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Bioelectrocatalytic Dechlorination of Trichloroacetic Acid by Hemoglobin Modified Graphite Electrode in Aqueous Solutions of Different pH and Temperature

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The bioelectrochemical mechanism was investigated in this study, in which several experiments were conducted to trichloroacetic acid (TCA) using a hemoglobin-multiwalled carbon nanotubes-graphite composite electrode (Hb-MWCNT-GE). The electrons transfer from enzyme in the electrode to COCs was the key step, which determined the average current efficiency and was influenced by the pH and temperature of the systems. Specifically, the effect of temperature (288-318 K) and pH (2-11) was examined. The results showed that high temperature and acid conditions were beneficial to the catalytic dechlorination of TCA. Favorable degradation conditions for TCA by Hb-MWCNT-GE were found to be pH 3 and 310 K. The activation energy for TCA of the Hb-MWCNT-GE was calculated to be 19 kJ·mol^{-1}. Total mass balance (on molar basis) of the reactant and the products was in the range of 95-104% during the bioelectrochemically reductive reaction. Based on the intermediates detected, a pathway was proposed for TCA degradation in which it undergoes dechlorination process. A reduction by sequential reactions is not a main degradation mechanism for the dechlorination of TCA in Hb-MWCNT-GE system but a reductive dechlorination by parallel reaction mechanism can rather be the one for the TCA degradation. Such studies provide relevant information about the applicability of bioelectrocatalytic systems for remediation of raw wastewaters.

Keywords: trichloroacetic acid; bioelectrochemical dechlorination; activation energy; average current efficiency; hemoglobin

1. INTRODUCTION

Chlorinated organic compounds (COCs) are widespread soil and water contaminants which have been widely used as refrigerants, agricultural fumigant, and solvents in industrial processes for

several decades [1-4]. They have been attracted an attention due to their carcinogenic and mutagenic characteristics [5] and persistence in natural environments [6]. The toxicity of these compounds is related with the halogen content, and increase with increasing number of halogen atoms [7]. Therefore, it is highly desirable to develop efficient and safe technology for the dechlorination of COCs.

Recently, many methods have been developed to treat COCs effluents [8-10]. The electrochemical activation of carbon-chloride bonds is a widely explored field in the electrochemistry. The great interest for this process is due to the important roles it plays in environmental applications [11], especially the abatement of COCs. The reductive cleavage of the carbon-chloride bond has also attracted much interest in the investigation of dissociative electron transfer processes [12]. A major drawback to the use of organic halides in reduction is that electrochemical decomposition of carbon-halogen bonds, especially chlorides, requires application of quite negative potentials.

Bioelectrochemically reductive dechlorination by enzyme has been considered as a promising way for the treatment of polluted waters because it maintains a relatively high current efficiency in dechlorination of COCs [13]. The enzymatic electrode in a bioelectrochemical system is consisting of an inner layer, an intermediate layer, and an outer layer [14]. During the reaction, electrons transport from the inner layer *via* the intermediate layers to the active centres in the outer layer, in which the charge transfer will take place between the enzyme and the COCs [15]. The activity of the active centres in the enzymes is the key factor, which determines the dechlorination performance of bioelectrochemical system.

For the bioelectrochemical systems, high activity of the active centres is necessary to enhance the charge transfer from the enzyme to the COCs for efficient current utilization. High activity of enzyme always requires proper environments [16]. For example, it has been reported that the activities of enzyme are strongly depends on the temperature and pH of the systems [17-19]. The electrostatic force, one of the most crucial interactions between the enzyme and support surfaces, and also between themselves, is sensitive to the pH environment [20]. Moreover, in a given system, the enzyme can only exhibit efficient activity under a very narrow temperature range [21]. Therefore, optimization of the system environment becomes a feasible way to achieve high efficient current utilization.

In order to obtain the higher current efficiency of the bioelectrochemical system, it is important to investigate the effect of pH and temperature change on the dechlorination reactions. Bioelectrochemical dechlorination of COCs by hemoglobin (Hb) with the composite materials exhibits extraordinary electrocatalytic activities towards the reduction process [15]. Surprisingly, although the temperature and pH in a bioelectrochemical reaction plays an important role, very little information concerning the temperature effect on the heterogeneous bioelectrochemical dechlorination of pollutants in aqueous solutions is available. In the present work, we have carried out bioelectrocatalytic reductive decomposition of TCA in aqueous solution using hemoglobin modified graphite electrode. Cyclic voltammetry and electrolysis are conducted to examine the effect of pH and temperature of the system on the activity of the enzymatic electrode. Relationships between the conditional variables and performance parameters will be discussed in this paper.

2. EXPERMENTAL

2.1. Reagents

Hb and didodecyldimethylammonium bromide (DDAB) were purchased from Sigma. Graphite electrode was obtained from Hangzhou Cell Electrochemistry Technology CO., Ltd. MWCNT was purchased from Shenzhen Nanotech Port CO., Ltd. All other chemicals, including TCA, dichloroacetic acid (DCA), and monochloroacetic acid (MCA), acetic acid (AA), $C_6H_8O_7$, $C_6H_5Na_3O_7$, Na_2HPO_4, NaH_2PO_4, $Na_2B_4O_7$, and NaOH, were analytical grade and were purchased from Huadong Medicine Hangzhou Co., Ltd.

2.2. Preparation and electrochemical characterization of the electrode

The detailed procedure for the preparation of the hemoglobin-multiwalled carbon nanotubes-graphite composite electrode (Hb-MWCNT-GE) had been described in our previous work [15]. Briefly, the electrode included three layers: the graphite substrate inner layer, the MWCNT intermediate layer, and the Hb outer layer.

The cyclic voltammograms (CV) test was done with an electrochemical workstation (PAR 2273 potentiostat). A three-electrode cell system with the Hb-MWCNT-GE (ø 2 mm) as working electrode was used. The counter electrode and the reference electrode were a platinum sheet (2×2.5 cm^2) and an aqueous saturated calomel electrode (SCE), respectively.

2.3. Bulk electrolysis experiments

The preparative electrolyses were carried out in a two-compartment cell, divided by the cation exchange membrane (Nafion-117). The cathode consisted of the Hb-MWCNT-GE (ø 15 mm) and a platinum sheet (2×2.5 cm^2) was positioned at the center of the anode compartment. During each run, the catholyte was stirred by a magnetic follower and electrolysis was done at a constant current in a temperature-controlled bath (288, 298, 303, 310, and 318 K, respectively). Aqueous solutions of TCA 10 mM were always used. Bioelectrochemical experiments at controlled pH were carried out using six different 0.1 M aqueous buffer solutions ($C_6H_8O_7/C_6H_5Na_3O_7$, $C_6H_8O_7/C_6H_5Na_3O_7$, $C_6H_8O_7/C_6H_5Na_3O_7$, NaH_2PO_4/Na_2HPO_4, $Na_2B_4O_7$/NaOH and Na_2HPO_4/NaOH associated with pH 2, 3, 5, 7, 9 and 11, respectively).

The concentration of TCA and intermediate products in electrolyzed solutions were analyzed with a Dionex model ICS 2000 ion chromatograph (IC).

3. RESULTS AND DISCUSSION

3.1. Cyclic voltammetry

The electrochemical behaviors of Hb-MWCNT-GE and MWCNT-GE (ø 2 mm) were studied by cyclic voltammetry (CV). As it can be seen from Fig. 1, there is no peak at the CV curve of

MWCNT-GE in 0.1 M pH 7.0 phosphate buffer solutions (PBS) (curve 1). While a redox peaks are observed on the Hb-MWCNT-GE with the potentials at cathode peak potential (*E*pc= -0.315 V) and anode peak potential (*E*pa= -0.177 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 [22], indicating that direct electron transfer between Hb and GE was realized in the microenvironment formed by 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 MWCNT-GE was quasi-reversible [23].

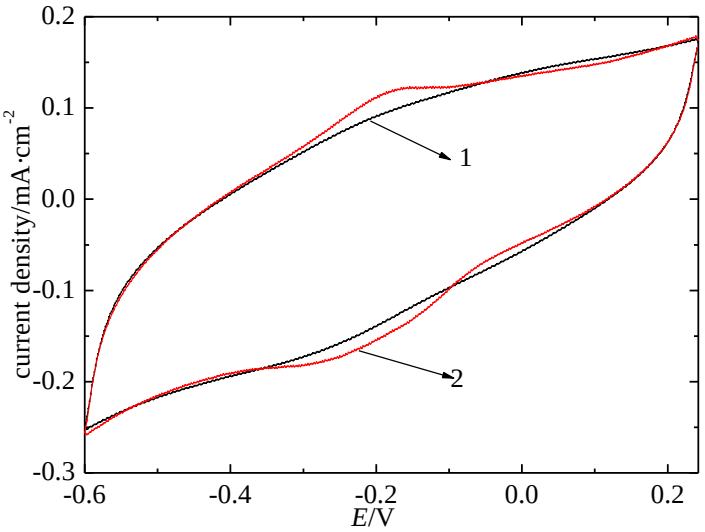

Figure 1. Cyclic voltammograms of the MWCNT-GE (curve 1), and Hb-MWCNT-GE (curve 2) in 0.1 M pH 7.0 PBS at a scan rate of 300 $mV \cdot s^{-1}$.

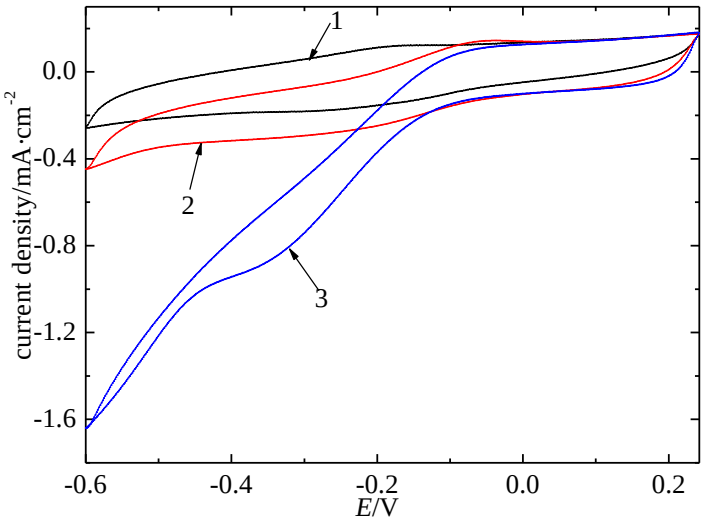

Figure 2. Cyclic voltammograms at a scan rate of 300 $mV \cdot s^{-1}$ in 0.1 M pH 7.0 PBS: Hb-MWCNT-GE with no TCA (curve 1), Hb-MWCNT-GE containing 0.1 M TCA (curve 2), Hb -MWCNT-GE containing 0.2 M TCA (curve 3).

Electrocatalytic reduction of TCA by the Hb-MWCNT-GE was also tested by CV (Fig. 2). Taking Hb-MWCNT-GE as an example, when TCA is added into PBS, a significant increase in the Hb reduction peak at about -0.32 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 without the help of Hb at the potential more negative than -1.24 V, a decrease of 0.92 V of the TCA reduction overpotential for Hb-MWCNT-GE was achieved [15]. 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 [24].

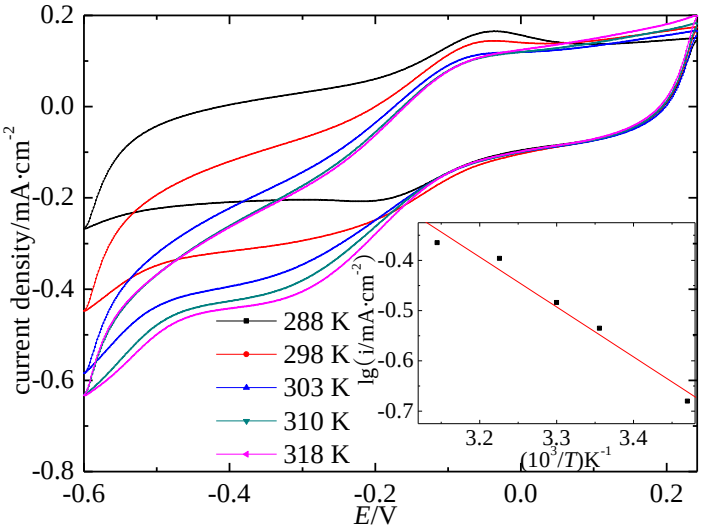

Figure 3. Cyclic voltammograms of the Hb-MWCNT-GE at pH 7 in different temperature with a scan rate of 300 mV·s^{-1} containing 0.1 M TCA. Insert graph: variation of lg *i* vs. 1/T.

Typical examples of CV recorded for Hb-MWCNT-GE 0.1M TCA at pH 7.0 were separately conducted under the temperature of 288, 298, 303, 310 and 318 K (Fig. 3). The reduction peak of TCA grew with increasing temperature and peak potentials shifted to more positive value (Fig. 3).

Electrochemical rate can be given as fallows:

$$i = nFkCe^{-\alpha F\eta/RT} \qquad (2)$$

Reaction activation energy calculated from Arhenius equation. The dependence of rate constant on temperature over a limited range can usually be represented by an empirical equation proposed by Arhenius.

$$k = Ae^{-E_a/\mathbf{R}T} \qquad (3)$$

where A is the frequency factor and E_a is activation energy.

Substituting (2) into (3) gives:

$$\lg i = \lg(FKC) - E_a/(2.3RT) \quad (4)$$

When the logarithm of the peak current is plotted against the reciprocal of the absolute temperature (Inset of Fig. 3) a straight line is obtained and the activation energy value calculated from the slope of this line is 19 kJ·mol^{-1}.

3.2. Preparative electrolysis experiments

3.2.1 Effect of pH

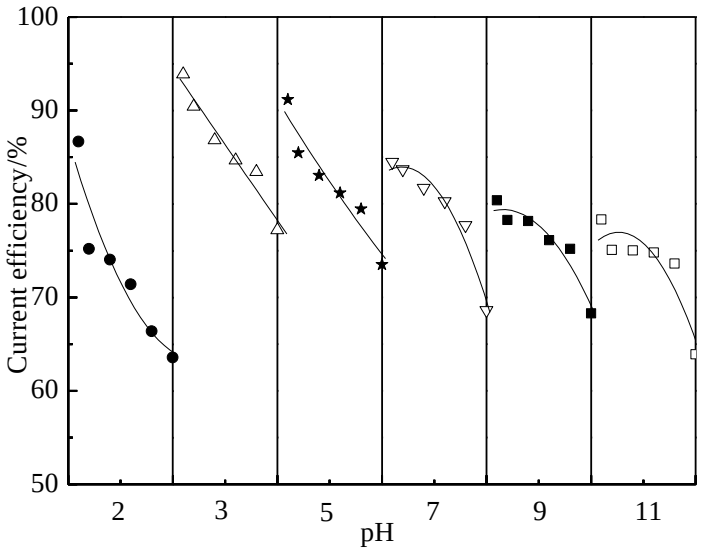

Figure 4. The average current efficiency for the reduction of 0.01 M TCA on the Hb-MWCNT-GE at 298 K in different pH, using applied current 2 mA, as a function of the time of electrolysis/h.

Since the catalytic activities of Hb-MWCNTs-GE is critically dependent on the solution pH, the optimization of the reaction pH is crucial to obtain the maximum average current efficiency (CE) on the degradation of TCA. In order to investigate the influence of pH value on the TCA dechlorination, electrochemical reductive dechlorination of 10 mM TCA at the Hb-MWCNTs-GE was performed in constant current (2.0 mA) in aqueous solutions with different buffer solutions (pH 2-11).

As shown in Fig. 4, the CE of TCA was higher at pH 3.0 than in other pH conditions. According to the principle of heterogeneous catalysis, the concentration of H^+ ions is critical for the generation of HbFe(II) [15]. Thus, decreasing the pH of solution was proposed to enhance the number of H^+ ions on the Hb-MWCNTs-GE surface and promote the bioelectrochemical degradation. The isoelectric point of hemoglobin is about 6.8 [25]. This means that the Hb-MWCNTs-GE surface would be positively charged in an acidic solution, negatively charged in an alkaline medium, and neutral when the transition solution pH value is around 6.8. Due to the anionic property of TCA, the acid Hb-MWCNTs-GE surface seems beneficial for the adsorption of TCA. Considering the combined effect of the generation of HbFe(II) and the interaction between TCA and the surface of Hb-MWCNTs-GE, the

bioelectrochemical degradation of TCA was most efficient in acid medium. The results demonstrated that the acid condition favors the dechlorination of Hb-MWCNTs-GE. Additionally, lower pH conditions favor higher activities of both proton and electron and hence speed up the overall reaction rates of TCA. However, as more intensive corrosion of Hb-MWCNTs-GE could result in the loss of Hb at the pH value of 2.0, and inhibition of the reaction as the surface becomes covered by H_2 becomes apparent at the lower pH. Therefore, pH 3.0 is the most suitable for Hb-MWCNTs-GE dechlorination of TCA.

3.2.2 Effect of temperature

Optimum temperature is very important since the enzyme activity will increase with temperature, but on the other hand at high temperatures there may be thermal deactivation of the enzyme. The temperature range for achieving maximum Hb-MWCNTs-GE activity for the bioelectrochemically reductive dechlorination is conducted under various temperature conditions ranging from 288 to 318 K.

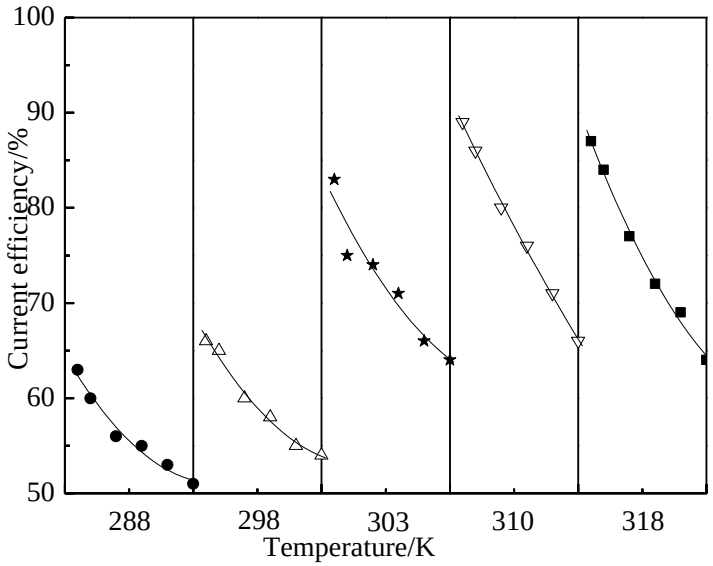

Figure 5. The average current efficiency for the reduction of 0.01 M TCA on the Hb-MWCNT-GE pH 7 in different temperature, using applied current 2 mA, as a function of the time of electrolysis/h.

Representative results of the degradation reaction at different temperature conditions are shown in Fig. 5. As depicted in Fig. 5, looking at CE as a function of temperature, an increase of CE is achieved until 310 K, while temperatures higher than 310 K lead to a decrease of the average current efficiency. The CE of the Hb-MWCNTs-GE was above 83% at 4 h, except at the lower temperature (288 and 298 K) where less than 66% average current efficiency was directly achieved. The CE is clearly limiting at low temperature. Increasing temperature usually causes a significant increase in reaction rate depending on the reaction processes. Rising the temperature then leads to higher amounts

of TCA reaching the surface of the electrode and a reaction occurred. However, increasing the reaction temperature may increase the reduction rate of organic compounds at the interface between the catalyst and the solution, but it also reduces the adsorptive capacities associated with the organics. In addition, it could be the denaturalization of Hb in the case of the temperatures above 310 K. A factor of great importance because it regulates the bioelectrochemical mechanism by capturing the electrons, as well as the increase of the desorption of the reactants from the catalyst surface [26]. Taking into consideration the above-mentioned multisided reasons, we considered 310 K as the optimum temperature for the study of TCA on the Hb-MWCNTs-GE.

3.5. Mechanism and production

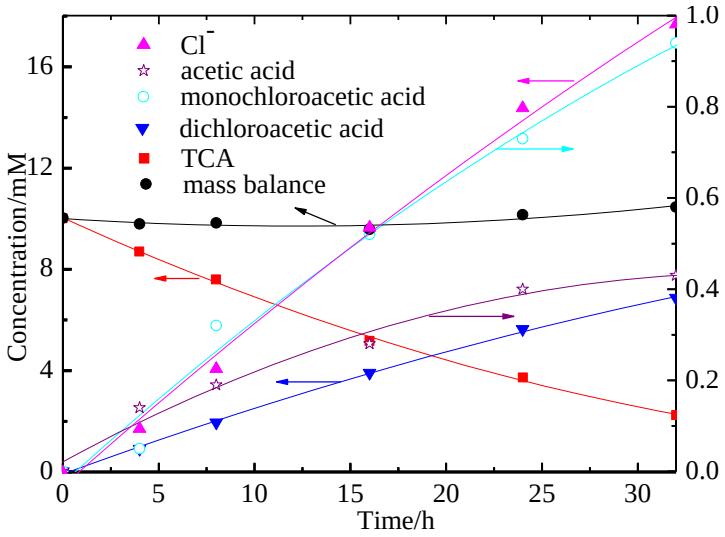

Figure 6. Variation of the concentration of related organic intermediates and anions during electrochemical dechlorination of TCA with the Hb-MWCNT-GE. Conditions: TCA, 0.01 mM; T, 310 K; current, 2 mA; supporting electrolyte, 0.1 M aqueous buffer solutions of pH 3.0.`

As shown in Fig. 6, it presented a typical concentration profile of the reactant and the final products versus time for the batch systems of Hb-MWCNT-GE tested at pH 3 and 310 K. These intermediates were unequivocally identified by comparing their retention time with those of known congeners in analytical reference standards by IC.

Fig. 6 showed that the concentration of TCA gradual decreased until the end of the experiment. The concentrations of MCA and AA increased gradually with time, whereas the DCA concentration increased rapidly. Compared with TCA, the dechlorination rate of DCA and MCA were much slower. Apparently, highly chlorinated chloroacetic acids were more readily dechlorinated than lightly chlorinated ones. This phenomenon was in good agreement with previous literature [27, 28]. Additionally, the concentration of chloride ion released from the compounds during bioelectrochemical reduction was measured to evaluate the dechlorination degree. It is the chlorine substitution that mainly account for the toxicity of COCs. Chlorinated chloroacetic acids are much resistant to biodegradation compared to non-chlorinated ones. Therefore, dechlorination of such

compounds is greatly desirable prior to using biological processes to treat them. Along with the removal of TCA, the chloride ions released and their concentration increased gradually within the time scales investigated. This attributed to the fact that the chloride ions could diffuse to the solution for the electro-static repulsion from the negatively charged cathode. High values of chloride ion and AA yield were observed. This means that the chloride component of TCA was completely detached from organic molecules. Moreover, the total mass balance (on molar basis) of the reactant and the products was in the range of 95-104% during the bioelectrochemically reductive reaction.

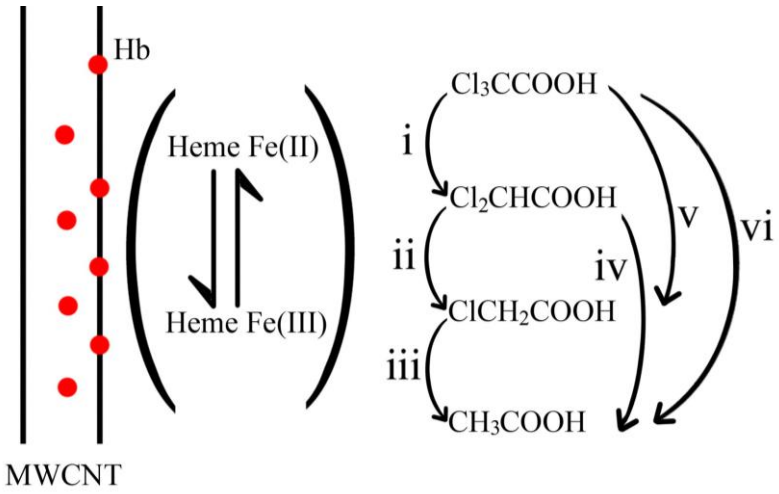

Figure 7. Illustrative diagram of TCA dechlorination on the surface of Hb-MWCNT-GE.

Based on the proposed intermediates, a possible pathway of the dechlorination of TCA by the Hb-MWCNT-GE system was proposed as shown in Fig. 7. One pathway assumed that the dechlorination of chloroacetic acids proceeded by six parallel reactions (TCA→DCA, TCA→MCA, TCA→AA, DCA→MCA, DCA→AA and MCA→AA) and the other indicated that the degradation of TCA occurred by three sequential reactions (TCA→DCA→MCA→AA). The consecutive model predicted that the concentration of DCA should decrease over time and that the concentration of AA should continue to increase over time. However, as seen in Fig. 6, the DCA concentration increased rapidly during the whole time period, whereas the concentration of AA increased slightly. Therefore, the parallel reaction mechanism appeared to be a better way of explaining the reductive transformation of TCA by Hb-MWCNT-GE as shown in Fig. 7 [12]. It was different with several reduction studies of chlorinated compounds with electrochemical dechlorination [29]. The reductive dechlorination by successive loss of chloride atoms was confirmed as the main pathway of the degradation of COCs in the literature [30].

Taken together, a conceptual model that explained the catalytic behavior of Hb-MWCNT-GE in the dechlorination of chloroacetic acid was presented in Fig. 7. The degradation pathway was proposed as follows:

$$HbFe(III) + H^+ + e^- \leftrightarrow HbFe(II) \quad (5)$$

$$MWCNT + CCl_3COOH \leftrightarrow (CCl_3COOH)_{ads}MWCNT \quad (6)$$

$$2HbFe(II) + (CCl_3COOH)_{ads}MWCNT \rightarrow 2HbFe(III) + (CHCl_2COOH)_{ads}MWCNT + H^+ + Cl^- \quad (7)$$

$$4HbFe(II) + (CCl_3COOH)_{ads}MWCNT \rightarrow 4HbFe(III) + (CH_2ClCOOH)_{ads}MWCNT + 2H^+ + 2Cl^- \quad (8)$$

$$6HbFe(II) + (CCl_3COOH)_{ads}MWCNT \rightarrow 6HbFe(III) + (CH_3COOH)_{ads}MWCNT + 3H^+ + 3Cl^- \quad (9)$$

$$2HbFe(II) + (CHCl_2COOH)_{ads}MWCNT \rightarrow 2HbFe(III) + (CH_2ClCOOH)_{ads}MWCNT + H^+ + Cl^- \quad (10)$$

$$4HbFe(II) + (CHCl_2COOH)_{ads}MWCNT \rightarrow 4HbFe(III) + (CH_3COOH)_{ads}MWCNT + 2H^+ + 2Cl^- \quad (11)$$

$$2HbFe(II) + (CH_2ClCOOH)_{ads}MWCNT \rightarrow 2HbFe(III) + (CH_3COOH)_{ads}MWCNT + H^+ + Cl^- \quad (12)$$

$$(CHCl_2COOH)_{ads}MWCNT \leftrightarrow CHCl_2COOH + MWCNT \quad (13)$$

$$(CH_2ClCOOH)_{ads}MWCNT \leftrightarrow CH_2ClCOOH + MWCNT \quad (14)$$

$$(CH_3COOH)_{ads}MWCNT \leftrightarrow CH_3COOH + MWCNT \quad (15)$$

4. CONCLUSIONS

An experimental study was conducted to characterize the bioelectrochemical dechlorination of TCA and to investigate the degradation mechanisms of TCA in the Hb-MWCNT-GE system. Information related to the effect of environmental parameters such as pH and temperature on CE of TCA reduction is vital to assess the applicability of bioelectrochemistry for the treatment of groundwater or raw industrial wastewaters. The CE of TCA was higher at pH 3.0 than in other pH conditions. With an increase in temperature, an increase of the average current efficiency is achieved until 310 K, while temperatures higher than 310 K lead to a decrease of the average current efficiency. The activation energy was determined to be 19 $kJ \cdot mol^{-1}$. The process was attended via TCA, DCA, MCA, AA, and chloride ion measurements. Total mass balance (on molar basis) of the reactant and the products was in the range of 95-104% during the bioelectrochemically reductive reaction. The conversion of TCA to AA was better described by a parallel reaction model than by a consecutive reaction model. The pathway assumed that degradation proceeded by six parallel reactions (TCA→DCA, TCA→MCA, TCA→AA, DCA→MCA, DCA→AA and MCA→AA).

Hb-MWCNT-GE shows extraordinary electrocatalytic properties toward the reductive cleavage of C-Cl bonds in TCA. The most important outcome of this study is that highly toxic chlorinated congeners can be converted to AA under fairly mild conditions and this certainly is a result of high environmental relevance as it affords the bases for developing reductive destruction methods in combination with biological removal of TCA from contaminated wastewaters. These results can also provide basic knowledge to understand the bioelectrochemical dechlorination of COCs in the Hb-MWCNT-GE system and show an example how to apply the Hb-MWCNT-GE system to the treatment through reductive degradation mechanisms depending on the contaminant type.

ACKNOWLEDGEMENTS

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