mg IM-HRP (0.36 g/L), and no obvious enhancement of phenol removal was observed with further increase in IM-HRP dosage. At optimal catalyst dosage, the phenol removal percentage was reached 92.75% under initial phenol concentration of 16 mg/L. It was because that oxidative degradation of organics was restricted at low concentration of enzymes, consequently leading to a slow reaction rate and poor phenol removal. Moreover, it should be noted that an errors of experimental replications (see in Fig. 8), might be attributed to the uneven distribution of HRP enzymes during HRP immobilization process on hydrate titanium coatings.

3.5.4 Effect of Reaction time on phenol removal

Fig.9 showed the change of phenol concentration with the reaction time. It was found more than 92.34% of phenol was removed within only 15 min, no significant improvement was observed with time proceeded further. The phenol concentration was reduced from 16.00 mg/L to 1.23 mg/L after 15 min of reaction time.

As showed in Table 1, phenol degradation could be fitted well with both the pseudo second-order kinetic and pseudo

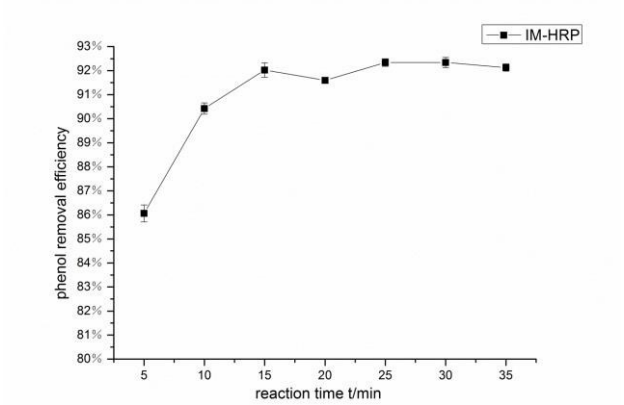


Fig. 9 - Phenol removal efficiency of the reaction time under the condition of 37 °C (phenol concentration of 16 mg/L; 1.0 ml H_2O_2 (6 g/L, 30%); 15 mg of IM-HRP)

first-order kinetic equations. The apparent phenol degradation rate constant (k) was 0.02499 L/(mg.min) and 0.54659 (min^{-1}), and the linear correlation coefficient was 0.95 and 0.93. The fitting results revealed that the degradation of phenol was likely dependent on the concentrations of substrate and H_2O_2.

For the substrate, the degradation of phenol rate was decreased under the condition of low initial substrate concentrations. In addition, degradation of phenol was not linear to the concentration of H_2O_2, the excessive H_2O_2 might also exhibited oxidative capacity on phenol molecules. As for the effect of IM-HRP dosage (seen in Fig.8), phenol removal efficiency was around 90% at different IM-HRP dosages revealing that the dosage of IM-HRP had no obvious influence

on phenol degradation. Hence, the concentrations of both substrate and H_2O_2 had important in phenol degradation and substrate concentration might be more crucial factor.

Table 1 - Degradation rate of phenol in different time

	K_{obs} *	R^2 **
Pseudo-1st $c/c_o = e^{-kt}$	0.54659(min^{-1})	0.9321
Pseudo-2nd $c/c_o = \frac{1}{ktc_o+1}$	0.02499(L/(mg.min))	0.95452
Pseudo-3rd $c/c_o = \frac{1}{\sqrt{2ktc_o^2-1}}$	0.0066(L^2/(mg^2.min))	0.54084

* k is the coefficient of determination for the fit of oxidation or adsorption curves to kinetic models.
**R^2 is the pseudo first-order rate constant, pseudo second-order rate constant and pseudo third-order rate constant of the oxidation kinetics.

3.6 Reaction mechanism of degradation of phenol by IM-HRP in present of H_2O_2

The cycle of phenol removal from aqueous solutions by IM-HRP with H_2O_2 were summarized in Fig.10. At first, phenol and H_2O_2 molecules were adsorbed to the surface of porously IM-HRP, and then it was involved to the enzyme catalytic process. It was reported that reactions occurred during process of enzyme catalysis were involved in one-electron oxidation of phenol compounds [30]. It could be indicated that the free enzyme (IM-HRP, E) was oxidized to a state of an active intermediate enzyme product called compound E_1 by hydrogen peroxide (H_2O_2). Compound E_1 then oxidized one phenol molecule to form free phenoxy radical (PhO·) through one-electron transferring. Then, another phenol molecule was oxidized into free phenoxy radical. Meanwhile, compound E_2 was converted into base enzyme E. Finally, the free phenoxy radicals were released to the water environment through desorption from surface of IM-HRP, and the free phenoxy radicals may couple to each other to form insoluble polymers through non-enzymatic reaction process at certain concentration [31].

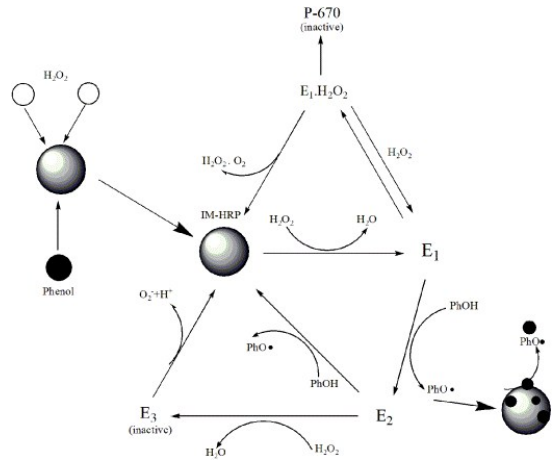


Fig. 10 - Model of IM-HRP catalysis progress on phenolic compounds in present of H_2O_2, resulting in free phenol radical. Enzyme inactive production was ending in forming P-670 or E_3.

For the enzyme catalytic progress, several side reactions may also take place in enzyme-catalytic reactions. This could result in denaturation of native enzymes and reduce the activity of IM-HRP enzymes. E_1 could be converted to P-670 which was inactive product through irreversible reaction process. Or it would transform into E_3 which could undergo further decomposition into native state E. However, the formation reaction of P-670 was very likely to be inhibited in the present of hydrous-titanium carriers, which contributed to catalytic stability of HRP enzyme. Hence, HRP enzymes with the protection of hydrous-titanium can avoid the direct contact of enzymes to strong acid/alkaline solution, or can protect its enzymes from denaturation under condition of high temperature to a certain degree.

4. Conclusions

In this study, the feasibility of using a biomimetic process to immobilize the enzymes on hydrous titanium and its application to the phenol removal were investigated. The experiment results revealed the immobilized process could improve HRP enzymes stability in harsh conditions, while the free-HRP enzymes were easy to be affected at strong alkaline or high temperature conditions. Immobilization process had very limited effect on catalytic activity of HRP enzymes. And it also demonstrated the degradation of phenol was dependent on different reaction conditions (e.g. temperature, dosage of IM-HRP and H_2O_2, reaction time), 90% of phenol could be removed under optimal reaction conditions. In addition, the phenol was initially adsorbed on the surface of porously hydrous-titanium and then oxidative degradation occurred with addition of H_2O_2. In conclusions, this work has developed a novel enzyme immobilization method which could effectively enhance the stability of HRP enzymes at harsh water quality conditions. It is a very promising technology for stable abatement of organic contaminant in drinking and/or waste water.

Acknowledgments

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Abstract In this work, hydrous-titanium was utilized to immobilize horseradish peroxidase (HRP) in order to improve its stability and adaptability under different water qualities by biomimetic titanification process. The catalytic performance of immobilized HRP for phenol removal from aqueous solution was evaluated. The physicochemical properties of immobilized HRP (IM-HRP) were characterized by means of scanning electron microscopy (SEM), transmission electron microscopy (TEM), and Fourier transform infrared spectroscopy (FT-IR). The effects of reaction conditions (H_2O_2 and IM-HRP dosage, initial phenol concentrations) on phenol removal were investigated. Enzymatic activity analysis indicated that immobilization process has no adverse effects on catalytic performance of HRP, and it was observed a slightly increase in enzymatic catalytic kinetic (k_m) of IM-HRP when compared to its free enzymes. It was found that in a synthetic condition of 37±3 ^{o}C, the IM-HRP exhibited a perfect phenol removal over 90% after reaction 15 min. In addition, the enzyme activity of IM-HRP was very stable over a wide reaction conditions, e.g. temperature from 20 to 90 ^{o}C and pH from 3.0 to 11.0.Therefore, HRP enzymes loaded on hydrous-titanium was very effective to improve stability of enzymes and can alleviate the adverse effects of environmental and varying water conditions on contaminants water.

Graphic abstract