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Electroenzymatic oxidation of bisphenol A (BPA) based on the hemoglobin (Hb) film in a membraneless electrochemical reactor

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ABSTRACT

This paper presents a novel electroenzymatic method for the treatment of bisphenol A (BPA) in a membraneless electrochemical reactor. The electrochemical reactor was arranged with a stainless steel and an enzymatic film as anode and cathode, respectively. The enzymatic film was formed by immobilizing hemoglobin (Hb) on carbon fiber. In the membraneless electrochemical reactor, hydrogen peroxide (H_2O_2) was generated *in situ* in cathode and BPA was oxidated and removed by the combining Hb with H_2O_2. The experimental conditions for electrogeneration of H_2O_2 and electroremoval of BPA were optimized. Experimental results showed that in supplied voltage 2.4 V, pH 5.0 and oxygen flow rate 25 mL/min, the electrogeneration of H_2O_2 and the electroenzymatic removal of BPA were highest. Under optimal operation conditions, the removal efficiency of BPA reached 50.7% in 120 min and then kept constant when further prolonging the period of reaction. Compared with electrochemical and biochemical methods, the removal of BPA through electroenzymatic method was comparatively favorable.

1. Introduction

Bisphenol A [BPA, 2,2-bis(4-hydroxyphenyl)propane] is an important organic chemical as an intermediate in the industrial manufacture of polycarbonate plastics, epoxy resins flame retardants, and other products [1]. In recent years, the use of such plastic products became increasingly extensive which directly resulted in the increased release of BPA into the environment [2]. It was found that BPA has biological toxicity and estrogenic activity in specific responses [3–6], and the wide existence of BPA will constitute threats to human health and safety of the environment. For these instances, a considerable amount of attention has been focused on effective removal of BPA.

The removal of many estrogenic phenolic compounds challenges traditional water and wastewater treatment technologies [7]. The characteristic that phenols are relatively hydrophilic, make them easily escape physical/chemical treatment processes designed to remove hydrophobic contaminants [8,9]. For these instances, researchers put forward various physical, chemical or biological treatments to eliminate BPA [10,11]. Physical methods only transfer BPA from the aqueous phase to a second phase, but there is no destruction of the pollutant. Biological processes require a long treatment period and application is limited to very low pollutant concentrations, while chemical treatments such as chlorination processes can lead to products (e.g., 3-ClBPA and 3,3′-diClBPA) with higher EDE and/or toxicity [12–14]. Therefore, a rapid, simple and economic wastewater treatment for the removal of BPA is now highly in demand.

The use of oxidoreductases, i.e., horseradish peroxidase (HRP, EC 1.11.1.7) and tyrosinase (Tyr, EC 1.10.3.1) [15,16] to catalyze the removal of aromatic compounds from wastewaters has been extensively investigated. For example, Huang and Weber [7] utilized the soluble HRP to catalyze the removal of BPA in aqueous solution. However, these native enzymes are expensive and show limited stability [17], which dramatically increased the cost of an enzyme-based process for organopollutants removal. Therefore, it cannot be widely used in practice. In recent years, the use of stable and cheap mimetic enzymes such as hemin [17], hematin [18] and the porphyrin complexes of Mn, Co, Fe and Mo [19] has become an attractive research area. However, these simple natural or synthetic metalloporphyrins, lacking the spatial structure of the natural enzyme for the special inclusion behavior between the enzyme and the substrate, do not show satisfactory activity and selectivity [20].

Hemoglobin (Hb), a natural macromolecular protein, has the natural quaternary structure. It contains four subunits of polypeptide and each polypeptide chain contains a heme group that serves as the active center [21]. Hb plays an important role in carrying oxygen in the blood of vertebrate animals. It is a model protein for studies on structure-function relationship of proteins due to its nice stability, commercial availability and well-documented structure information [22,23]. Hb is known to have some intrinsic peroxidase

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activity owing to its close structural similarity to peroxidase [24], and these characteristics make it possible to catalyze the removal of BPA with the presence of hydrogen peroxide (H_2O_2). However, to the best of our knowledge, there is no report based on Hb film to catalyze the removal of organic contaminant from wastewaters. Furthermore, due to its high stability and low cost [21,24], Hb is a preferred material for wastewaters treatment.

In the presence of H_2O_2, peroxidase can catalyze the oxidation of a variety of phenolic compounds to generate phenoxy radicals [25]. The supply of H_2O_2 is necessary to maintain the activity of enzyme. But due to the instability, it is limited by difficulties in the storage and transport of H_2O_2. To overcome these drawbacks, Lee [26,27] used an electrode reaction to produce H_2O_2 at the cathode, and then the peroxidases such as horseradish peroxidase (HRP) and lignin peroxidase (Lip) catalyzed a sequential oxidation reaction of 2,4,6-trinitrotoluene with the H_2O_2 generated *in situ*. In the electric field, the consumption of dissolved oxygen leads to the formation of H_2O_2 or H_2O at the cathode followed by a series oxidization catalyzed by enzyme.

Accordingly, electrogeneration of H_2O_2 is an attractive approach since it does not require additional chemicals, and electricity is readily available [28]. Based on this, some electrochemical reactors with native peroxidases were fabricated to electroenzymatically oxidize a variety of phenolic compounds such as pentachlorophenol [28] and phenol [29]. Nevertheless, few investigations for electroenzymatic degradation of BPA were reported.

The aim of this study is to assess the feasibility of the electroenzymatic degradation of BPA in a membraneless electrochemical reactor with Hb film. Effects of experimental parameters such as supplied voltage, pH, temperature and oxygen flow rate were investigated. In addition, the differences between the electrochemical and electroenzymatic degradation of BPA were also examined.

2. Experimental

2.1. Materials

Hemoglobin (Hb) from bovine blood (MW, 64500) was obtained from Beijing Biodee Biotechnology Company Ltd. (China). Bisphenol A (BPA) and 4-aminoantipyrine (4-AAP) and other chemicals were obtained from Shanghai Jingchun Reagent Company (China). Chitosan (CS) was obtained from Sigma–Aldrich Company. Carbon fiber was purchased from Jilin Carbon Materials Company Ltd. (China). All other chemicals were of analytical reagent grade and used as received without further purification.

2.2. Immobilization of Hb on the carbon fibers

A 0.2 wt% chitosan (CS) solution was prepared by dissolving chitosan in a 1 wt% acetic acid solution. A carbon fiber was cut into 2 cm × 5 cm and dipped into 10 mL CS solution containing 5 mg Hb for 10 min. Then, it was exposed to the air for 30 min at room temperature. After repeating this procedure three times, the final carbon fiber was allowed to dry for 2 h at room temperature to build up a rigid Hb film. The amount of Hb immobilized on the carbon fiber surface was calculated by the difference between amount of Hb initially adsorbed and that detected in the final solution by UV spectroscopy. Under the optimal conditions, the amount of retained Hb was 44% (0.183 mg/cm^2).

2.3. Membraneless electrochemical reactor design and removal of BPA

The electroenzymatic oxidation of BPA was studied in a membraneless electrochemical reactor. As shown in Fig. 1, a jacketed beaker with an effective volume of 35 cm^3 was used for the membraneless electrochemical reactor and the electrochemical reactor was arranged with stainless steel (2 cm × 5 cm) as anode and a prepared Hb film as cathode. Oxygen was supplied into the membraneless electrochemical reactor for the saturation of the dissolved oxygen. BPA was dissolved in a 100 mM phosphate buffer solution (PBS, pH 5.0). The experiments were conducted under potentiostatic conditions using a WLS power supply (Sangli, Nanjing).

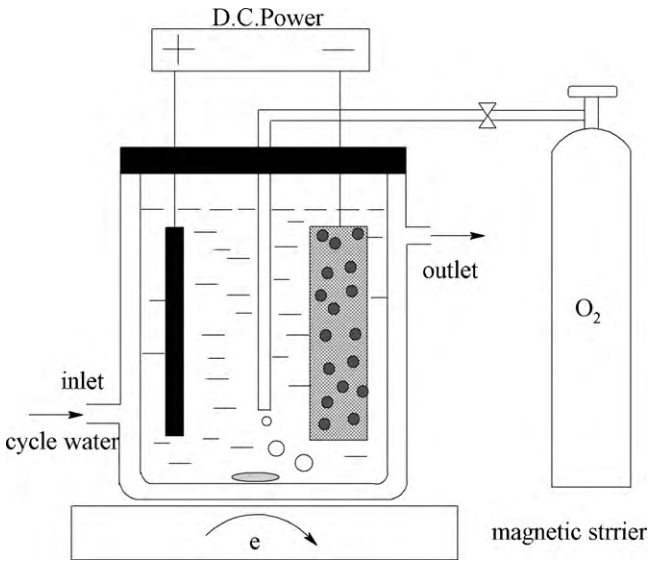

Fig. 1. Schematic diagram of a membraneless electrochemical reactor with three cell pairs.

For comparison, the removal of BPA by electrochemical method and biochemical method was as follows: The electrochemical reactor was arranged with pure carbon fibers without immobilized Hb film and stainless steel as anodes and cathodes, respectively. Other supply conditions such as supplied voltage, pH, temperature and oxygen flow rate were same as that in electroenzymatic method. The biochemical reactor was constructed with a jacketed beaker. In 100 mM PBS (pH 5.0), the concentration of the H_2O_2 added to biochemical reactor was about 2-fold higher than that of BPA. And then, the conversion of BPA was initiated by adding H_2O_2 and reaction allowed to proceed under the same pH, temperature and Hb dose with the electroenzymatic method.

2.4. H_2O_2 concentration assay

The concentration of electrogenerated H_2O_2 in the membraneless electrochemical reactor was measured using copper (II) ion and 2,9-dimethyl-1,10-phenanthroline (DMP) at 453 nm in UV-5301 visible spectrophotometer (Shimadzu, Japan) [30].

2.5. Bisphenol A concentration assay

BPA concentrations were determined using a colorimetric assay [31]. Under alkaline conditions, the primary amine of 4-AAP exerts an electrophilic attack on the phenolic compound to form an intermediate compound which is subsequently oxidized by potassium ferricyanide to a red quinone-type dye with an absorption peak at 506 nm (max wavelength for BPA) upon completion of the reaction.

The absorbance is proportional to BPA concentration in the assay. Reagents were added in the following order: 6 mL of 0.25 M $NaHCO_3$ buffer, 2 mL of aqueous BPA sample, 1 mL of 20.8 mM 4-AAP and 1 mL of 83.4 mM potassium ferricyanide. After reacting for 10 min, the absorbance was recorded at 506 nm and the BPA concentration was determined from a standard curve. The maximum

concentration of BPA in the assay mixture was ≤100 mg/L in order to maintain a linear relationship between the formed color and BPA concentration.

3. Results and discussions

3.1. Electrogeneration of hydrogen peroxide

To characterize the ability of the system to accumulate H_2O_2, electrolyses were carried out in a jacketed reactor with PBS (100 mM, pH 5.0) as electrolyte. In the experiments, oxygen was continuously sparged into the electrolyte for the saturation of the dissolved oxygen and the H_2O_2 concentration was measured periodically. Fig. 2 shows the formation of H_2O_2 with time in the electrolyte. It was observed that the accumulated H_2O_2 reached a steady concentration of 2.5 mM after 120 min, implying that H_2O_2 is electrogenerated and simultaneously degraded in the system at the same rate [27].

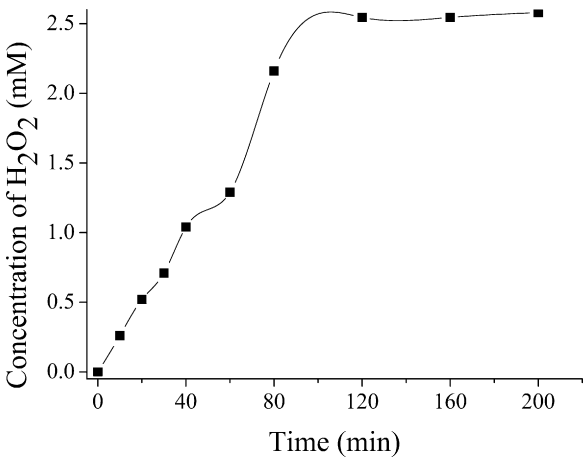

Fig. 2. The formation of H_2O_2 at different times.

3.1.1. Effect of the supplied voltage

Electrolytic experiments were carried out for 120 min to investigate the effect of the applied potential on the electrogeneration of H_2O_2. As shown in Fig. 3(A), the power density was increased slightly with the supplied voltage changes below 2.0 V, and then improved steeply above 2.4 V due to the charge transfer resistance. The charge transfer resistance was caused by the excess of protons (H^+) and hydroxide ions (OH^-) generated from water dissociation [29].

At anode:

$$2H_2O \rightarrow O_2 + 4H^+ + 4e^- \quad (1)$$

At cathode:

$$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2 \quad (2)$$

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O \quad (3)$$

In addition, the production rate of H_2O_2 increased initially, reaching the maximum 3.09×10^{-5} mM s^{-1} V^{-1} at 2.4 V, and then

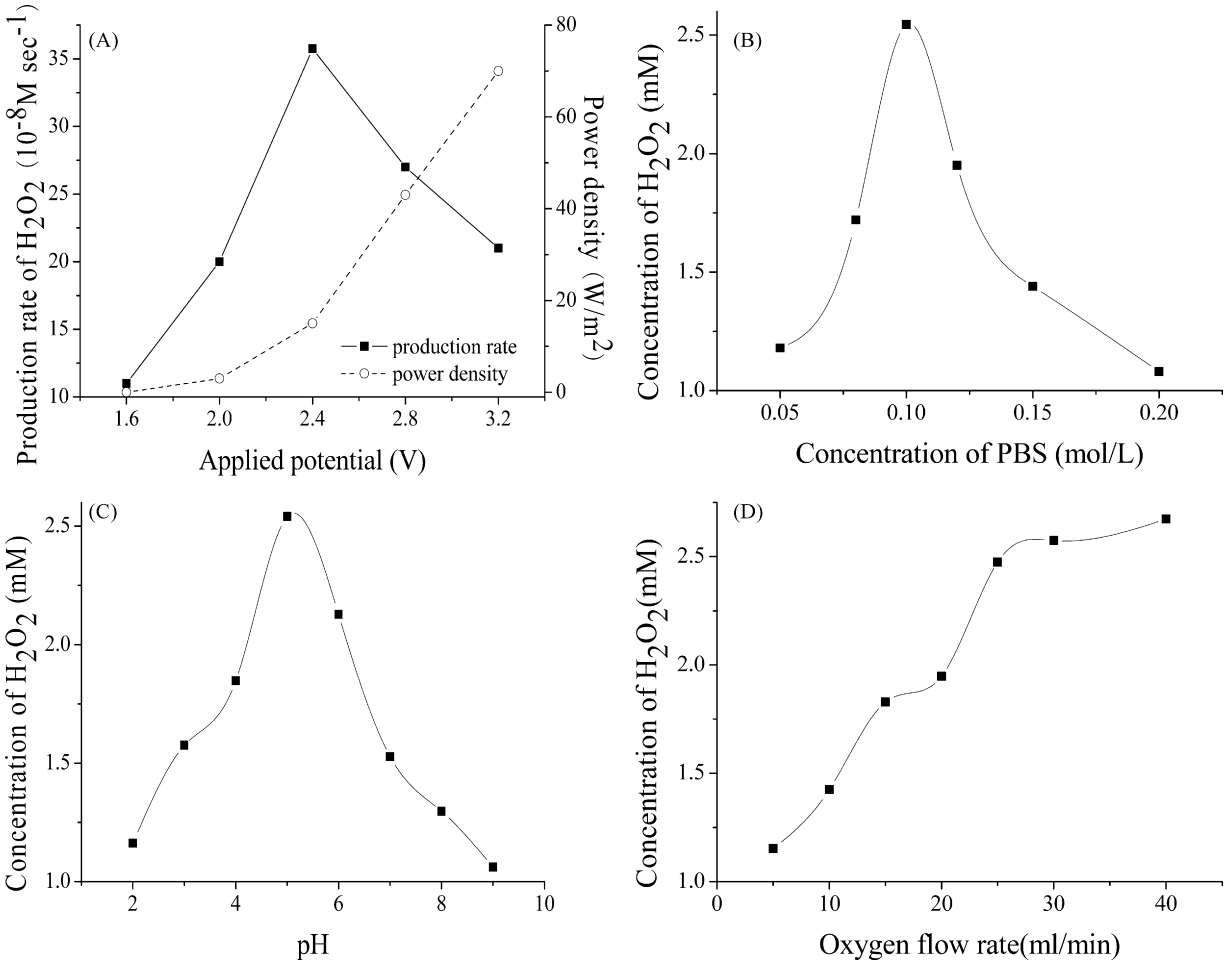

Fig. 3. Effects of conditions on the electrogeneration of H_2O_2. Applied potential (A), concentration of PBS (B), pH (C) and oxygen flow rate (D).

decreased. This is because protons were vigorously generated at the anode surface by water dissociation as shown in Eq. (1). And then the generated protons were further reduced to H_2O_2 at the cathode surface by combination with oxygen through a two-electron reaction as shown in Eq. (2). Above 2.4 V, however, oxygen was converted to water molecules via a four-electron reaction as shown in Eq. (3) [32]. Hence, the applied voltage was fixed at 2.4 V, where the dominant reaction was a two-electron reaction.

3.1.2. Effect of the electrolyte concentrations

As shown in Fig. 3(B), the generation of H_2O_2 increased first and then decreased with the increase of PBS concentration. The concentration of H_2O_2 decreased when the PBS concentration decreased from 100 to 50 mM, which resulted from the conductivity decay [33]. While in a high electrolyte concentration solution, the interactions of the electrolyte ions were enhanced which would interfere with the electrogeneration of H_2O_2. For these reasons, 0.1 M PBS was selected as the electrolyte.

3.1.3. Effect of pH

The effect of the solution pH on the electrogeneration of H_2O_2 is shown in Fig. 3(C). The generation of H_2O_2 first increased and then decreased with the increase of pH, and it reached maximum at pH 5.0. The result is consistent with the report from Cho et al. [34]. The result implied that the electrogeneration of H_2O_2 was dependent on the pH and the weakly acidic condition was favorable for the production of H_2O_2 because the conversion of dissolved oxygen to H_2O_2 consumes protons, according to Eq. (2).

$$H_2O_2 + 2H^+ + 2e^- \rightarrow 2H_2O \quad (4)$$

$$2H^+ + 2e^- \rightarrow H_2 \quad (5)$$

However, according to Eqs. (4) and (5), a very low pH of electrolyte also promotes hydrogen evolution which reduces the number of active sites for generating H_2O_2. Therefore, an optimal solution pH was 5 determined by the experimental results.

3.1.4. Effect of oxygen flow rate

O_2 is the main material to electrogenerate H_2O_2, and the amounts of dissolved oxygen and H_2O_2 generated in the system are controlled by the flow rate of O_2. As shown in Fig. 3(D), the yield of H_2O_2 increased with the oxygen flow rate increasing from 5 to 25 mL/min, and then stabilized. The results indicated that above 25 mL/min, the effect of oxygen flow rate on the electrogeneration of H_2O_2 could be neglected. Clearly, an oxygen flow rate of 25 mL/min was adequate for the electrogeneration of H_2O_2 in this work.

3.2. Electroenzymatic degradation of BPA

3.2.1. Effect of enzyme dosage on the removal of BPA

Fig. 4 illustrated that the dosage of Hb immobilized on carbon fiber influenced the removal of BPA. As expected, the removal percentage of BPA increased quickly along with an increase of the enzyme dosage, from 24.5% to 50.7%. When the enzyme dose was beyond 0.183 mg/cm^2, the removal efficiency of BPA was almost constant. These results are similar to those obtained by Cheng et al. [35]. At present, the cost of enzyme had always been the bottleneck of application of enzymatic process on the treatment of wastewater. Therefore, the dose of immobilized Hb was set at 0.183 mg/cm^2 to obtain a higher catalytic efficiency as far as possible.

3.2.2. Effects of pH and temperature

The effects of pH and temperature on the degradation efficiency were examined in the pH range from 3 to 8 at 15, 25 and 35 °C for 2 h, respectively. From Fig. 5, it was found that the removal efficiencies of BPA were all showing the trend of increasing initially and then decreasing with pH at any temperatures. The maximum degradation efficiencies were obtained at pH 5.0 and 25 °C. Similar result was also reported by Kim and Moon [28]. In this course, a reducing substrate such as BPA was degraded and the degradation efficiency was highly dependent on the enzyme activity [28]. The maximum amount of H_2O_2, obtained at pH 5 (Fig. 3C), can achieve the highest activity of Hb.

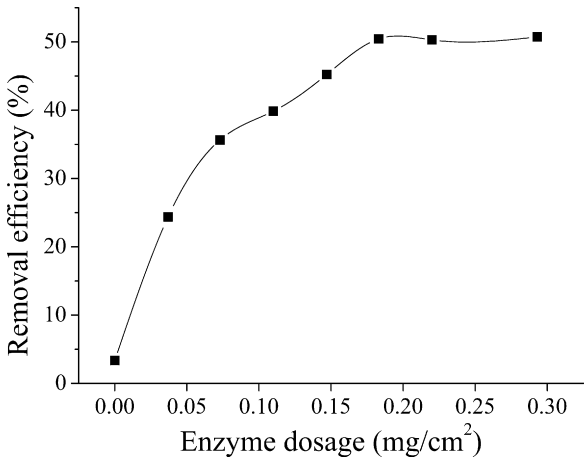

Fig. 4. Effect of enzyme dosage on BPA removal percentage.

The negative influence of temperature was that the increase in temperature led to the lower concentration of dissolved oxygen and the self-decomposition of H_2O_2 [36]. In addition, the catalytic activity of Hb was also affected by temperature. Therefore, the optimal pH and temperature for the catalytic removal of BPA were pH 5 and 25 °C.

3.2.3. Influence of the type of electrolytes

It was reported that the activity or the stability of the enzyme depends on the electrolytes [37,38]. Although it is not widely recognized, this behavior is also common to HRP [39]. To determine the effect of electrolyte on the activity of Hb, the degradation of BPA was evaluated in the presence of PBS (0.1 M), sodium sulphate solutions ($NaSO_4$, 0.1 M) and deionized water. As shown in Fig. 6, it is clear that the BPA removal percentage was higher in PBS than that in sodium sulphate solution and deionized water. Therefore, 0.1 M PBS was selected as an electrolyte for the catalytic oxidation of BPA.

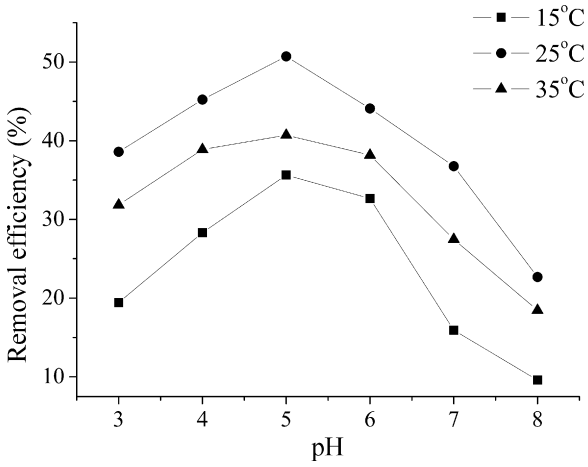

Fig. 5. The effects of pH and temperature on the degradation of BPA in 0.1 M PBS.

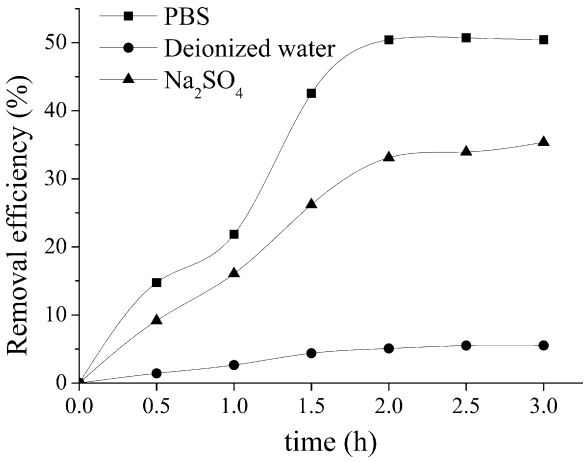

Fig. 6. Influence of type of the electrolytes on BPA degradation.

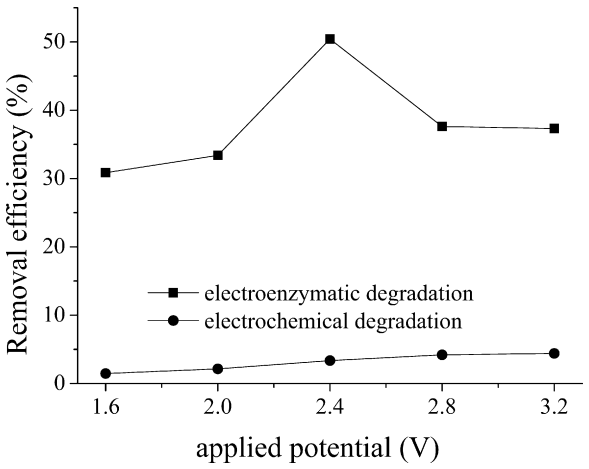

Fig. 8. The removal of BPA through electrochemical and electroenzymatic methods.

3.2.4. Effect of initial concentrations of BPA on BPA degradation

In enzyme-catalyzed reaction, substrate concentration had important influence on the degradation efficiency. To study the effect of BPA concentration in the process, initial BPA concentration was varied in Section 2, and the result was shown in Fig. 7. The removal efficiency of BPA was increased from 15% to 50.7% within the concentration range 20–100 mg/L. These results are similar to those obtained by Zhang et al. [40]. The possible reason could be explained as that the reaction velocity of the enzyme-catalyzed reaction has close relationship with the initial concentration of BPA. Under the same condition, the increase in substrate concentration can increase the probability of contact between enzyme and substrate [40].

3.2.5. The electroenzymatic and electrochemical degradation of BPA

Under the optimal conditions, experiments for the removal of BPA were carried out using two different methods—electrochemical and electroenzymatic methods. Fig. 8 shows that BPA was degraded through the electrochemical and electroenzymatic methods in a membraneless electrochemical reactor with the help of Hb film immobilized on the carbon fiber. Although the carbon fiber could adsorb part BPA during the experiment, this effect was negligible, because the removal efficiency of BPA by adsorption was low. As shown in Fig. 8, the removal efficiency of BPA attributed by electrochemical degradation was very low and it increased slightly with the supplied voltage. This is due to the effects of the oxidants that had been produced in the membraneless electrochemical reactor [29]. The removal efficiency in electroenzymatic degradation was higher than that of electrochemical oxidation in all the tested voltages, and the highest removal efficiency was obtained at 2.4 V. The result implied that at 2.4 V, the highest production of H_2O_2 was obtained and the immobilized Hb was without inactivation or denaturation. Therefore, the highest removal percentage of BPA was also obtained at 2.4 V.

3.2.6. Degradation for the different procedures

Fig. 9 presents the degradation of BPA under different conditions. BPA was not degraded without an applied potential even in presence of enzyme and O_2, due to the absence of H_2O_2. Even with the applied potential, the removal of BPA was insignificant through electrochemical method. When Hb was added with the applied potential, BPA degraded rapidly. The result implied that Hb together with H_2O_2 played important role in the electroenzymatic degradation of BPA, which could also be proved by Fig. 8.

Although the removal efficiency of BPA in biochemical treatment was high during the initial 40 min period, the overall removal efficiency was much lower than that of the electroenzymatic method due to the depletion of active H_2O_2. The H_2O_2 supply strategy (electrogeneration or externally supplied) had a significant effect on the rate and on the overall degradation efficiency. An instantaneous addition at the beginning of the experiment caused a rapid approach to limited degradation. However, when H_2O_2 was

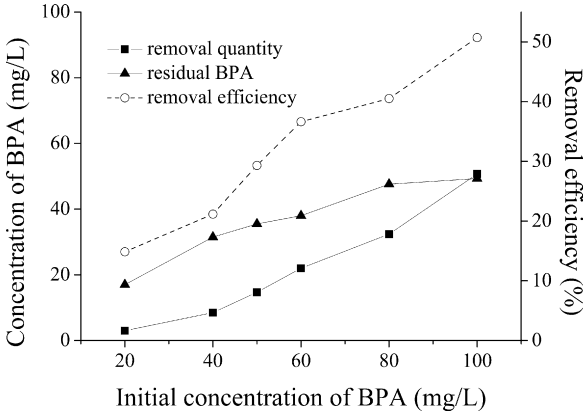

Fig. 7. The effect of initial concentration of BPA on BPA degradation.

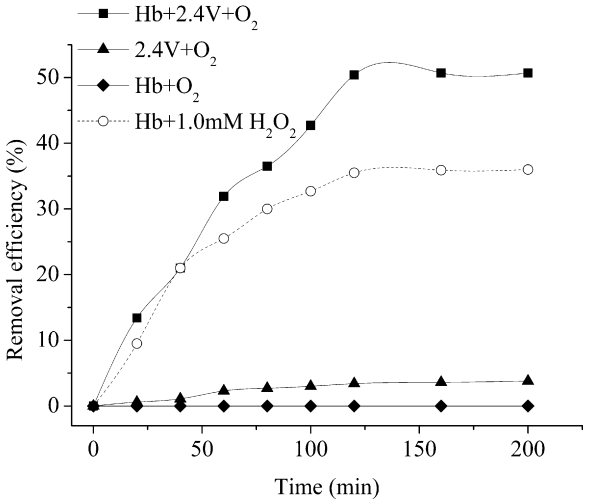

Fig. 9. The degradation of BPA for the different procedures.

supplied continuously, a constant activity was observed throughout the experiment, which made it possible to obtain a much greater degradation [26]. A similar result was reported by Lee et al. [27].

4. Conclusions

In this paper, the removal of bisphenol A by using a membraneless electrochemical reactor with hemoglobin (Hb) film was investigated. Hb film was formed by dipcoating the carbon fiber in a chitosan (CS) solution containing enzyme and then the membraneless electrochemical reactor was arranged with the modified carbon fiber as cathode and stainless steel as anode, respectively. In the membraneless electrochemical reactor, the optimal conditions for *in situ* generated H_2O_2 were applied with potential 2.4 V, 0.1 M PBS, pH 5.0 and oxygen flow rate 25 mL/min, respectively. Under the optimality conditions, the highest removal efficiency was 50.7% obtained after 2 h degradation through the electroenzymatic treatment, which was comparatively higher than that of biochemical and electrochemical methods in this study. Consequently, the combination of Hb film with *in situ* generated H_2O_2 in a membraneless electrochemical reactor is a relatively useful method for the catalytic removal of BPA.

Acknowledgements

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