Immobilization of Horseradish Peroxidase on Modified Electrospun Nanofibrous Membrane for 2,4-Dichlorophenol Removal

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Abstract: In this study, a facile and simple method was used to modify the hydrophobic PAN/PVdF membranes into hydrophilic ones. Results showed that the PAN/PVdF membranes have been modified successfully and the water contact angle changed from 87.135° to 0°. Horseradish peroxidase (HRP) was immobilized onto the modified PAN/PVdF membranes through covalent binding and the maximum enzyme loading was approximately 440 mg/g under optimal conditions (after 8 h at pH 8.0 and 25 ºC). The effects of pH and temperature on the relative activity of free and immobilized HRP were studied. Under the optimum conditions of pH and temperature, immobilized HRP was greatly improved in operational and storage stability. 2,4-DCP removal experiments showed that the immobilized HRP and free HRP had a similar removal efficiency (87% and 93%, respectively). However, the immobilized HRP had an excellent reusability. HRP-PAN/PVdF could still remove 47.6% of 2,4-DCP after 7 repeated runs, which could overcome the inherent drawbacks of free enzymes-hard separation and non-reusability.

1 INTRODUCTION

Phenols, especially chlorinated ones, are considered as persistent organic pollutants among different kinds of pollutants in aquatic ecosystems mainly due to their harmful effects on organisms even at very low concentrations (Antizar-Ladislao and Galil, 2004) (Khenifi et al., 2009). Chlorophenols are one of the most important industrial materials and widely used in the production of insecticides, herbicides, wood treatment agents and flame retardants (Zhang et al., 2004). A typical example is 2,4-dichlorophenol (2,4-DCP), which is regarded as a priority pollutant by European Union (No, 2001) and United States Environmental Protection Agency (Keith and Telliard, 1979). It is mainly used in the applications of higher chlorophenols manufacturing and other productions of Cl-based herbicides (Ormad et al., 2001). People who drink water containing 2,4-DCP for long time might suffer from headache, hyperpyrexia, sicchasia, anepithymia, and even death. The fate and transport of 2,4-DCP in aqueous media are rather complicated mainly due to its high solubility and low air-water partition coefficients (Khenifi et al., 2009). Biochemical technology, mainly including activated sludge process, biomembrane process and biological fluidized bed process, is the most widely used method for the treatment of organiccontaminations in water. However, some disadvantages which cannot be overcame still exist in the methods above. For example, production of huge amount of sludge, poor ability to resist impact load and high cost greatly limits the application of activated sludge technology. In addition, secondary pollution and operation difficulties may also be the restrictions on the methods above. However, enzyme immobilized on nanofibrous membranes, which may overcome those shortcomings to a certain extent, was considered to be a great candidate for the removal of chlorophenols due to their excellent properties of large surface area, easy separation and reutilization as well as high removal efficiency towards organic pollutants. In our previous studies, we have found that the removal of the organic pollutants by immobilized enzyme on nanofibrous membranes was mainly attributed to two aspects: the adsorption of the membranes and the biodegradation by the

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enzyme (Xu et al., 2015c). Meanwhile, the organic pollutants could be removed from water in a short time. For example, horseradish peroxidase (HRP) immobilized on chitosan/poly (vinyl alcohol)-nanocrystalline cellulose nanofibrous membrane could remove 98.34% 3,3',5,5'-tetrabromobisphenol in 2 h (Xu et al., 2015b). In another case, 24% of diclofenac was absorbed by the electrospun carriers while the total removal efficiency is 100% after 6 h (Xu et al., 2015c). Bio-enzymes are considered to be great candidates for pollutants removal due to their high specificity, high efficiency and eco-friendliness, in which HRP is a typical representative. It is characterized by its tolerance to pH, temperature and other outside interferences and is adopted as the experimental enzyme (Nicell et al., 1993).

Polyacrylonitrile (PAN) (Qin et al., 2007；Liu et al., 2014；Selloum et al., 2014；Pan et al., 2015) and polyvinylidene fluoride (PVdF) (Kim et al., 2004) (Gao et al., 2006)are two commonly used polymers to prepare electrospun nanofibers. Studies (Yin et al., 1998) (Yang and Liu, 2003) found that a suitable incorporation of PVdF will increase the toughness of the PAN nanofiber, thus increasing the applicability of the nanofibrous membranes for future application. However, the PAN/PVdF membrane is not easy to absorb aquatic pollutants for its hydrophobicity.

In this study, we aimed to use a simple method to modify the commonly used PAN/PVdF membrane in order to change its property from hydrophobicity to hydrophilicity and apply it as a carrier for HRP to remove 2,4-DCP in water with operational and storage stability. The immobilized HRP was used for the treatment of 2,4-DCP in water. The research had important scientific and practical significance for the application of enzyme catalytic technology in environmental engineering.

2 MATERIALS AND METHODS

2.1 Materials

Polyacrylonitrile (PAN, Mw=150,000), N,N-dimethylformamide (DMF), Coomassie brilliant blue (G250), citrate phosphate buffer solution (CPBS), 2,2'-azinobis-(3-ethylbenzthizaoline-6-sulphonate) (ABTS), 1,1'-carbonyldiimidazole (CDI), were obtained from Sigma-Aldrich. Polyvinylidene fluoride (PVdF) was purchased from Arkema, China. Sodium hydroxide (NaOH), horse radish peroxidase (HRP, RZ~3), tetrahydrofuran (THF) were obtained from Sinopharm Chemical Reagent Co. Ltd, China. Deionized water was used in all experiments. All chemicals used were of analytical grade.

2.2 Preparation of PAN/PVdF Nanofibrous Membranes by Electrospinning

PAN (8 g) was dissolved in DMF(92 g) and stirred for 8 h at 60 ºC, meanwhile PVdF(5 g) was dissolved in DMF(50 g) and stirred for 5 h at 60 ºC. Then, 1.5 g of 10 % PVdF was added into 4.5 g of 8 % PAN solution and stirred for 2 h at 60 ºC to obtain the spinning solution, and the mixture solution was poured into a 10 mL plastic injector with a stainless steel spinneret of 1.2 mm inner diameter after the air bubbles were completely removed. The electrospinning conditions were controlled as follows: a high voltage of 16 kV, a flow rate of 1.5 mL/h, a tip-to-target distance of 18 cm and a relative humidity of 45 ± 5%. Finally, the PAN/PVdF nanofibrous membranes were collected for 4 h on a rotating cylinder wrapped with aluminum foiland then dried in a vacuum drying oven for 12 h to get a non-woven format. The membranes were water insoluble.

2.3 Measurement and Characterization

Scanning electron microscopy (SEM) was used to observe the morphology of the nanofibers on a field emission XL-30 SEM system at 20 kV. Fourier transform infrared-attenuated total reflectance (FTIR-ATR) spectroscopy equipped with a germanium crystal was used to assay the functional groups of the original and modified nanofibers. The immobilization efficiency and residual activity of free and immobilized HRP were measured by Shimadzu UV-1700 spectrophotometer.

2.4 Immobilization of HRP on the PAN/PVdF Nanofibrous Membranes

10 mg PAN/PVdF nanofibrous membrane was immersed into the mixture solution of 10 mL 0.8 M NaOH and 2 mL ethanol for 2 h to convert part of the cyano groups into carboxylate. Then take out the membrane and wash it with anhydrous THF to remove any residue water. The reaction of carboxyl groups with CDI was controlled under non-aqueous conditions. CDI was dissolved in anhydrous THF at

a concentration of 0.3 M. The reaction lasted for 12 h at 25 °C. Afterwards, the activated nanofibers were washed 3 times with THF to remove excess CDI and by-products, when THF vanished under natural drying, the membranes were stored before use.

The activated membranes were immersed into an HRP solution at a concentration of 1 mg/mL (pH 6.0 CPBS) at 25 °C for 12 h. The effects of time (2, 4, 6, 7, 8,10,12 h) and pH (3,4,5,6,7,8,9) on HRP immobilization were analyzed. After enzyme immobilization, the membranes were removed from the enzyme solution and rinsed with CPBS until no HRP was detected in the washings, then the membranes immobilized with HRP were stored at 4 °C to protect the immobilized HRP from losing activity for later use.

2.5 Assays of Immobilization Efficiency and Activity of Free and Immobilized HRP

The immobilization efficiency of HRP was calculated by subtracting the loading of HRP remained in the supernatants and washing buffer from the total HRP initially added into the solution. The loading of HRP (mg) and immobilization efficiency (mg/g) were estimated at 595 nm by a UV-1700 spectrophotometer from Shimadzu.

Determination of HRP activity was calculated spectrophotometrically by monitoring the absorbance change of ABTS at 420 nm on a UV-1700 spectrophotometer (Shimadzu) (Xu et al., 2015c). The formula for calculating the HRP activity is as follows:

$$U = \frac{\Delta A \times V \times 10^6}{\varepsilon \times M \times L \times t} \quad (1)$$

Where ΔA is the change in absorbance before and after the reaction; V is the volume (L) of the liquid added to the cuvette; M is the mass of the added HRP (mg); L is the cuvette path (cm); t is Reaction time (min).

In the immobilization experiment of HRP, the concept of relative enzyme activity is often used. In the horizontal gradient experiment, the enzyme activity under the optimal conditions was taken as 100%, and the enzyme activities of other horizontal gradients were relative to the activity of 100% enzyme.

2.6 Stabilities of Free and Immobilized HRP

Free HRP (1mg/mL) and a certain amount of immobilized HRP were stored in CPBS at 4 °C. The effects of temperature on the activity of free and immobilized HRP were examined by evaluating the enzyme activity at pH 4.0 from temperature 20 to 50 °C. The effects of pH on the activity of free and immobilized HRP were obtained by evaluating the enzyme activity at 25 °C from pH 3.0 to 9.0.

The reusability of immobilized HRP was determined as follows: the immobilized HRP was used 10 times within a day at the optimum conditions.

The storage stability of immobilized HRP was determined as follows: After each reaction, the immobilized HRP was washed with CPBS (pH 4.0) to remove any residual substrate. The storage stabilities of free and immobilized HRP were obtained by calculating the residual activity of the enzyme every 3 days at 4 °C within 30 days.

2.7 Removal of 2,4-DCP by Free and Immobilized HRP

Fifty mL of 20 mg /L 2,4-DCP in PBS was used as substrate, 5 mg of free or immobilized HRP and 0.8 mmol/L H_2O_2 were used for the removal of 2,4-DCP. After 3 h, the upper solution was filtered through syringe filters (membrane of nylon) with the size of 0.45 μm and the residue concentration of the 2,4-DCP was measured using UV-1700 based on the standard methods for the assay of phenols.

The effect of pH on the removal of 2,4-DCP was investigated at 25 °C, pH 3-8. The effect of initial H_2O_2 on the 2,4-DCP removal was carried out with the concentration range of 0.2 mM to 1.2 mM. A PAN/PVdF membrane modified by NaOH was used as a pure carrier to study the adsorption capacity of the membrane.

The removal of 2,4-DCP by HRP-PAN/PVdF includes two aspects: adsorption of the membrane and biodegradation of HRP immobilized on PAN/PVdF. The biotransformation by the immobilized HRP was calculated by the following Eq. (2):

$$C_b = C_r - C_a \quad (2)$$

C_r, C_a, and C_b are the concentrations of the 2,4-DCP removed, absorbed, and biotransformed by HRP-PAN/PVdF NFM, respectively.

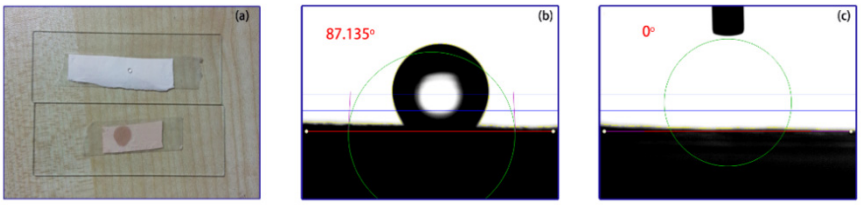

Figure 1: Water contact performances (a) comparison of PAN/PVdF membrane before and after treatment (b) water contact angle of PAN/PVdF membrane (c) water contact angle of PAN/PVdF membrane after treatment.

2.8 Data Analysis

Non-linear regression analysis using first-order model, Eq. (3)-(5) (Xu et al., 2015c), was used to estimate the first order rates (k), the time required to obtain 50% of substrate degradation/adsorption (t1/2), and the 2,4-DCP removal efficiency after (REt).

$$C_t = C_0 \exp(-kt) \quad (3)$$

C_0 and C_t are the substrate concentrations at the beginning of the run and at the time (t), and k is the first-order rates (k), and the time required to obtain 50% of the substrate degradation/adsorption ($t_{1/2}$), and the 2,4-DCP removal efficiency after t (RE_t).

$$t_{1/2} = \ln 2/k \quad (4)$$

$$RE_t=(C_0-C_t)/C_0\times 100 \quad (5)$$

3 RESULTS AND DISCUSSION

3.1 Characterization of the Electrospun PAN/PVdF Nanofibrous Membranes

The hydrophobicity of the pristine PAN/PVdF membrane was a great limitation to its application. Therefore, the PAN/PVdF membranes were treated with 10 wt% NaOH and a certain amount of ethanol beforehand to improve its hydrophilic performance. Figure 1(a) shows the different water contact performances of pristine PAN/PVdF and PAN/PVdF membrane after treated with NaOH. It can be seen that the color of the nanofiber membrane changes from white to reddish brown after modification with an aqueous solution of sodium hydroxide and ethanol. It was found the water contact angle of PAN/PVdF membrane was 87.135° (see Figure 1b), while that of the membrane after NaOH treatment was 0° (see Figure 1c). The smaller the water contact angle, the greater the hydrophilicity. It means the membrane surface had been changed from hydrophobic to totally hydrophilic after NaOH treatment. This change could be attributed to the existence of carboxyl groups derived from cyano groups.

The membranes were activated under non-aqueous condition by CDI to form N-acylimidazoles of high reactivity, followed by enzyme immobilization through conjugation with amino groups of HRP.

Figure 2 shows the SEM photographs of the nanofibrous membranes before and after HRP immobilization. The nanofibers' surface was smooth and their average diameter ranged from 200 to 300 nm. The HRP immobilized on the membranes made the surface of the fiber coarser than those without HRP. Several big beads appeared in the image may be some fragments of the fiber or salt crystals.

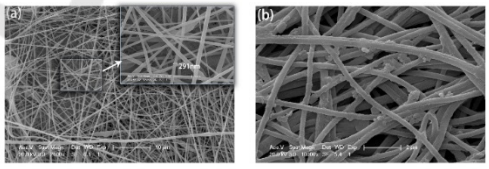

Figure 2: SEM images of the nanofibrous membrane (a) before and (b) after immobilization.

FTIR was used to characterize the PAN/PVdF nanofibrous membrane (NFM), NFM after NaOH treatment, and NFM immobilized with HRP. Figure 3a shows peaks at 1402.8, 1276.7 and 474.6 cm^{-1}, which are corresponding to CF_2 bending, CF_2 stretching and CF_2 wagging, respectively. Compared Figure 3b with Figure 3a, new peaks at 1561.8 cm^{-1} and 1665.7 cm^{-1} indicate the existence of carboxylic and formamide groups. The appearance of these hydrophilic groups can also support the change of

membrane surface from hydrophobic to totally hydrophilic after NaOH treatment. which depicted in Figure 1. It can be seen that Figure 3c has a strong adsorption band at 1692.5 cm^{-1}, representing the stretching vibration of C=O. Meanwhile, a peak at 1277.0 cm^{-1} appeared which could be due to the combination of C-N stretching vibration and N-H bending vibration. In addition, the peak at 3201.0 cm^{-1} indicates the stretching vibration of N-H. These new peaks are produced during the immobilization of HRP on the surface of the fiber membrane. As a result, it can be confirmed that HRP molecules has been successfully immobilized on PAN/PVdF NFMs through chemical bonding.

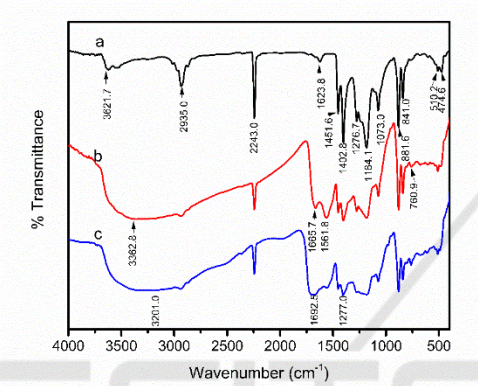

Figure 3: FTIR graphs of (a) pristine PAN/PVdF membrane (b) membrane after NaOH treatment (c) membrane immobilized with enzyme.

3.2 Effects of pH and Time on HRP Immobilization

Figure 4 shows the effects of time and pH on the immobilization efficiency of HRP. Both pH and time have significant influences on the immobilization. It can be seen from Figure 4 that extreme pH conditions greatly restricted the immobilization efficiency. This is not only because extreme pH condition will destroy the structure of the enzyme, but more importantly because the mode of interaction between the enzyme and the carrier (eg, the orientation of the enzyme) depends on the ionic strength and pH (Xu et al., 2016). The optimum pH for HRP immobilization was between 7.5 and 8.0. It was in accordance with the results in our previous study (Xu et al., 2015a). The immobilization efficiency improved with the increase of time until it levelled off after 8 h. It might be resulted from the saturation of immobilized HRP owing to steric constraints because the enzyme occupies a certain volume of space (Cristóvão et al., 2011). The maximum HRP loading was 440.2 mg/g after 8 h at pH 8.0 and 25 °C. This immobilization efficiency was relatively higher than that reported before (Takahashi et al., 2001) (Lai and Lin, 2005) (Xu et al., 2013). The high HRP loading may be attributed to the high specific surface area of the PAN/PVdF membrane and the suitable immobilization method which may retain high residue activity of HRP.

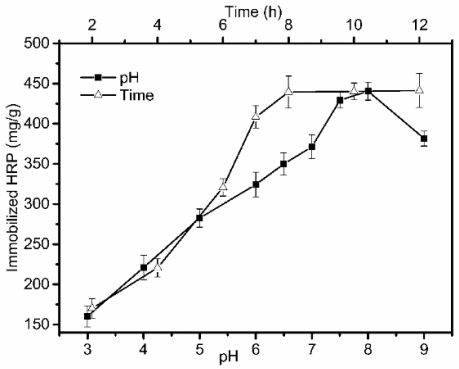

Figure 4: Effect of pH (12 h at 25 oC with pH 3,4,5,6,7,8,9) and time (2, 4, 6, 7, 8,10,12 h at 25 oC with pH 6.0 CPBS) on the HRP immobilization efficiency.

3.3 Characterization of Free and Immobilized HRP

Stabilities are important indexes to measure the properties of the immobilized enzymes for their further industrial applications. Figure 5(a) and (b) shows the effects of pH and temperature on the relative activity of free and immobilized HRP. Free HRP reached optimum activity at pH 4.0 and 30 °C while immobilized ones shifted to pH 7.0 and 40 °C. The enzyme activity of immobilized HRP was higher than that of free ones in the pH range from 3.0 to 3.5 and5.0 to 9.0, demonstrating that the immobilized HRP was less sensitive to the pH conditions during the testing period. For example, the relative activity of immobilized HRP at pH 9 was 50.2%, which was significantly higher than that of free HRP (10.1%). It could be attributed to the buffering effect provided by the support of membrane (Liu et al., 2013). Additionally, the immobilized HRP showed greater relative activity than free HRP, especially at temperature higher than 40 °C. As temperature increased, the relative activity of immobilized HRP decreased significantly slower than that of free HRP, indicating that the immobilized HRP had a higher temperature stability. It may be attributed to the protecting effect provided

by the immobilization support at high temperatures when enzyme deactivation occured (Osma et al., 2010). The enzyme rigidity increased through the immobilization process demonstrated by an increased thermal stability against denaturation (Abdel-Naby, 1993).

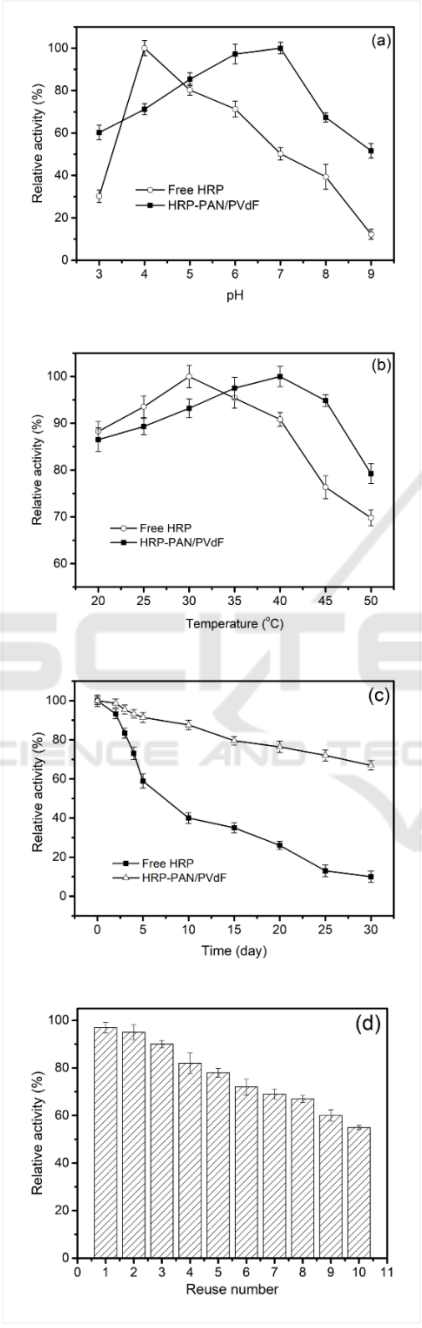

Figure 5: pH (a) (at 25 °C with pH 3-9), temperature (b) (at 20-50 °C with pH 4), storage (c) (optimum conditions) and operational stabilities (d) (optimum conditions) of free HRP and HRP-PAN/PVdF.

Generally, enzymes are unstable in solution, and their activities would decrease as storage period increases. In contrast, immobilized enzyme can overcome this disadvantage. Figure 5c shows the activity of free HRP decreased significantly faster than immobilized HRP ($p<0.05$). For example, after 30 days, the immobilized HRP still retained an activity of 66.9%, while for free HRP, the relative activity was only 10.0%. Therefore, immobilized HRP was more stable than free HRP. It was attributed to the limited conformational changes in enzyme molecules in the matrix of fibrous membrane (Xu et al., 2015b).

Immobilized enzyme can be more easily separated from the reaction solution compared with free enzyme, which would greatly decrease the cost of enzyme for further application (Quintanilla-Guerrero et al., 2008). It was found from Figure 5d that HRP-PAN/PVdF retained 55% of its initial activity after 10 cycles of reuse. The loss in enzyme activity may be related to the inactivation of enzyme caused by denaturation of the protein as well as the breakage of the membrane (Huang et al., 2008).

3.4 Removal of 2,4-DCP by Free and Immobilized HRP

Figure 6 shows the effect of pH and H_2O_2 initial concentration on the removal efficiency of 2,4-DCP by free HRP, PAN/PVdF NFM as well as HRP-PAN/PVdF. As illustrated in Figure 6(a), the adsorption of the PAN/PVdF membrane removed approximately 20% of 2,4-DCP in the experiment about the effect of pH, and no significant variation ($p>0.05$) was observed as pH value changed, which could due to the strong stability of the PAN/PVdF membrane under different pH conditions. The removal efficiency of 2,4-DCP reached a maximum of 90% at pH 7.0 by immobilized HRP and 89.2% by free HRP at pH 4.0. The difference in optimum pH conditions for 2,4-DCP removal by free and immobilized HRP was consistent with the pH stability results. Furthermore, immobilized HRP showed high removal efficiency towards 2,4-DCP than free HRP, especially under alkaline conditions. It may be explained by the protection of the carrier. Figure 6(b) shows that the maximum 2,4-DCP removal efficiency reached as high as 94.9% by free HRP at an initial H_2O_2 concentration of 0.4 mM and 94% by immobilized HRP at an initial H_2O_2 concentration of 0.6 mM. As initial H_2O_2 concentration changed, there was no significant change ($p>0.05$)in adsorption rate of 2,4-DCP by

PAN/PVdF NFM. In addition, superabundant H_2O_2 had adverse effect on the removal of 2,4-DCP. It may be attributed to the following reasons: Firstly, excess HRP may react with the intermediates and result in a less active form of HRP during the process of 2,4-DCP catalytic oxidation (Cai and Tien, 1992). Secondly, an overdose of H_2O_2 may participate in irrelevant reactions (Maloney et al., 1986).Therefore, a suitable amount of H_2O_2 is a key factor for 2,4-DCP removal in an reaction system.

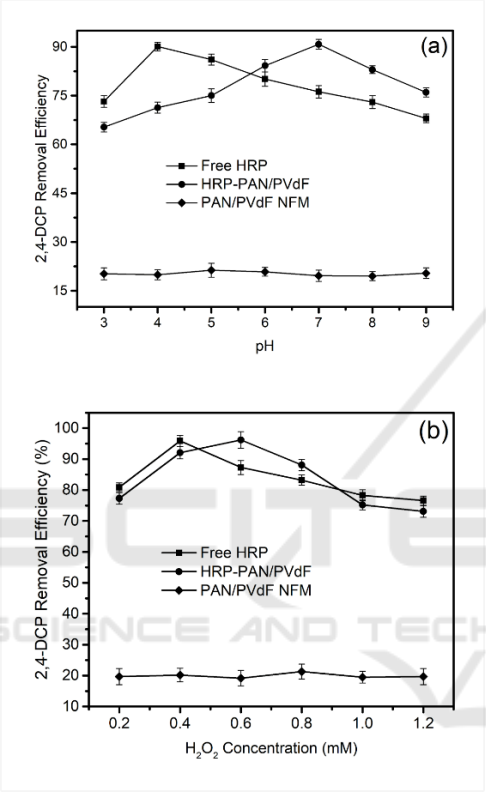

Figure 6: Effect of pH (a) (4h with 20 mg/L 2,4-DCP, 5 mg of free or immobilized HRP and 0.8 mmol/L H2O2) H2O2 initial concentration (b) on the removal efficiency of 2,4-DCP by free HRP and HRP-PAN/PVdF. (4h with 20 mg/L 2,4-DCP, 5 mg of free or immobilized HRP and a constant pH)

Figure 7 shows the removal efficiency of 2,4-DCP in a 4 h batch experiment. The degradation efficiency of 2,4-DCP by free HRP reached approximately 83% in the first 30 min and 93% after a 3 h treatment. By contrast, the removal efficiency of 2,4-DCP by HRP-PAN/PVdF reached 77% in the first 30 min and 87% after 3 h. According to the Eq. (2), we could easily calculate that about 68% of 2,4-DCP was biotransformed by HRP-PAN/PVdF. Although the enzyme molecules immobilized on PAN/PVdF NFM were relatively less than that of free HRP, the removal efficiency towards 2,4-DCP did not show significant difference. It was due to the combined effects of biodegradation and adsorption by HRP-PAN/PVdF NFM. The adsorption of 2,4-DCP on the nanofiber membrane carrier was concentrated in 1 hour after the start of the reaction, and there was no significant increase after 1 hour. This indicates that the adsorption of 2,4-DCP on the carrier is in the initial stage of the reaction, after which the adsorption-desorption equilibrium is reached. Figure 7 also shows that the kinetics of 2,4-DCP removal followed a first-order reaction.

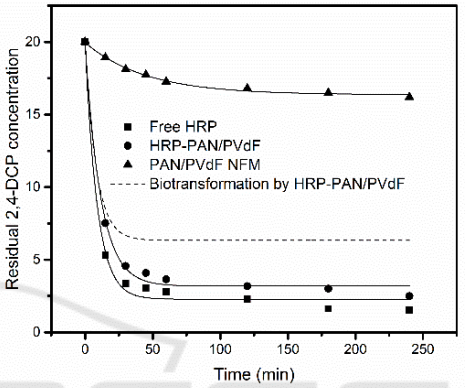

Figure 7: Removal kinetics of 2,4-DCP by free HRP (4h at 25 °C with pH 4.0 and 0.4 mM H_2O_2 initially) and HRP-PAN/PVdF (4 h at 25°C with pH 6.0 and 0.6 mM H_2O_2 initially).

Table 1 showed the biodegradation rate of HRP immobilized on NFMs was slower than that of free HRP. It may be explained by spatial limitations for substrate diffusion and protein flexibility after enzyme immobilization on the carrier (Bai et al., 2006) (Sari et al., 2006), as well as the fact that the immobilized HRP was partly inactivated during the immobilization process.

Table 1: Value of k, $t_{1/2}$, and RE_{240} of free HRP, HRP-PAN/PVdF NFM, and NFM towards 2,4-DCP.

Sample	k/(min)	$t_{1/2}$ (min)	RE_{240} (%)
Free HRP	0.122	5.68	92.35
HRP-PAN/PVdF NFM	0.087	7.97	87.55
PAN/PVdF NFM	0.022	31.51	19.00
Biotransformation by HRP-PAN/PVdF NFM	0.112	6.19	68.55

3.5 Reusability of HRP-PAN/PVdF NFM

Immobilization of the enzyme can overcome the inherent drawbacks of free enzymes-hard separation and non-reusability. Figure 8 shows that HRP-PAN/PVdF could still remove 47.6% of 2,4-DCP after 7 repeated runs, which is much higher than some other researches (Magri et al., 2007). The decrease of the 2,4-DCP removal efficiency could be explained by the adsorption of the reaction products as well as the appearance of some undissolved substances like the damaged components of the PAN/PVdf membrane. The adsorption prevented the contact between enzyme and substrate and the undissolved substances slowed down the flow velocity (Durán et al., 2002).

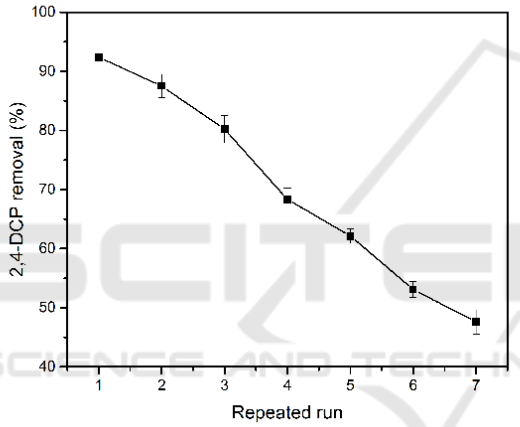

Figure 8: Variation of the 2,4-DCP removal rate by HRP-PAN/PVdF. (at 25°C with pH 6.0 and 0.6 mM H_2O_2 initially)

4 CONCLUSIONS

One novel method for horseradish peroxidase immobilization was developed in this study. The PAN/PVdF membranes fabricated by electrospinning were converted from hydrophobic into hydrophilic ones and were successfully applied for HRP immobilization to retain high activity. Under the optimum immobilization conditions, the maximum enzyme loading of PAN/PVdF nanofibrous membranes were 440 mg/g. Meanwhile, the enzyme could retain high relativity after immobilization. Compared with free HRP, the immobilized HRP has better pH, thermal, storage and operational stability. The work range of pH and temperature was extended as well. Free HRP and the immobilized HRP were applied in the removal of 2,4-DCP. Results showed that the removal efficiency of the immobilized HRP for 2,4-DCP was 87%, while that of free HRP was 93%. The removal efficiency of the immobilized enzyme as good as free HRP. It could be concluded from the experiments that the degradation of immobilized HRP was first-order reaction and the removal of the pollutants could be attributed to the adsorption of nanofibrous membrane and the biodegradation of HRP. HRP immobolized on PAN/PVdF membranes also had better reusability. Therefore, HRP immobilized on modified PAN/PVdF membrane could be deemed as a promising material for future applications in aquatic organic pollutants removal.

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