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Reductive dehalogenation of haloacetic acids by hemoglobin-loaded carbon nanotube electrode

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ABSTRACT

Hemoglobin (Hb) was immobilized on carbon nanotube (CNT) electrode to catalyze the dehalogenation of haloacetic acids (HAAs). FTIR and UV measurements were performed to investigate the activity-keep of Hb after immobilization on CNT. The electrocatalytic behaviors of the Hb-loaded electrode for the dehalogenation of HAAs were studied by cyclic voltammmetry and constant-potential electrolysis technique. An Hb-loaded packed-bed flow reactor was also constructed for bioelectrocatalytic dehalogenation of HAAs. The results showed that Hb retained its nature, the essential features of its native secondary structure, and its biocatalytic activity after immobilization on CNT. Chloroacetic acids and bromoacetic acids could be dehalogenated completely with Hb catalysis through a stepwise dehalogenation process at −0.400 V (vs. saturated calomel electrode (SCE)) and −0.200 V (vs. SCE), respectively. The removal of 10.5 mM trichloroacetic acid and dichloroacetic acid is ca. 97% and 63%, respectively, with electrolysis for 300 min at −0.400 V (vs. SCE) using the Hb-loaded packed-bed flow reactor, and almost 100% of tribromoacetic acid and dibromoacetic acid was removed with electrolysis for 40 min at −0.200 V (vs. SCE). The average current efficiency of Hb-catalytic dehalogenation almost reaches 100%.

1. Introduction

Haloacetic acids (HAAs) and other halogenated disinfection byproducts (DBPs) are formed upon the addition of chlorine to water for disinfection purposes (Chen and Weisel, 1988; Singer, 1994; Dojlido et al., 1999) and through the use of C2-chlorocarbon and chlorofluorocarbon (CFC) replacement compounds in various industrial applications (Lifongo et al., 2004). HAAs tend to accumulate in surface water and pose threats to humans and the ecosystem due to their toxicity and high stability. Many researches have demonstrated that HAAs are also carcinogenic to humans (Kühn and Pattard, 1990). Therefore, the United States Environmental Protection Agency began to regulate HAAs concentration in its *Disinfectants and Disinfection Byproducts Rule of 1998* (USEPA, 1998).

The accepted treatment technologies of drinking water, e.g., adsorption by activated carbon, may not perform well with HAAs due to their very high hydrophilicity. Photodegradation is an advanced technology to remove HAAs from drinking water, but it is found that the photodegradation of HAAs might produce chloroform of higher toxicity (Spangenberg et al., 1996; Wu et al., 2001). Electrochemical treatment seems to achieve the goal without producing toxic byproducts or adding any toxic chemicals. However, reductive dehalogenation of HAAs happens at potentials more cathodic than −1.0 V where profuse molecular hydrogen would evolve, and

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parallel electrolysis of water would lower current efficiency as well. Though some bacteria can aerobically degrade HAAs (McRae et al., 2004), biodegradation by microorganisms is not a suitable method for the treatment of drinking water, considering new pollution caused by the added nutrients and microorganisms. In contrast to microbiological process, enzymatic treatment does not need those conditions which maintain the growing of bacterial culture, and thus it appears to be more advantageous for the treatment of drinking water. However, enzymes are readily inactivated and their obtainment is not easy, dramatically increasing the cost. It has been reported that some heme proteins might be involved in microbial reductive dechlorination in the environment (Castro et al., 1985; Li and Wackett, 1993), and dehalogenation of organic halides by reduced heme proteins has thus recently aroused increasing interest (Connors et al., 1988; Helvenston and Castro, 1992). Some of these heme proteins are enzymes or redox-active cofactors that can catalyze reductive dehalogenation, and others also have enzyme-like catalytic activity. A few heme proteins, e.g., haemoglobin, myoglobin, etc, become ideal model enzymes due to their known structure and low cost of commercial availability. Electrochemical reduction may be the preferred method for regeneration of reduced heme proteins due to its cleaner process, but the electron transfer between the proteins and electrodes is really difficult. Furthermore, there are many difficulties in immobilization of the enzyme-like proteins and their activity-keep.

In this paper, hemoglobin (Hb) was immobilized on carbon nanotube (CNT) electrode for electrochemical dehalogenation of HAAs. An Hb-loaded packed-bed flow reactor was then constructed for reductive dehalogenation of HAAs. The activity of Hb was investigated by FTIR and UV measurements, and the electrochemical behaviors of Hb-loaded electrode for reductive dehalogenation were studied by cyclic voltammetry and constant-potential electrolysis techniques. The reduction kinetics and current efficiency were also investigated.

2. Materials and method

2.1. Chemicals and solutions

Hb, didodecyldimethylammonium bromide (DDAB), tribromoacetic acid and dibromoacetic acid were purchased from Sigma Chemical Company (USA). Pyrolytic graphite was purchased from Shanghai Carbon Materials Company (China). Multi-wall CNTs were obtained from the Chemical Engineering Department of Tsinghua University and purified by nitric acid. All other chemicals were of analytical grade and were purchased from Beijing Chemical Company (China). All solutions were prepared with Milli-Q water.

2.2. Immobilization of Hb on CNT-modified electrode

2.2.1. Hb-loaded electrode for cylic voltammetric measurements

5.0 mg multi-wall CNTs were dispersed in 5.0 ml of aqueous DDAB dispersion (1 mg ml^{-1}) by ultrasonication for about 10 min to obtain a stable black suspension. 5 μl of CNT dispersion was cast onto the surface of a polished pyrolytic graphite electrode and a uniform CNT film was formed after the water evaporated. Finally, 5 μl of 2×10^{-5} M Hb solution was cast onto the CNT film and then water was evaporated overnight in air. If not used immediately, the Hb-loaded electrode was stored in phosphate buffer (pH 7.0) at 4 °C.

2.2.2. Hb-loaded electrode for flow reactor

A pyrolytic graphite cube was cut into many small pyrolytic graphite cubes ($10 \times 20 \times 5$ mm). The pyrolytic graphite cubes were abraded with metallographic sand paper, and then polished on a clean billiard cloth with water. The polished pyrolytic graphite cubes were ultrasonicated in water for about 30 s and then rinsed. The treated pyrolytic graphite cubes were immersed in CNT dispersion as described in Section 2.2.1 and then taken out to dry slowly. The CNT-modified pyrolytic graphite cubes were immersed in 2 mg ml^{-1} Hb solution and tumbled at intervals of 20 min for about 4 h. Finally, the Hb-loaded pyrolytic graphite cubes were washed with phosphate buffer (pH 7.0) and then packed in the working electrode (cathode) compartment of a flow reactor.

2.3. Construction of a packed-bed flow reactor

The schematic diagram of the packed-bed flow reactor is shown in Fig. 1. The reactor was composed of two glass compartments separated by a cationic exchange membrane (Aldrich Nafion 450). The working electrode (cathode) compartment was packed with Hb-loaded pyrolytic graphite electrodes, as described in Section 2.2.2, with a working volume of 25 ml. A pyrolytic graphite electrode was inserted into the packed bed to collect the current, a saturated calomel

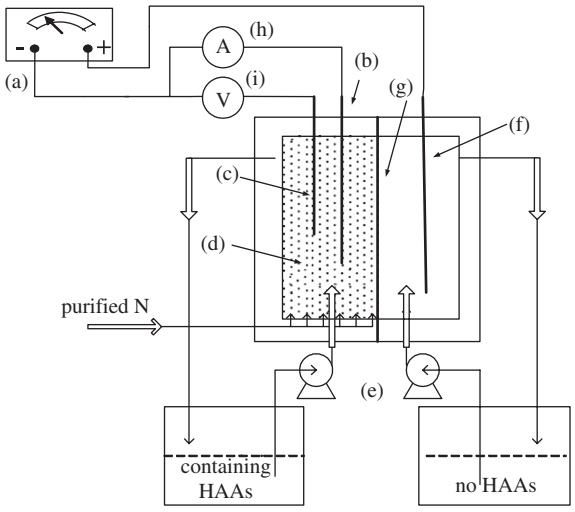

Fig. 1 – Schematic diagram of the two-compartment packed-bed flow reactor: (a) voltage-adjustable direct current power; (b) current collector (pyrolytic graphite electrode); (c) reference electrode (SCE); (d) Hb-loaded electrodes matrix; (e) pump; (f) platinum electrode (anode); (g) cationic exchange membrane; (h) ampere meter; (i) voltage meter.