Enzymatic removal of paracetamol from aqueous phase: horseradish peroxidase immobilized on nanofibrous membranes

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Abstract Paracetamol is a widely used as an analgesic and an antipyretic that can easily accumulate in aquatic environments. This study aimed to enhance paracetamol removal efficiency from water by combining the biocatalytic activity of horseradish peroxidase (HRP) with the adsorption of nanofibrous membrane. Poly(vinyl alcohol)/poly(acrylic acid)/SiO_2 electrospinning nanofibrous membrane was prepared with fiber diameters of 200 to 300 nm. The membrane was made insoluble by the thermal cross-linking process. HRP, which was previously activated by 1,1′-carbonyldiimidazole, was covalently immobilized on the surface of nanofibers. Immobilized HRP retained 79.4 % of the activity of free HRP. The physical, chemical, and biochemical properties of the immobilized HRP and its application in paracetamol removal were comprehensively investigated. Immobilized HRP showed better storage capability and higher tolerance to the changes in pH and temperature than free HRP. Paracetamol removal rate by immobilized HRP (83.5 %) was similar to that of free HRP (84.4 %), but immobilized HRP showed excellent reusability. The results signify that enzyme immobilized on nanofibers has great application potential in water treatment.

Keywords Fibrous membrane · Horseradish peroxidase immobilization · Enzymatic catalysis · Paracetamol

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Introduction

Pollution by pharmaceuticals has recently been documented and reported as an emerging environmental issue (Jørgensen and Halling-Sørensen 2000). Hundreds of tons of pharmaceuticals, including anti-inflammatory drugs, analgesics, beta-blockers, lipid regulators, antibiotics, antiepileptics, and estrogens, are globally prescribed annually and are consequently being discharged in sewage effluents. Pharmaceuticals and their metabolites or modified derivatives have been found in surface and groundwaters, even in drinking waters (Heberer 2002; Wu et al. 2012). Paracetamol is a widely used over-the-counter analgesic and antipyretic drug. The toxicity of this drug accounts for at least 42 % of acute liver failure cases and one third of the deaths at tertiary care centers in the USA (Larson et al. 2005). Paracetamol and structural analogs are ubiquitous in the natural environment and can easily accumulate in aquatic environments. Paracetamol has been found at a concentration of up to 10 μg/L in US natural waters (Kolpin et al. 2002), and more than 65 μg/L was recorded in the Tyne River in the UK (Gros et al. 2006). Although no specific indications of paracetamol toxicity toward test organisms have been documented, the drug should still be removed from water sources as a precautionary measure.

Paracetamol could be incompletely and inefficiently removed by conventional sewage treatment plants (STPs) (Onesios et al. 2009) or non-mechanical STPs, such as wetlands (Ranieri et al. 2011). Some activated carbons made from wastes and commercialized carbonaceous adsorbents had been studied for paracetamol removal from water (Cabrita et al. 2010) as well. However, the absorption could not achieve the goal of paracetamol transformation or detoxification.

Enzymes are well-known green catalysts with a high degree of specificity. The mildness and specificity of enzymes provide them with a high efficiency for applications in

environment protection (Mrozik et al. 2008). Horseradish peroxidase (HRP) has been successfully used to transform many industrial toxic compounds, such as phenols (Liu et al. 2002) and anilines (Husain and Ulber 2011), effectively. As a kind of enzyme, HRP has such drawbacks as low stability, short lifetime, high price, and difficulty in recycling, which limit its practical applications (Kim et al. 2013). Enzyme immobilization technique can be used to improve the economic efficiency and practical application of HRP (Mohamed et al. 2013; Pramparo et al. 2010).

Numerous materials and techniques, such as mesoporous silicas, Eupergit® C and porous acrylic resins (Sheldon 2007), can be used for enzyme immobilization. We have used several electrospun fibrous membranes (EFMs) for enzyme immobilization, such as polyacrylonitrile EFM (Xu et al. 2013a) and chitosan/poly(vinyl alcohol) composite EFM for laccase immobilization (Xu et al. 2013b). The experimental results demonstrated that enzyme immobilized on nanofibrous membranes has high enzyme loading, catalytic activity, and reusability. Additionally, the high specific area and porous structures of nanofibrous membranes are beneficial for contaminant adsorption and mass transfer enhancement between substrate and the active sites of enzymes (Huang et al. 2003; Lin et al. 2012). Therefore, HRP immobilization on the nanofibrous membranes may improve its catalytic transformation of pollutants in practical applications and thus expand its application in water treatment.

This study aims to use immobilized HRP on poly(vinyl alcohol)/poly(acrylic acid)/SiO$_2$ (PVA/PAA/SiO$_2$) nanofibrous membranes (PPSiNFM-HRP) to biotransform paracetamol catalytically. This is the first to report on the removal of paracetamol by immobilized HRP. The physical, chemical, and biochemical properties of immobilized HRP are comprehensively studied. Likewise, the factors influencing paracetamol removal by immobilized HRP are investigated.

Experimental section

Materials

PVA (molecular weight (M_W=88,000–97,000), PAA (M_W=450,000), tetraethyl orthosilicate (TEOS), 1,1′-carbonyldiimidazole (CDI), tetrahydrofuran (THF), hydrogen peroxide (H$_2$O$_2$), 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), paracetamol, fluorescein isothiocyanate (FITC) isomer I, and Brilliant Blue G were provided by Aldrich. HRP was purchased from the Sinopharm Chemical Reagent Company, China. Deionized (DI) water was used throughout this work. All chemicals used were of analytical grade.

Preparation of PVA/PAA/SiO$_2$ nanofibrous membranes by electrospinning

A PVA/PAA/SiO$_2$ gel for electrospinning was prepared through the following procedures: first, PVA was dissolved in deionized water and gently stirred for 5 h at 90 °C to prepare 5 % w/w solution. PAA was dissolved in DI water and stirred for 8 h at room temperature to prepare 10 % w/w solution. Second, 10 g aqueous solutions of PVA and 1.5 g aqueous solutions of PAA (5.45 OH/COOH ratio) were mixed and stirred for 24 h at room temperature. Third, 0.8 mL of HCl (1 M) was dropped into the mixture of 1 g of TEOS, 1 g of water, and 2 g of ethanol. A silica gel was obtained after mild stirring for 2 h at 60 °C. Finally, the mixture of PVA and PAA was slowly added to the silica gel and stirred for another 30 min at 60 °C. The gel of PVA/PAA/SiO$_2$ composite was obtained.

Thereafter, 10 mL of fresh PVA/PAA/SiO$_2$ gel mixture was loaded in a fitted syringe. The solution was pumped through a thin stainless needle with an inner diameter of 1.2 mm attached to a power supply. Electrospinning conditions were set as follows: 15 kV, flow rate of 1 mL/h, and nozzle-collector distance of 15 cm. The fibers were collected on a glass plate covered with an aluminum foil. A few hours were needed to collect sufficient membrane samples. After electrospinning, the nanofibrous membranes were heated at 140 °C for 1 h in a vacuum oven. The membranes were insoluble in water.

Characterization

Scanning electron microscopy (SEM) measurements were performed on a field emission XL-30 SEM system at 30 kV. The residual concentration and activity of HRP were assayed using a UV-1700 spectrophotometer from Shimadzu. Fourier transform infrared–attenuated total reflectance (FTIR-ATR) spectroscopy equipped with a germanium crystal was used to detect the functional groups of nanofibers before and after enzyme immobilization. High-performance liquid chromatography (HPLC) was used to measure paracetamol concentration. To verify whether HRP had been immobilized by the nanofibers, HRP labeled with FITC (HRP-FITC) was used for immobilization and characterized by laser confocal scanning microscopy (LCSM; Leica TCS-SP5, Germany). HRP labeling was conducted through the following procedures: (1) HRPs were fluorescence labeled with FITC: first, 1 mg/mL HRP solution was prepare by phosphate-buffered solution (PBS) (pH=8.0) at 4 °C; second, FITC was dissolved into 3 % sodium carbonate solution, and the amount of FITC was determined by 15 μg/mg HRP; and third, two kinds of solutions above were mixed for 12 h at 4 °C in the dark. (2) The labeled enzyme solution was dialyzed at 4 °C in the dark for 36 h to ensure that no free FITC remained in the enzyme solution, and then, the FITC could be labeled on HRP. (3) The

FITC-labeled HRP was used for immobilization, and the immobilized FITC-HRP was washed with DI water for five times before LCSM observation to ensure that there is no free FITC-HRP remained on the membrane.

Immobilization of HRP on PVA/PAA/SiO$_2$ nanofibrous membranes

CDI was chosen as the activator in this study. The hydroxyl groups of the PVA/PAA were reacted with CDI, and the final products contained amine-reactive imidazolyl carbamate groups. Then, 2 g of cross-linked PVA/PAA/SiO$_2$ nanofibrous membranes were washed several times with anhydrous THF to eliminate any remaining traces of water and then placed in a 100-mL conical flask with 65 mL of anhydrous THF containing CDI at a concentration of 50 mg/mL (0.3 M). The reaction lasted for 24 h at room temperature. The nanofibrous membranes were washed five times with anhydrous THF to remove excess CDI and reaction by-products. THF was distilled off, and the activated nanofibrous membranes were stored at 4 °C before use.

The activated nanofibrous membranes were mixed with an HRP solution (1 mg/mL in pH=6.0 PBS) at 25 °C for 12 h. The nanofibrous membranes immobilized with HRP were washed ten times using PBS to ensure that no HRP remained.

Assays of enzyme activity

The activities of free and immobilized HRP were determined by monitoring the oxidation of ABTS in a reaction mixture under standard conditions. The method of activity determination has been previously described in detail (Xu et al. 2013a).

The assay reaction contained 20 mL 1 mM ABTS in 0.1 M PBS (pH 4.5), 0.8 mM H$_2$O$_2$, and 1 mg free or immobilized enzyme. For both the free and immobilized HRP, the reaction time was 3 min, and the absorbance was monitored at 420 nm on a UV spectrophotometer.

Stabilities of free and immobilized HRP

Thermal, pH, storage, and operational stabilities of free and immobilized HRP were studied. The tested pH ranged from 3.0 to 9.0 in PBS, and the temperature ranged from 20 to 50 °C.

Residual activities of PPSiNFM-HRP were measured periodically for 1 month to assess storage stability. Before activity assays, PPSiNFM-HRP was stored in PBS (pH=5.0) at 4 °C. Determination of operational stability referred to the study of Dai et al. (2010) PPSiNFM-HRP was separated from the reaction system after each run, washed thrice with PBS, and then transferred to the fresh ABTS solution for observation of any the change in UV absorbance.

To examine reusability and stability, the membranes were removed from the reaction mixture and washed with the PBS/ethyl alcohol (1:1 w/w) solution until no paracetamol was detected in the washings after each run. The membranes were then transferred to treat paracetamol and to evaluate operational stability.

All stability tests were performed in quintuplicate.

Paracetamol removal by free and immobilized HRP

Paracetamol removal by HRP was performed in a mixture containing 50 mL of 20 mg/L paracetamol in PBS, 0.4 mM H$_2$O$_2$, and 5 mg of free or immobilized HRP. After 90 min, the upper solution was filtered, and the residual concentration of paracetamol was measured using HPLC based on standard methods for the examination of water and wastewater.

The effect of pH on paracetamol removal was investigated at 25 °C within the pH range of 3.0 to 9.0, and the effect of initial H$_2$O$_2$ concentration was investigated within the range of 0.4 to 1.6 mM. A blank carrier was used in place of PPSiNFM-HRP to determine the effect of carrier absorption on paracetamol removal. The reusability of PPSiNFM-HRP was evaluated using paracetamol as a substrate, and the removal efficiency was used as the index of reusability. After each reaction run, PPSiNFM-HRP was washed with PBS until no paracetamol was detected in the washings.

HPLC analysis

HPLC (Agilent Technologies 1200 Series) equipped with a reverse phase column (DiKMA, C18, 250×4.6 mm, 5 μm) was used to analyze the paracetamol concentration in the samples. The separated components were detected at 250 nm and 35 °C. The mobile phase was composed of CH$_3$OH/50 mM ammonium acetate (ratio of 17:83) with a flow rate of 0.8 mL/min. The paracetamol retention time was 10.61 min. All the analyses were completed in triplicate.

Data analysis

One-way ANOVA was used to determine statistical significance of HRP immobilization efficiency and paracetamol removal performance. Differences among multiple groups of data were determined by multiple comparisons using Tukey's procedure at a family error rate of 5 %. Data were deemed significantly different between two values if $p<0.05$. All statistical analyses were performed using Design Expert Version 8.0.5b.

The first-order rates (k), the time required to obtain 50 % of substrate adsorption/biotransformation ($t_{1/2}$), and the paracetamol removal rate after t (RE$_t$) were estimated by nonlinear

regression analysis using the first-order model, as shown in Eqs. (1) to (3).

$$C_t = C_0 e^{(-kt)} \tag{1}$$

C_0 and C_t are the substrate concentrations at the beginning of the run at the time (t), and k is the first-order reaction constant. From the k values, two efficiency ($t_{1/2}$ and RE_t) factors can be calculated.

$$t_{\frac{1}{2}} = \frac{\ln 2}{k} \tag{2}$$

$$RE_t = \frac{C_0 - C_t}{C_0} * 100 \tag{3}$$

where C_0 and C_t are the paracetamol concentrations at the onset and time (t) of reaction.

Results and discussion

Morphology and structure of nanofibrous membranes

In this study, nanofibrous membranes were generated from the mixtures of PVA and PAA by thermally induced cross-linking. Consequently, all membranes became insoluble in water because of the thermally induced esterification reaction between PAA and PVA (Li and Hsieh 2005). Figure 1 shows a schematic of the PPSiNFM composite nanofiber membranes.

Figure 2a illustrates the morphology of nanofibrous membranes before enzyme immobilization. Continuous fibers with diameters of 200 to 300 nm were formed. The nanofibers were bead-free, uniform, and randomly arrayed. The surfaces of fibers were rough, which may be attributed to the pore formation in the fibers caused by polymer surface tension, rapid solvent evaporation, and phase separation during the electrospinning processes (Selvakannan et al. 2013; Teng et al. 2011). The formed pores could enhance the specific surface area of the fibers for contaminant capture and enzyme immobilization (Greiner and Wendorff 2007; Sill and von Recum 2008). Therefore, PVA/PAA/SiO$_2$ nanofibrous membranes prepared in this work may have advantages for enzyme immobilization and pollutant adsorption.

Immobilization of HRP

The covalently immobilization program has two steps. First, the hydroxyl groups on the nanofiber membrane surfaces react with CDI to form imidazole carbamates. Second, the amino groups of enzyme react with the imidazole carbamates, and enzyme molecules bond to the fiber surfaces.
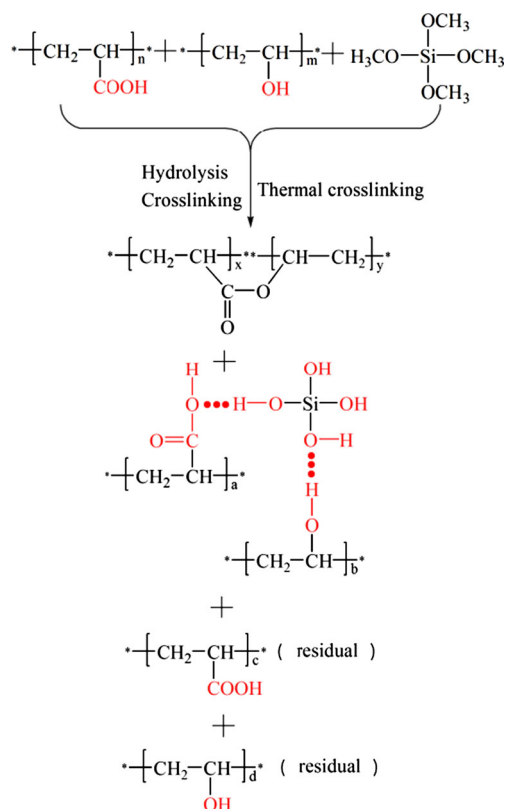

Fig. 1 The schematic synthesis route of PPSiNFM composite nanofiber membranes

The results showed that the HRP-FITC immobilized on nanofibers activated by CDI radiated much stronger green fluorescence (Fig. 2b) compared to the non-activated ones (Fig. 2c). The faint fluorescence radiated from the non-activated ones may be due to the physical adsorption of a small number of HRP-FITC. The strong fluorescence emitted by the activated ones should be caused by the combined effect of covalently binding and physical adsorption of HRP-FITC to the fibers. In summary, the LCSM observation showed that HRP immobilization was carried out successfully after PPSiNFM activated by CDI.

The variation of functional groups of membranes after immobilization can be deduced from the results of FTIR (Fig. 3). The characteristic IR bands included Si–O (H) (964 cm^{-1}), Si–O–Si (1096, 806, and 468 cm^{-1}), and O–H (3212 cm^{-1}). The appearance of an N–H (1559 cm^{-1}) adsorption peak in Fig. 3(b) indicated the reaction between enzyme and –OH on the membranes.

Several studies about HRP immobilization showed that the binding capacity of the support materials differed owing to the characteristic properties of the materials, activator, and reaction environment (Basturk et al. 2013; Caramori and Fernandes 2004; Monier et al. 2010). In this study, the HRP loading was approximately 343 mg/g membranes and retained 79.4 % activity of free HRP, higher than some reported results, such as those immobilized on Eupergit® C
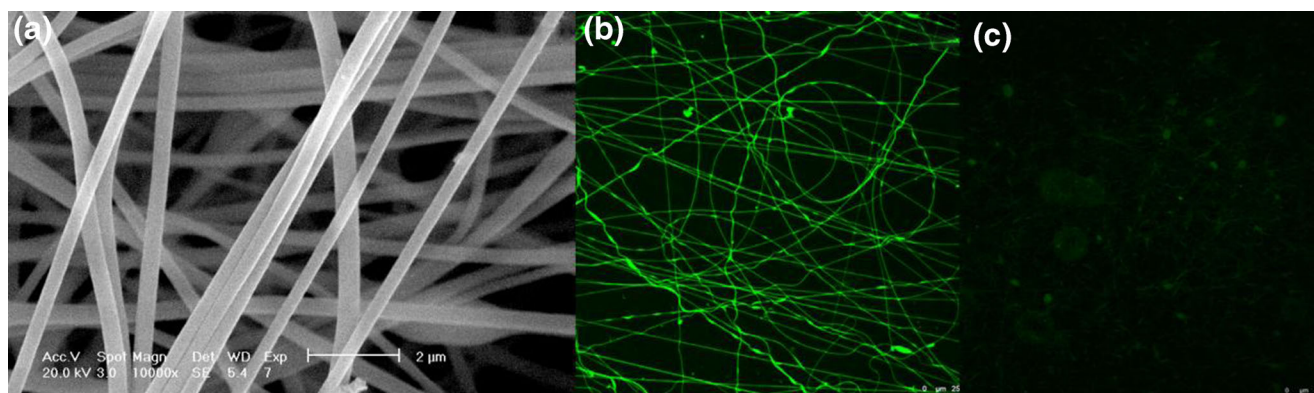
Fig. 2 **a** SEM image of PPSiNFM and LSCM images of **b** CDI activated and **c** non-activated PPSiNFM immobilized with HRP-FITC

(70.4 mg/g and 40 %, respectively) (Pramparo et al. 2010) and modified chitosan beads (80.2 mg and 77.3 %, respectively) (Monier et al. 2010).
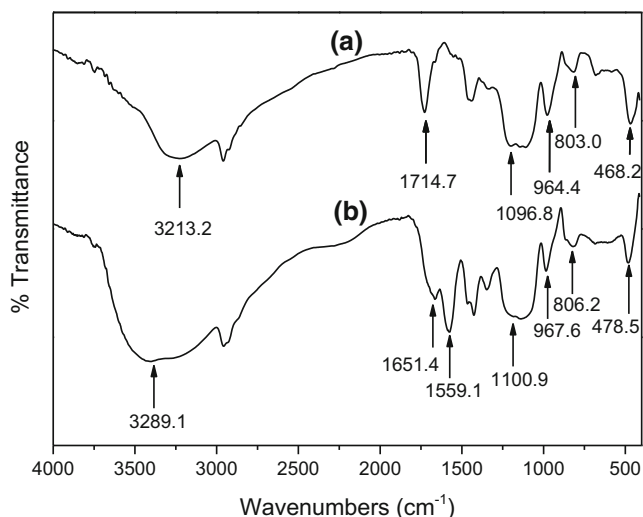
Effects of pH and time on HRP immobilization experiments were determined at room temperature, and the results are shown in Fig. 4. HRP immobilization was significantly affected by pH and time ($p < 0.05$). The optimum pH and time for enzyme immobilization were 8.0 and 10 h, respectively. Figure 4 shows that the immobilized HRP showed high residual activity at high pH, possibly because the enzyme proteins easily combined with imidazole carbamates at high pH (Hermanson 2013). The relative activity of immobilized HRP showed no obvious change after 10 h because HRP immobilization reached saturation owing to the steric constraints (Cristóvão et al. 2011).

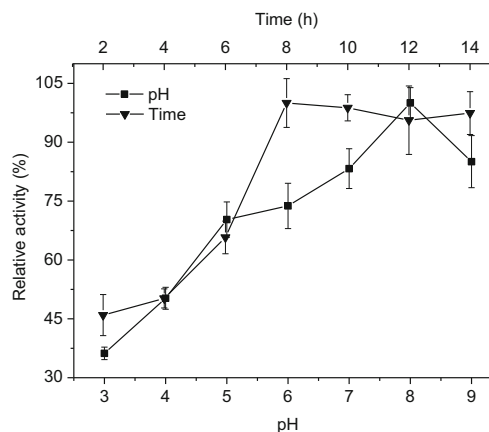Stabilities of free and immobilized HRP

Stabilities are important assessment indexes for the application of immobilized enzyme in diverse biotechnological field. The pH and thermal stabilities of free and immobilized HRP are shown in Fig. 5. Free HRP showed optimum activity at pH

4.0 and 30 °C. By contrast, immobilized HRP showed optimum pH and temperature in broader ranges, namely pH 5.0 to 7.0 and 35 to 45 °C, respectively. Immobilized enzyme maintained a significantly higher ($p < 0.05$) relative activity than the free enzyme throughout the range of pH tested except pH 4, indicating that immobilized enzyme is less sensitive to pH changes than free HRP. This condition can be attributed to the buffering effect provided by the support (Liu et al. 2013). In addition, the relative activity of immobilized HRP was greater than the free HRP at high temperature and decreased slower compared with free HRP. Osma et al. (2010) found that support has a protective effect when high temperature-induced enzyme deactivation occurs.

Enzymes are generally unstable in solution, and their activities could decrease during storage. Nevertheless, immobilization procedures can significantly improve storage stability ($p < 0.05$) (Fig. 5c). The activity of free HRP decreased significantly faster than that of PPSiNFM-HRP ($p < 0.05$). For instance, after 15 days of storage, free HRP lost approximately 73.0 % its activity, whereas the PPSiNFM-HRP lost only about 30.0 %. Therefore, immobilized HRP was more stable than the free HRP. The results can be attributed to the limited conformational changes of HRP molecules in the matrix of the support (Wan et al. 2008).

Fig. 3 FTIR spectra of *a* PPSiNFM and *b* PPSiNFM-HRP

Fig. 4 Effect of pH and time on HRP immobilization efficiency
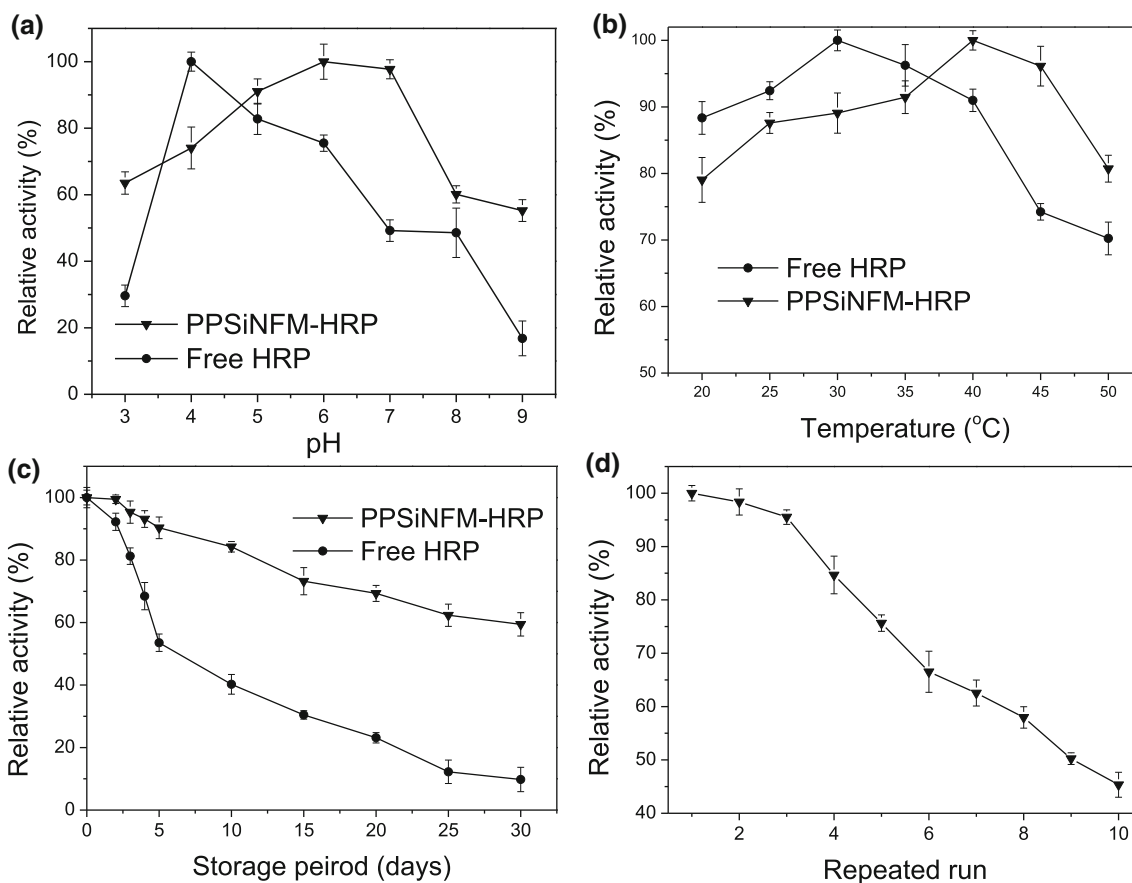
Fig. 5 **a** pH, **b** temperature and **c** storage stabilities of free HRP and PPSiNFM-HRP, **d** operational stability of PPSiNFM-HRP

Operational stability is one of the significant indexes used to evaluate enzyme properties. Figure 5d clearly illustrates that PPSiNFM-HRP retained 53 % of the initial activity after 10 cycles of use. The decrease in activity after repeated use can be attributed to the enzyme protein denaturation and membrane damage (Huang et al. 2008).

Removal of paracetamol by free and immobilized HRP

Effects of pH and initial concentration of H_2O_2

Paracetamol can be transformed to dimers, trimers, and tetramers via the process of H_2O_2-dependent oxidation and polymerization catalyzed by HRP (Potter et al. 1985). Paracetamol can then be removed from water as a precipitate.

Figure 6 shows the effects of pH on paracetamol biotransformation by free HRP as well as its removal by PPSiNFM and PPSiNFM-HRP. Paracetamol adsorption by PPSiNFM was approximately 20 % in this experiment, and no significant changes ($p>0.05$) were observed at different pH values because the fibers had no functional groups that could be significantly affected by pH.

Paracetamol removal by free and immobilized HRP generally occurred high under acidic conditions. This condition may be explained by the non-dissociated state of paracetamol and the net positive charge on PPSiNFM surface under acidic conditions. In this case, the hydroxyl groups on the fiber surface could provide protons for paracetamol. In addition, the HRP exhibited high catalytic activity under acidic conditions. The results were consistent with those reported by Meloun et al. (2005).

Paracetamol removal rates reached a maximum of 77.5 % at pH 4.0 by free HRP and 73.3 % at pH 6.0 by PPSiNFM-HRP. The difference in optimum pH conditions for paracetamol removal by free and immobilized HRP was consistent with the pH stability results. In addition, PPSiNFM-HRP was less sensitive to the change of pH than free HRP because the immobilization of HRP improved its pH stability.

Figure 6b shows that the maximum paracetamol removal rates reached the maximum of 84.4 % by free HRP and 83.5% by PPSiNFM-HRP at 0.8 mM of initial H_2O_2 concentration. However, the adsorption of paracetamol by PPSiNFM remained significantly unchanged with the variation of initial H_2O_2 concentration ($p>0.05$) because the membrane had no functional groups that could be affected by H_2O_2. Figure 6b shows that excessive H_2O_2 was unfavorable for paracetamol

Fig. 6 Effect of **a** pH on the removal of paracetamol by free HRP and PPSiNFM-HRP (60 min at 25 °C with 0.4 mM H$_2$O$_2$ initially) and **b** initial H$_2$O$_2$ concentration on the removal of paracetamol by free HRP (60 min at 25 °C with pH 4.0) and PPSiNFM-HRP (60 min at 25 °C with pH 6.0)
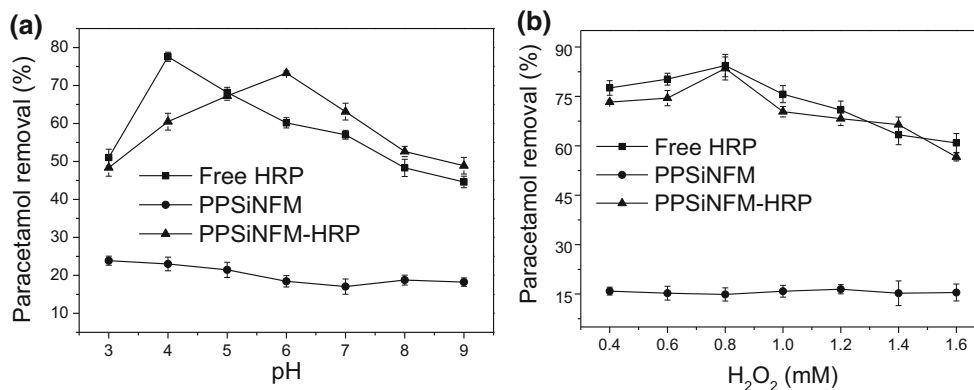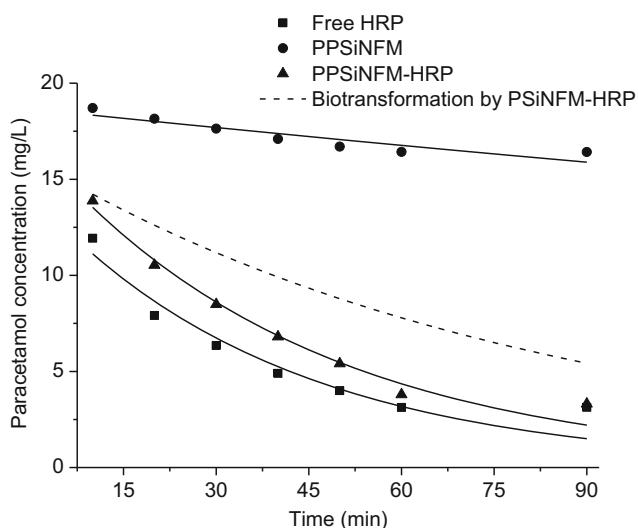

removal. First, H$_2$O$_2$ served as an inhibitor in enzymatic reaction when the ratio of H$_2$O$_2$ to enzyme was high. Second, H$_2$O$_2$ was involved in the unrelated reaction during paracetamol catalytic oxidation process (Maloney et al. 1986). Therefore, an appropriate dose of H$_2$O$_2$ to reaction system is very important for paracetamol removal.

Effect of time

Figure 7 shows the variation of paracetamol concentration with time. At the end of 60 min experiments, 84.4, 83.5, and 16.9 % of paracetamol were removed by free HRP, PPSiNFM-HRP, and PPSiNFM, respectively. PPSiNFM-HRP had two functions on the removal of paracetamol: the adsorption by the PPSiNFM and the biotransformation by HRP immobilized on PPSiNFM. The biotransformation of paracetamol by the immobilized HRP was calculated using Eq. (4)

$$C_b = C_r - C_a \qquad (4)$$

C_r, C_a, and C_b are the concentrations of paracetamol removed, adsorbed, and biotransformed by PPSiNFM-HRP, respectively.

According to the computation, 64.6 % paracetamol was biotransformed by PPSiNFM-HRP. Although relatively less enzyme molecules were found on PPSiNFM-HRP than on free HRP, their paracetamol removal efficiencies were similar. This condition may be explained by the combined effects of paracetamol biotransformation and adsorption by PPSiNFM-HRP.

The nonlinear fitting results demonstrated that the kinetics of paracetamol removal followed a first-order reaction. Table 1 shows that the paracetamol biotransformation rate of PPSiNFM-HRP was slower than that of free HRP, which may be attributed to the partial inactivation of PPSiNFM-HRP caused by spatial limitations for substrate diffusion and protein flexibility after enzyme immobilization (Bai et al. 2006; Sari et al. 2006).

Traditional biological biotransformation by organisms in municipal wastewater treatment took more than 72 h to transformed completely 1000 μg/L paracetamol (De Gusseme et al. 2011) and 2.4 h for 1 μg/L paracetamol (Joss et al. 2006). In addition, it took laccases hours to transformed similar amount of paracetamol to polymer (Ba et al. 2014). By contrast, the HRP catalysis treatment in this study showed a high removal efficiency of paracetamol. Therefore, enzyme catalytic transformation may become a novel approach for paracetamol removal from water in the future.

Fig. 7 Removal kinetics of paracetamol by free HRP (60 min at 25 °C with pH 4.0 and 0.8 mM H$_2$O$_2$ initially) and PPSiNFM-HRP (60 min at 25 °C with pH 6.0 and 0.8 mM H$_2$O$_2$ initially)

Table 1 k, $t_{1/2}$, and RE_{120} of free HRP, PPSiNFM-HRP, and PPSiNFM toward paracetamol

Sample	K (/min)	$t_{1/2}$ (min)	RE_{120} (%)
Free HRP	0.025	27.7	95.0
PPSiNFM-HRP	0.023	385.1	93.6
PPSiNFM	0.002	30.1	19.4
Biotransformation by PPSiNFM-HRP	0.012	57.8	76.3

Biotransformation by PPSiNFM-HRP: biotransformation by bonded HRP on the PPSiNFM
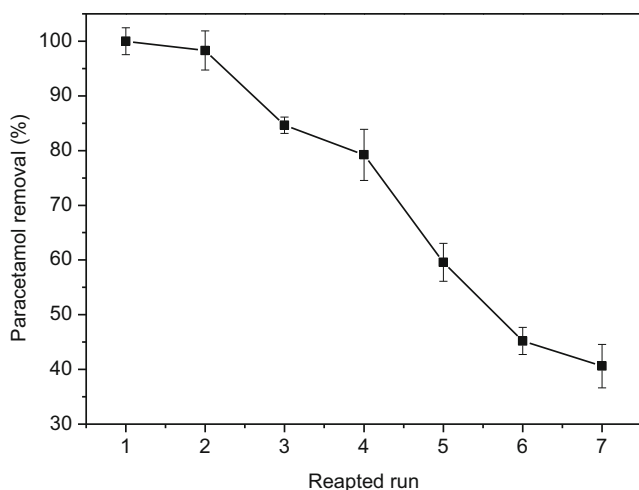
Fig. 8 Transformation of paracetamol removal rate of PPSiNFM-HRP during repeated runs

Reusability of PPSiNFM-HRP

The immobilized enzyme was superior to the free HRP in terms of reusability. Figure 8 shows that the paracetamol removal rate of PPSiNFM-HRP was approximately 40.6 % after seven batch operation runs, possibly because of the high HRP relative activity that remained after immobilization. The result showed that PPSiNFM-HRP provided higher reusability than some reported results, such as soybean peroxidase (SBP) immobilized on soybean seed coats (only be reused once) (Magri et al. 2007). Meanwhile, the loss and inactivation of the enzyme by polymeric products and the breakage of the membranes during the reaction could explain the decrease in removal efficiency.

Conclusion

PPSiNFM was successfully fabricated by electrospinning. These membranes were made to be water insoluble by thermo-cross-linking. This membrane can serve an enzyme support for HRP to retain high catalytic activity. The stability and reusability of the immobilized HRP on PPSiNFM were significantly improved compared with those of free HRP. The paracetamol removal rate of PPSiNFM-HRP (83.5 %) was similar to that of free HRP (84.4 %). PPSiNFM-HRP possessed the capabilities of both biocatalysis and adsorption because of its unique nanofibrous structure. The excellent reusability of this membrane makes it applicable to the field of wastewater treatment.

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