

Using immobilized enzymatic membrane reactor to treat synthetic phenol polluted drinking water

Yingying Li², Haitao Li^{1,*}, Yixin Yang^{2,**}, Jie Liu¹, Gengzhi Yu²

¹CECEP Engineering Technology Research Institute, Beijing 100082, China

²Beijing key laboratory of fuel clean and efficient catalytic emission reduction technology, School of Chemical Engineering, Beijing Institute Petrochemical Technology, Beijing 102617, China

Email: *lihaitao103904@126.com, **0020150026@bipt.edu.cn

Abstract. The catalyzed polymerization of phenol from the synthetic polluted drinking water using immobilized enzymatic membrane reactor (MER) was investigated. Horseradish peroxidase (HRP) immobilized microporous filter was prepared by porous calcium alginate method. The immobilized HRP demonstrates better performance for degradation of phenol. In addition, the experimental parameters including reaction time, pH, HRP dosage, and H₂O₂ concentration were optimized. In the condition of 0.1 mmol/L initial concentration of phenol, 4.78 u/ml HRP, n_{H₂O₂}: n_{phenol}=2:1 and reaction time 10 min at 20 °C and pH 7, the degradation of phenol reached 73.57%. Therefore, the immobilized enzymatic membrane reactor is feasible to treat synthetic phenol polluted drinking water.

1. Introduction

Due to enzymes' unique performances including high stability, selectivity, catalytic activity and low toxicity, it has been used in a wide range of applications including biochemistry, chemistry, pharmaceutical applications, medicine and industry^[1]. Meanwhile, many researchers have extensively investigated about enzymatic degradation of phenolic compounds from water. It has been indicated that peroxidases are able to react with aqueous phenolic compounds and form insoluble substance that could be easily degraded from the aqueous phase^[2]. But these processes suffer from enzyme inactivation^[3] and the loss of enzyme. Therefore, attention came on immobilization of peroxidases for the purpose of phenolic compound removal. Horseradish peroxidase (HRP) is the most widely used biocatalyst for the polymerization of phenols, anilines, their derivatives and a series of new polyaromatic compounds^[4].

In this work, we attempt to use a porous calcium alginate method^[5] for immobilization of HRP for the purpose of phenol removal from a synthetic drinking water. We aim to evaluate the property of immobilized enzyme to degrade phenol on reaction time, pH, HRP dosage, and H₂O₂ concentration.

2. Materials and methods

2.1. Materials and reagents

HRP (lyophilized powder, 250 u/mg); Phenol (AR≥99.0%); 4-Aminoantipyrine (AR≥99.0%); Potassium ferricyanide (AR≥99.0%); Ammonium chloride (AR≥99.5%); Ammonia water (AR≥99.0%); Hydrogen peroxide 3% (w/w); Hydrochloric acid (AR≥99.0%); Sodium hydroxide (AR≥99.0%); Calcium chloride (AR≥99.0%); Sodium alginate (AR≥99.0%); Sodium dihydrogen phosphate



(AR \geq 99.0%); Disodium hydrogen phosphate (AR \geq 99.0%).

Electronic balance (ME204/02); pH acidity meter (pHS-3C), UV-Vis spectrophotometer (UV, BlueStar A); Pipette gun (200 μ L and 5000 μ L), Microporous filter (ϕ 100mm, membrane pore size 0.22 μ m).

2.2. Immobilization of HRP

The HRP was immobilized on the microporous filter using a porous calcium alginate method. The specific process was as follows: 0.25g of sodium alginate was dissolved into 100mL of distilled water, meanwhile 120 mg of horseradish peroxidase was dissolved in 10 mL phosphate buffer solution of pH 7. Next, 1.5 mL of sodium alginate solution and 1.5 mL of horseradish peroxidase solution were thoroughly mixed. Then, the whole mixture was evenly coated on the microporous filter and allowed to stand for 10 min. Finally, the microporous filter was placed in 0.1 mol/L calcium chloride solution and allowed to stand for 20 min, the prepared enzymatic membranes were stored in phosphate buffer solution for use.

2.3. Analysis and detection

Determination of phenol: it was determined at characteristic absorption wavelength of 510nm by UV-Vis Spectrophotometer ($R^2 = 0.9997$). According to the following formula to calculate the degradation rate of phenol: $\eta = (A_0 - A_t) / A_0 \times 100\%$, η is the degradation rate of phenol, A_0 is the initial absorbance of phenol and A_t is the absorbance of phenol after degradation.

2.4. Experimental procedure

The 100mL of phenol was pumped the reactor through the peristaltic pump. The part of the liquid flowed through the surface of the microporous filter and this liquid was the return water. The other part of the liquid passed through the microporous filter under pressure and this liquid was final water. Finally, these two parts of the liquid was returned to the beaker. The flow chart was as follows:

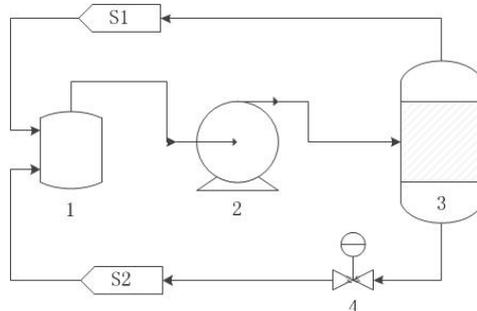


Fig. 1 The flow chart of enzymatic membrane reactor.

(1Beaker; 2 Peristaltic Pump; 3 Reactor; 4 Pressure Indicator; S1 Return water; S2 Final water)

3. Results and discussion

3.1. Effect of reaction time on degradation of phenol

The degradation of phenol with reaction time was conducted as shown in Fig. 2. With the increase of reaction time, the concentration of phenol decreased rapidly in initial 10 min and then slowly in 10 to 80 min. The removal rates of phenol were 46.0% in 10 min and 68.33% in 80 min, respectively. The results showed that the degradation of phenol gradually decreased with the increase of reaction time, and it tended to be steady in finally. It was determined that the reaction time was 10 min in next experiment.

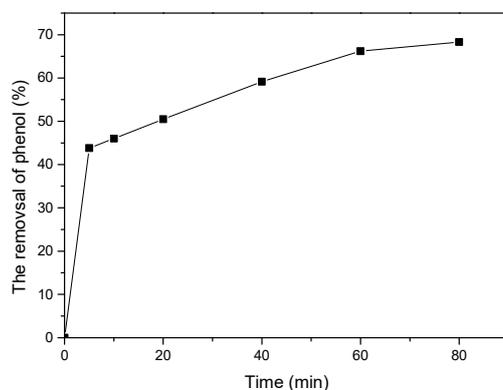


Fig. 2 The effect of the reaction time on the degradation of phenol in immobilized enzymatic membrane reactor system ($C_{\text{phenol}}=0.1\text{mmol/L}$, $n_{\text{H}_2\text{O}_2}:n_{\text{phenol}}=2:1$, the dosage of HRP =4.78u/mL, pH=7, T=20°C)

3.2. Effect of the dosage of H_2O_2 on degradation of phenol

As shown in Fig. 3, the degradation rate of phenol was only 45.85% when the mole ratio of H_2O_2 /phenol was 1:1 (the dosage of H_2O_2 was 3.4 mg/L). When the mole ratio of H_2O_2 /phenol increased to 2, the degradation rate of phenol reached 66.41%. When mole ratio of H_2O_2 /phenol was higher than 2, degradation efficiency of phenol had a tendency to decrease. The results indicated that the optimum H_2O_2 dosage was 6.8mg/L.

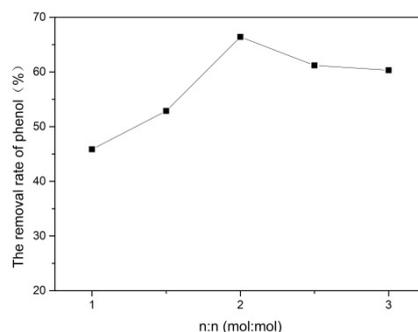


Fig. 3 Effect of H_2O_2 dosage on degradation of phenol in immobilized enzymatic membrane reactor system. ($C_{\text{phenol}}=0.1\text{mmol/L}$, reaction time of 10min, the dosage of HRP =4.78u/mL, pH=7, T=20°C)

3.3. Effect of the dosage of pH on degradation of phenol

The effect of pH on degradation of phenol was investigated in enzyme-catalyzed reaction system. As shown in Fig. 4, the optimum pH was 7 that the degradation rate of phenol reached 73.57%. The removal of phenol was inhibited in acid and alkaline conditions. It showed that the activity of HRP decreased significantly under the conditions of over-acid or over-alkaline.

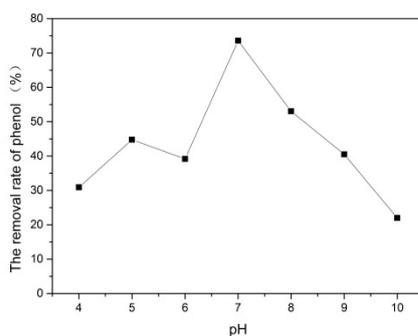


Fig. 4 The effect of pH on degradation of phenol in immobilized enzymatic membrane reactor system ($C_{\text{Phenol}}=0.1\text{mmol/L}$, reaction time of 10min, the dosage of HRP =4.78u/mL, $n_{\text{H}_2\text{O}_2}: n_{\text{Phenol}}=2:1$)

3.4. Effect of the dosage of HRP on degradation of phenol

As shown in Fig. 5, the degradation efficiency of phenol increased with the increase of HRP dosage, but the dosage of HRP was higher than 4.78u/mL that the degradation efficiency decreased. When HRP dosage increases to 4.78u/mL, the removal efficiency of phenol increased to 72.35%. The optimal HRP dosage could be controlled at 4.78 u/mL when 0.2 mmol of H_2O_2 was added with pH of 7.

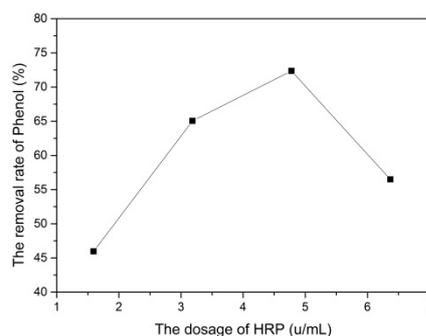


Fig. 5 Effect of HRP dosage on degradation of phenol for immobilized enzyme-catalyzed polymerization system. ($C_{\text{Phenol}}=0.1\text{mmol/L}$, reaction time of 10min, $n_{\text{H}_2\text{O}_2}: n_{\text{phenol}}=2:1$, pH=7, $T=20^\circ\text{C}$)

4. Conclusions

From the above experiments, the results manifest that immobilized HRP membrane reactor is feasible for degradation of phenol from synthetic drinking water. 4.78 u/mL of HRP dosage is economical and effective with the degradation of phenol reaching 72.35% in the optimal condition of reaction time 10 min, pH 7 and 6.8mg/L of H_2O_2 dosage.

Acknowledgement

The research was supported by the Program of Beijing Natural Science Foundation (No. 8152015) and CECEP Science and Technology Innovation Project (No. CECEP-KJ-2016-007).

References

- [1] Topal Yasemin, Tapan Senem, Gokturk Ersen, E Sahmetlioglu 2017 *J. J Saudi Chem Soc.* **21** 731
- [2] Turac Ersen, Sahmetlioglu Ertugrul 2010 *J. Synthetic Met* **160** 169.
- [3] Alemzadeh I, Nejati S 2009 *J. J Hazard Mater* **166** 1082.
- [4] Zhang Lei, Zhao Wenshan, Ma Zhanling, Guangrui Nie, Yuanchen Cui 2012 *J. Eur Polym J* **48** 580.
- [5] Kumbul Altug, Gokturk Ersen, Turac Ersen, Ertugrul Sahmetliolu 2015 *J. Polym Advan Technol* **26** 1123.