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Bioelectrochemically Reductive Dechlorination of Trichloroacetic Acid by Gel Immobilized Hemoglobin on Multiwalled Carbon Nanotubes Modified Graphite Electrode

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A gel immobilized method was used to prepare a sodium alginate/hemoglobin-multiwalled carbon nanotubes-graphite composite electrode (Hb/SA-MWCNT-GE) that provided a surface modification with catalytic properties for dechlorination of trichloroacetic acid (TCA). The results indicated that a film of SA hydrogel on the surface made the Hb tight and uniform. Compared with the SA-MWCNT-GE, the introduction of Hb lowered the reduction overpotential of TCA by at least 0.90 V. Moreover, the current efficiency decreased dramatically from 78% to 53% when the Hb-MWCNT-GE was used five cycles of dechlorination. In contrast, the current efficiency decreased from 83% to 74% at the Hb/SA-MWCNT-GE. Quantitative analysis of the intermediate products involving dichloroacetic acid, monochloroacetic acid, and acetic acid were also investigated.

Keywords: reductive dechlorination; hemoglobin; electrode stability; trichloroacetic acid; current efficiency

1. INTRODUCTION

Chlorinated organic compounds (COCs) are very common environmental contaminants found both in soil and water. Many of these compounds are of very poor degradability and tend to accumulate in the environment [1-3]. It is also known that most of their toxic properties are arising from the chlorine-containing groups in their structure [4]. Therefore, most of the treatment techniques

are focused on reductive dechlorination methods that transform chlorinated organic compounds into their nonchlorinated analogues and chloride ions [5].

Among the various methods, bioelectrochemical technique is an innovative and environmentally feasible way for the detoxification of COCs in water [6-16]. A relatively new approach to realize bioelectrochemical technique is to entrap enzyme into films that are formed on electrode surface [17]. Hemoglobin (Hb) becomes ideal model enzyme due to their known structure and low cost of commercial availability [18]. Pioneering works have reported on the bioelectrochemical degradation of COCs by Hb with the composite materials [19]. Nevertheless, the main obstacle to developing commercially available treatment technologies for bioelectrochemical reduction required electrode materials that are stable under cathodic polarization.

Considering the technique used to immobilize enzymes, encapsulation of the catalytically active components is regarded as one of the most effective methods, since the immobilization process can be achieved immediately, and the encapsulated enzymes usually has high operational stability because of avoiding direct exposure to toxic compounds [20, 21]. Among the encapsulation materials, sodium alginate (SA) has good biocompatibility for Hb immobilized on the electrode and can maintain their bioactivities [22].

In this work, Hb was immobilized on surface of multiwalled carbon nanotubes (MWCNT) modified electrode with or without the help of SA hydrogels for dechlorination of trichloroacetic acid (TCA), which is undesirably present in the drinking water as the result of chlorine disinfection [23]. Besides, the role of the Hb/SA in the Hb/SA-MWCNT graphite composite electrode (Hb/SA-MWCNT-GE) was characterized by cyclic voltammetry and galvanostatic electrolysis.

2. EXPERMENTAL

2.1. Reagents

Hb and didodecyldimethylammonium bromide (DDAB) were purchased from Sigma. Graphite electrode (GE) was obtained from Hangzhou Cell Electrochemistry Technology CO., Ltd. MWCNT were purchased from Shenzhen Nanoseason CO., Ltd. Other chemicals were of analytical grade and used without further purification.

2.2. Preparation of the modified electrodes

2 mg of MWCNT was added into 1 mL of DDAB aqueous solution (1 $mg \cdot mL^{-1}$) and the mixture was sonicated for 0.5 h at room temperature to obtain a homogeneous dispersion with MWCNT concentration of 1 $mg \cdot mL^{-1}$. In order to coat the GE with MWCNT, the dispersion was coated and spread onto the surface of the electrode with a level of 95 $\mu L \cdot cm^{-2}$. Then a uniform MWCNT film can be obtained on the GE after the water was evaporated in air.

SA was dissolved into 10 mL of pure water at 75 °C. Then, Hb was mixed with the SA (2 $mg \cdot mL^{-1}$) solution or water. After that, the Hb/SA or Hb solution was deposited onto the MWCNT-coated GE (64 $\mu L \cdot cm^{-2}$). Then the films were dried overnight in air.

2.3. Apparatus and procedures

Electrochemical measurements were carried out on a PAR 2273 potentiostat at 25±1 ºC. A three-electrode-cell composed of an aqueous saturated calomel electrode (SCE), a platinum sheet (2×2.5 cm^2) used as the counter electrode and the Hb/SA-MWCNT-GE or SA-MWCNT-GE (ø 2 mm) was used as the working electrode for voltammetric investigations. All solutions were purged with high-purified nitrogen for at least 30 min prior to each set of experiments, and the nitrogen environment was then maintained over the solutions in the electrochemical cell during the respective measurements.

The morphologies of the corresponding films were characterized by scanning electron microscopy (SEM) on a Hitachi S-4700 II electron microscope. The concentration of TCA and intermediate products in electrolyzed solutions were analyzed with a Dionex model ICS 2000 ion chromatograph (IC).

2.4. Bulk electrolysis

Preparative electrolyses ware carried out in a two-compartment cell, divided by the cation exchange membrane (Nafion-117), assembled with a magnetic stirring bar, the catholyte and anolyte volumes were approximately 100 mL, respectively. The cathode consisted of the Hb/SA-MWCNT-GE or Hb-MWCNT-GE (ø 15 mm), and a platinum sheet was positioned at the center of the anode compartment as a counter electrode. The electrolysis experiments were achieved by galvanostatic mode of PAR 2273, at 25 ºC.

3. RESULTS AND DISCUSSION

3.1. Characterization of MWCNT, Hb-MWCNT, and Hb/SA-MWCNT film

SEM was used to probe and compare the morphology of the MWCNT (Fig. 1 a), the Hb-MWCNT (Fig. 1 b) and the Hb/SA-MWCNT films (Fig. 1 c, d, e) [24]. As shown in Fig. 1 a, many twisted MWCNT bundles can be observed, suggesting it is a good biomaterial due to the high active surface area and good electrical conductivity. However, with the same magnification, the top view SEM image of Hb-MWCNT films (Fig. 1 b) shows very different morphology from that of MWCNT films. The change of morphology suggested that the interaction between MWCNT and Hb indeed occurred and might even influence the morphology of the dry films. This could be attributed to the adsorption of Hb molecules on the MWCNT. From the SEM image of surface morphology of Hb/SA-MWCNT films (Fig. 1 c), it can be seen that the SA hydrogel on the surface made the Hb more tight

and uniform. This interaction may also improve the retention activities of the Hb on the surface of electrode. Additionally, the most of incident electron beam in the SEM was converted into heat at the sample position, making the SA burst (Fig. 1 d, e). The smaller magnification will make it easier to break up the SA, and we also observed the gradual rupture of the SA process in the SEM (Fig. 1 d, e).

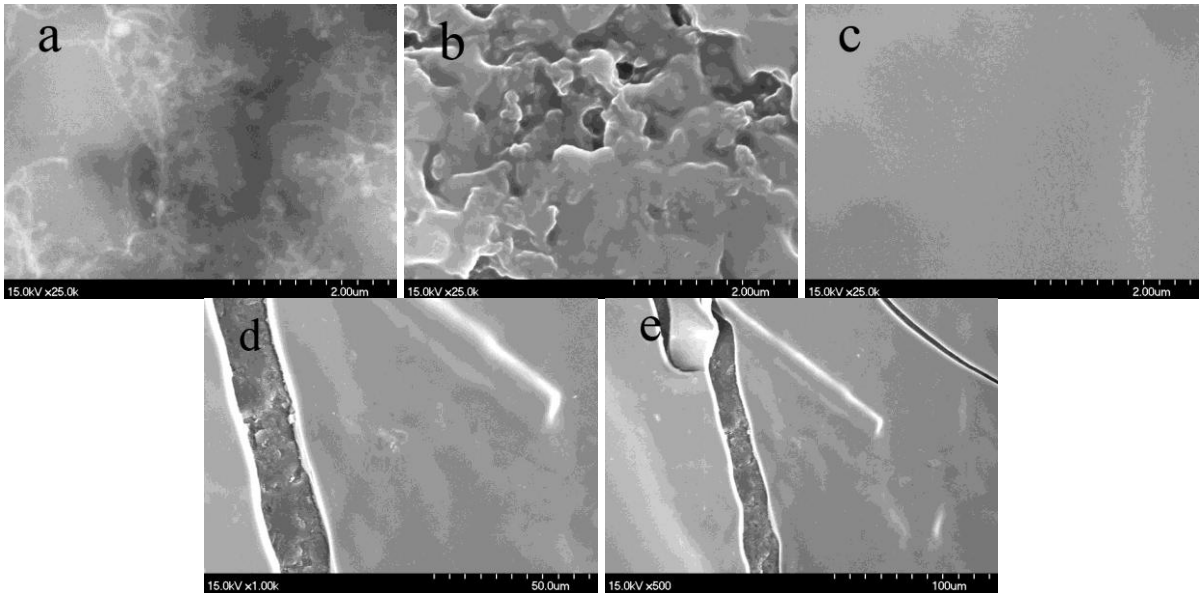

Figure 1. SEM image of MWCNTs (a), Hb-MWCNT (b), and Hb/SA-MWCNT (c, d, e).

3.2. Cyclic voltammetry

The electrochemical behaviors of Hb/SA-MWCNT-GE, Hb-MWCNT-GE and SA-MWCNT-GE (ø 2 mm) were studied by cyclic voltammetry (CV). As it can be seen from Fig. 2 a, there is no peak at the CV curve of SA-MWCNT-GE in 0.1 M pH 7.0 phosphate buffer solutions (PBS) (curve 1). While a redox peaks are observed on the Hb/SA-MWCNT-GE with the potentials at cathode peak potential (*E*pc= -0.336 V) and anode peak potential (*E*pa= -0.198 V) (curve 2), which can be attributed to the electrode process of electroactive center of heme Fe(III)/Fe(II) couples in the Hb molecule [19], indicating that direct electron transfer between Hb and GE was realized in the microenvironment formed by SA-MWCNT film. The peak potential separation $\Delta Ep = Epa - Epc$ corresponded to 0.138 V (larger than 0.059 V) with the peak current of $Ipa/Ipc \neq 1$, which indicated that the electrochemical process of Hb confined on SA-MWCNT-GE was quasi-reversible [25]. Compared with the Hb-MWCNT-GE (curve 3), Hb/SA-MWCNT-GE showed higher redox current density. Therefore, the SA could let the Hb maintain its suitable configuration and activity, made the direct electron transfer between the Hb and the electrode easier.

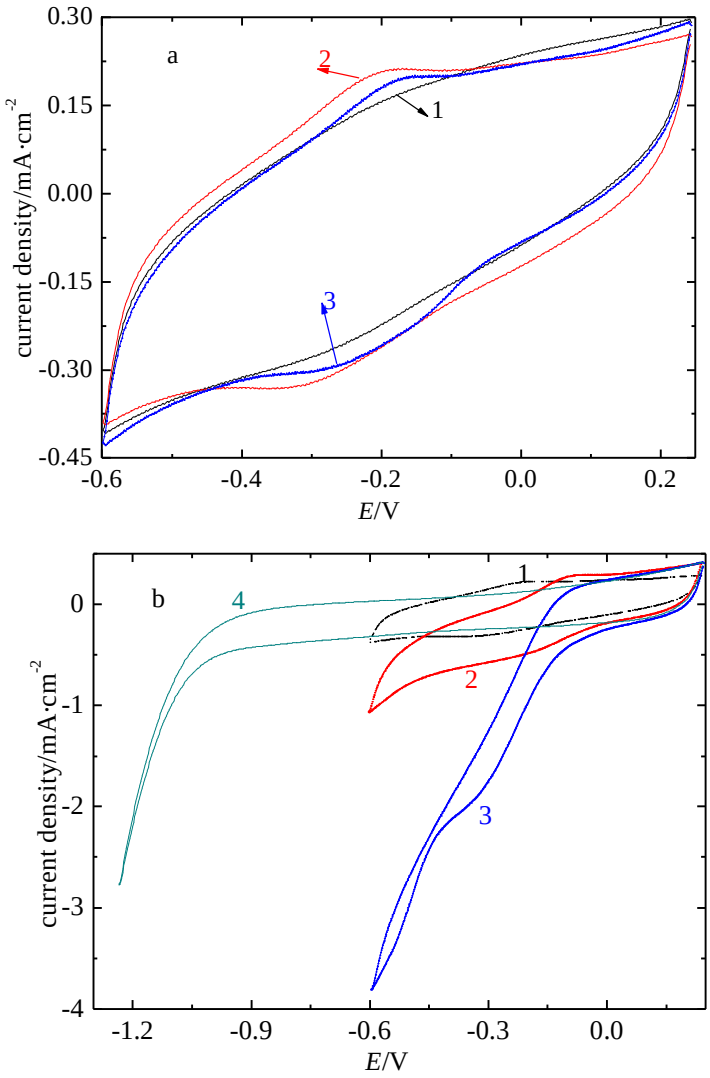

Figure 2. (a) Cyclic voltammograms of the SA-MWCNT-GE (curve 1), Hb/SA-MWCNT-GE (curve 2), and Hb-MWCNT-GE (curve 3) in 0.1 M pH 7.0 PBS at a scan rate of 300 mV·s^{-1}. (b) Cyclic voltammograms at a scan rate of 300 mV·s^{-1} in 0.1 M pH 7.0 PBS: Hb/SA-MWCNT-GE with no TCA (curve 1), Hb/SA-MWCNT-GE containing 0.1 M TCA (curve 2), Hb/SA-MWCNT-GE containing 0.2 M TCA (curve 3), SA-MWCNT-GE containing 0.1 M TCA (curve 4).

Electrocatalytic reduction of TCA by the Hb/SA-MWCNT-GE and SA-MWCNT-GE were also tested by CV (Fig. 2b). Taking Hb/SA-MWCNT-GE as an example, when TCA is added into PBS, a significant increase in the Hb reduction peak at about -0.34 V is observed, following accompanied by a decrease of the Hb oxidation peak (curve 2). The reduction peak current increases as the TCA concentration increased (curve 3). Compared with the direct reduction of TCA on SA-MWCNT-GE at the potential more negative than -1.24 V (curve 4), a decrease of 0.90 V of the TCA reduction overpotential for Hb/SA-MWCNT-GE was achieved. The specific interaction between the incorporated Hb and TCA suggest that a large decrease in activation energy for the reduction of TCA in the presence of Hb [26].

3.3. Preparative electrolysis experiments

To evaluate the stability of electrode, a constant current (2.0 mA) electrolysis of 10 mM TCA was conducted on both Hb/SA-MWCNT-GE and Hb-MWCNT-GE (ø 15 mm). The reductive dechlorination of TCA was repeated 5 times.

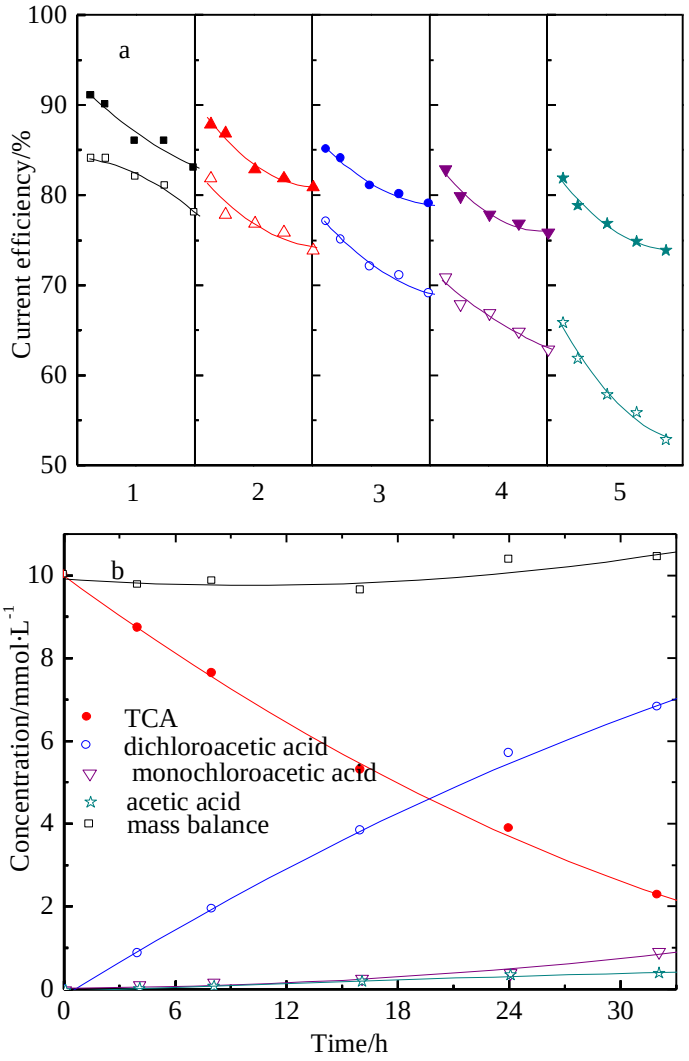

Figure 3. (a) Hb-MWCNT-GE (open symbols) and Hb/SA-MWCNT-GE (filled symbols) stability test registered during the reaction conduced one, two, three, four and five times using the same electrode corresponding respectively to (1), (2), (3), (4) and (5) in abscissa; (b) the intermediate products analysis of TCA dechlorination on Hb/SA-MWCNT-GE. Conditions: TCA, 10 mM; T, 25 ºC; current, 2 mA; supporting electrolyte, 0.1 M pH 7.0 PBS.

After each run of 32 h, the liquid phase was removed and the electrode was rinsed with pure water. Fig. 3a shows that both electrodes exhibited a decline in activity with reuse. The current efficiency decreased from 83% to 74% when the Hb/SA-MWCNT-GE was used 5 times, while it decreased from 78% to 53% at the Hb-MWCNT-GE. It can be seen that the Hb/SA-MWCNT-GE

exhibited higher stability than Hb-MWCNT-GE. The phenomena could be explained as follows. Hb was much easier desorbed from MWCNT-GE without the aid of SA hydrogels. However, a strong gel-support interaction between the films with the surface of the MWCNT was realized after deposition with Hb/SA. Such MWCNT with Hb/SA plays an important role in the improvement of bioelectrochemically reductive dechlorination of TCA. The results mentioned above reveal that the Hb/SA-MWCNT-GE is stable after being repeated experiments, which is probably due to the compact interaction of Hb/SA and MWCNT. Fig. 1 c showed the uniform dispersion of the Hb/SA-MWCNT films.

Intermediate products of TCA dechlorination at electrolysis current of 2 mA were detected by IC (shown in Fig. 3b). The concentration of TCA decreased gradually in the entire process. This indicated that TCA was degraded continuously. The dechlorination product concentrations of dichloroacetic acid, monochloroacetic acid, and acetic acid increased within the entire time. At the early stage, besides dichloroacetic acid, monochloroacetic acid and acetic acid were also found in the solution. The result implied that the most of generated dichloroacetic acid desorbed from the electrode surface and transferred to the solution, others was reduced to monochloroacetic acid, and acetic acid on the electrode further. The mass balances in the above IC measurements were close to 100% when TCA, dichloroacetic acid, monochloroacetic acid, and acetic acid were considered. The catalytic dechlorination of TCA by Hb could be expressed as follows:

$$HbFe(III) + H^+ + e^- \leftrightarrow HbFe(II) \quad (1)$$

$$2HbFe(II) + CCl_3COOH \rightarrow 2HbFe(III) + CHCl_2COOH + H^+ + Cl^- \quad (2)$$

$$2HbFe(II) + CHCl_2COOH \rightarrow 2HbFe(III) + CH_2ClCOOH + H^+ + Cl^- \quad (3)$$

$$2HbFe(II) + CH_2ClCOOH \rightarrow 2HbFe(III) + CH_3COOH + H^+ + Cl^- \quad (4)$$

4. CONCLUSIONS

In this study, a novel Hb/SA-MWCNT-GE was prepared by encapsulation method and was applied to dechlorination of TCA. The Hb/SA-MWCNT-GE showed a better performance for bioelectrochemically reductive dechlorination of trichloroacetic acid than Hb-MWCNT-GE. The current efficiency decreased from 78% to 53% when the Hb-MWCNT-GE was used five times, while it only decreased from 83% to 74% at the Hb/SA-MWCNT-GE. The catalytic dechlorination of TCA could be expressed as follows: TCA → dichloroacetic acid → monochloroacetic acid → acetic acid.

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