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Electrochemical inactivation of coliforms by in-situ generated hydroxyl radicals

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Abstract−Electrochemical disinfection is quite attractive as a promising alternative technology to chlorination. It is still debated whether conventional electrochemical disinfection, which electrolyzes the solution with very high chloride concentration to produce excess amounts of chlorine species, will generate toxic disinfection byproducts (DBPs) and have the same health risks as chlorination. To resolve this critical issue, we explored the possibility of electrochemical disinfection based on electrogenerated free radicals but not on active chlorine. The germicidal efficiency of 99.99% was achieved with a contact time of 5 min and current density of 7 mA cm^{-2} for a chloride-free model wastewater contaminated by coliforms. Electron spin resonance detection clearly confirmed that hydroxyl radicals were the major germicidal species responsible for efficient electrochemical disinfection. This process would not generate poisonous DBPs due to the avoidance of dangerous chlorine species. pH in the range of 5-9 has little effect on the bacteria inactivation. Formation mechanism of hydroxyl radicals was discussed.

Key words: Electrochemical Disinfection, ESR, Hydroxyl Radicals, Coliforms, Inactivation

INTRODUCTION

Disinfection is a significant treatment process in the water industry. Many strategies have been adopted to reduce microorganisms in drinking water and sewage water, including chlorination [1,2], ozonation [3], UV light irradiation [4] and the combinations of these approaches [5,6]. Chlorination is the most popular method, as it can provide both primary and residual disinfection. However, chlorination disinfection can produce potential carcinogens and there are safety problems in the handling, storage and transportation [7,8]. To date, more than 700 disinfection byproducts (DBPs) have been identified, among the most commonly formed DBPs are the trihalomethanes (THMs) [9]. Of these THMs, chloroform is often predominant as a recognized carcinogen for animals and a probable carcinogen for humans. The World Health Organization has a drinking water guideline for chloroform at 0.2 mg L^{-1} [10]. The United States Environmental Protection Agency has a maximum contaminant level for total trihalomethanes (TTHM) at 0.08 mg L^{-1} due to a two-stage disinfectants/disinfection-byproducts (D/DBP) rule implemented [11]. It is very urgent to develop green disinfection techniques without the generation of DBPs.

Electrochemical disinfection is quite attractive as a promising alternative to chlorination [12,13]. The process of electrochemical disinfection has several advantages: (i) environmental compatibility, (ii) versatility to kill a wide variety of microorganisms under the mild conditions, (iii) no need to add chemical medicines, and (iv) the benefits of *in-situ* generation and the avoidance of handling and storing hazards of disinfectants. The disinfection system is usually an electrochemical disinfector equipped with two electrodes. Electrode material, reactor configuration, the microorganism, current density and electrolyte composition are important factors that will affect the germicidal efficiency. Conventional electrochemical disinfection was carried out by the electrolytic production of germicidal active chlorine species [14]. Despite the prevalent acceptance in the water industry, it is still disputed whether electrochemical disinfection, which generates excess amounts of chlorine species, will replace chlorination. Moreover, will electro-chlorination have the same disadvantages of disinfection by-products risks as chlorination? If there is no chloride in the solution, can electrochemical systems still maintain great inactivation effectiveness? To answer these questions, a study of the mechanism of electrochemical disinfection is the crucial point. Several disinfection mechanisms have been proposed, including electro-chlorination, electric field destruction, and active intermediates sterilization [15-17]. Recently, increasing attention has been paid to free radicals, especially hydroxyl radical, which could be generated during electrochemical treatment [14,18]. Hydroxyl radical is critical active species and has excellent oxidizing power. Almost all polluting organics can be destroyed by hydroxyl radical. It is especially suitable for environmental application since hydroxyl radical is a green oxidant with short lifetime. In electrochemical disinfection, hydroxyl radical may play an important role on account of its stronger oxidizing capability than chlorine. More evidence is required for this hypothesis. However, few works have been done in this respect.

The objective of this work is to explore an efficient, green disinfection technology, which inactivates the bacteria by electrogenerated free radicals. The direct evidence of free radicals generation is shown by electron spin resonance (ESR), a powerful instrument for the investigation of free radicals. To eliminate the disturbing influence and achieve better repeatability, contaminated water was artificially synthesized and coliform was chosen as the model bacteria. Chloride-free electrolyte (Na_2SO_4) was used to avoid any confusion caused by the generation of chlorine. As a comparison, sodium chloride (NaCl) electrolyte was used to investigate the effect of chlorine. Here, the chloride content in natural derived water and

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drinking water was adopted as low as about several hundred milligrams per liter. It was quite different from conventional electro-chlorination, which electrolyzed the solution with very high chloride concentration (even saturated solution) to generate excess amounts of chlorine disinfectants. Effect of pH was discussed.

EXPERIMENTAL

1. Water Samples Containing Coliforms

The wastewater was artificially prepared by diluting the pure culture of coliforms to a cell density of 10^5-10^6 cfu ml^{-1} in stock electrolyte solutions, which was prepared using sterile deionized water and a certain concentration of Na_2SO_4 or NaCl (analytical grade reagents, concentration of 0-400 mg L^{-1} according to the test requirement). The coliforms culture was cultivated by inoculating seeds of coliform into a nutrient medium. The culture was grown on a shaker in a water bath for 24 h at 37 °C to reach its stationary growth phase. Stationary phase bacterial cultures were used in the experiments because exponentially growing cells have been shown to be more sensitive to inactivation by electric fields.

Total coliforms were enumerated by plate method. The microbial samples were diluted by using sterile deionized water. 1 mL of the treated dilutions and 15 mL of agar nutrient medium were aseptically transferred into the plates and rapidly mixed. The plates were reversed and incubated at 37 °C for 24 h before detection and enumeration.

2. Electrochemical Disinfection Tests

Disinfection experiments were carried out in an undivided cylindrical electrochemical cell. A DC power was applied to supply the electrode potential and current. A circulating pump was used to facilitate the diffusion of bacteria and generated species during electrochemical treatment. The anode used here was a β-PbO_2 electrode doped fluorine resin, which was deposited on the surface of ceramic tube. The cathode was a Ni-Cr-Ti alloy net concentrically assembled in the disinfector. The details of anode preparation have been given in previous work [19]. The choice of β-PbO_2 anode doped fluorine was principally based on the fact that it has high over oxygen potential and high electrocatalytic activity for effective anodic oxidation instead of the O_2 evolution. Moreover, PbO_2 is beneficial to be an electrode material for relative cheapness, good chemical inertness and large area for wide environmental application in industry. Due to the perfect chemical stability, no Pb^{2+} ion was detected in treated solution by atomic absorption spectrometry analysis, at least for the range of current density employed. The available anode area (Φ45×200 mm) was 250 cm^2. The effective volume of the disinfector was 500 mL.

Before beginning disinfection experiments, all necessary laboratory apparatus were sterilized in an autoclave (120 °C, 30 min). 500 mL of synthetic wastewater contaminated by coliforms was pumped through the electrochemical cell. All experiments were carried out at the temperature of 25 °C. The flowrate was controlled at the moderate rate. After 5 min of stable operation, a water sample was taken in sterile sample tube from the disinfector to represent time zero. Then a voltage was applied to the electrochemical cell and current density was adjusted to the desired value (0-7 mA cm^{-2}). Samples were taken at intervals from the sample point and $Na_2S_2O_3$ was immediately added to the samples to eliminate the oxidants present in the sample. After treatment, samples were stored at 4 °C before microbiological enumeration analysis.

3. Chemical Analysis

Applied voltage and current were read online from the stabilized voltage supply. pH was measured by METTLER TOLERDO 320-S pH meter. As a predominant representative of THMs, chloroform was analyzed by liquid/liquid extraction and by gas chromatography equipped with electron capture detection (GC/ECD) according to Unite State Environmental Protection Agency method 551 [20]. Chromatographic separation was performed on a DB-5 capillary column (30 m×0.25 mm i.d., 0.25 μm film thickness, J&W Scientific, USA).

Electron spin resonance (ESR) signals of radicals trapped by spin trapping agent 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) were obtained on a Bruker EMX-8 electron paramagnetic resonance spectrometer. The settings were center field 3490.000 G, microwave frequency 9.805 GHz, power 19.970 mW, receiver gain 1.00e+005, and modulation amplitude 1.00 G.

RESULTS AND DISCUSSION

1. Electrochemical Disinfection by In-situ Generated Radical Species

Electrochemical disinfection of the model wastewater containing coliforms and Na_2SO_4 electrolyte (no chloride ion) was carried out and the results are shown in Fig. 1. Disinfection efficiency increases significantly with the increasing current density. A germicidal efficiency of 99.99% was achieved with a contact time of 5 min and current density of 7 mA cm^{-2}. Obviously, the electrochemical method was greatly effective for wastewater disinfection even without the generation of chlorine. This indicated that coliforms were killed by other chemicals (except for chlorine) generated during electrochemical process. In previous work, β-PbO_2 electrode was used to degrade wastewater and it was found that active species were generated on the surface of electrode [21,22]. These active species

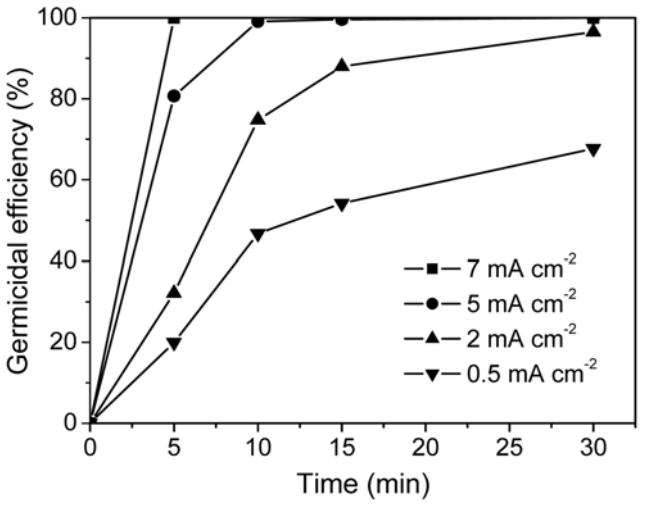

Fig. 1. Bacteria killing efficiency of electrochemical disinfection by radical intermediates. Operation conditions: Coliforms density 8.56×10^5 cfu ml^{-1}; Na_2SO_4 5 g L^{-1}; T 25 °C; pH 6.78; volume 500 mL.

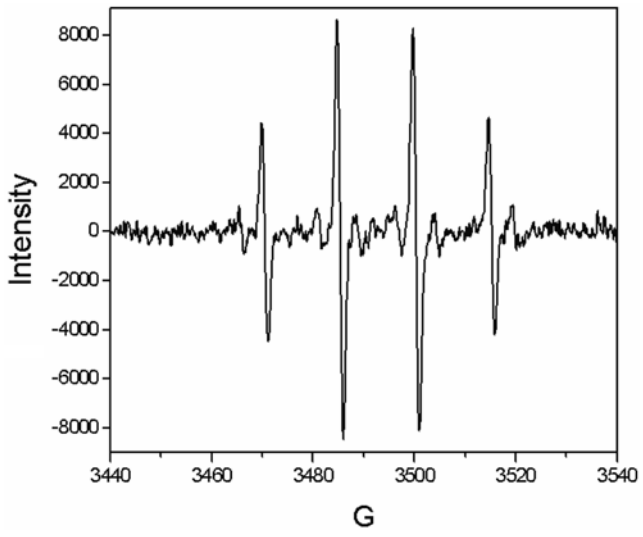

Fig. 2. Typical ESR spectra obtained during electrochemical disinfection without the presence of chloride ion. ESR parameters were as follows: Center field 3490.000 G; microwave frequency 9.805 GHz; power 19.970 mW; modulation amplitude 1.00 G; number of scans 5.

might play an important role in the effective electrochemical disinfection.

To determine whether active species were formed or which active species were generated in electrochemical disinfection, ESR measurements were applied for the detection of free radicals. During electrochemical disinfection, DMPO was added to the model wastewater and the power supply was immediately switched off to avoid DMPO from being oxidized. Fig. 2 shows the typical ESR spectra obtained during electrochemical disinfection in a chloride-free electrolyte. A four-line spectrum was observed and the quartet signal with an intensity ration is 1 : 2 : 2 : 1 (hyperfine coupling constants α_H= α_N=14.9 G). It is assigned to 5,5-dimethyl-2-hydroxypyrrolidine-*N*-oxyl (DMPO-•OH) spin adduct. This confirmed that hydroxyl radicals were really generated in electrochemical disinfection. Hydroxyl radical is a green active species and has a powerful oxidizing potential. Its lifetime is quite short and leaves no residue effects compared to other oxidants like chlorine. The direct confirmation of hydroxyl radicals generated in electrochemical disinfection provides favorable information for understanding the germicidal mechanism. It was obvious that hydroxyl radicals played a critical role in the effective electrochemical disinfection.

The mechanism of hydroxyl radical generation was considerably complex [23,24]. Here, it could be assumed that PbO_2 electrode was hydrated and there was equilibrium between the crystal and gel layer:

$$\underline{PbO_2+H_2O} \Leftrightarrow \underline{Pb^*O(OH)_2} \Leftrightarrow H_2PbO_3 \quad (1)$$

Crystal layer hydrated (gel) layer

The hydrated layer was an open system which interacts with the solution, exchanging cations, anions, and water molecules. Upon anodic polarization, there are electrochemical reactions proceeding in the $Pb^*O(OH)_2$ active centers:

$$Pb^*O(OH)_2 \Rightarrow Pb^*O(OH)^+(OH)^o+e^- \quad (2)$$

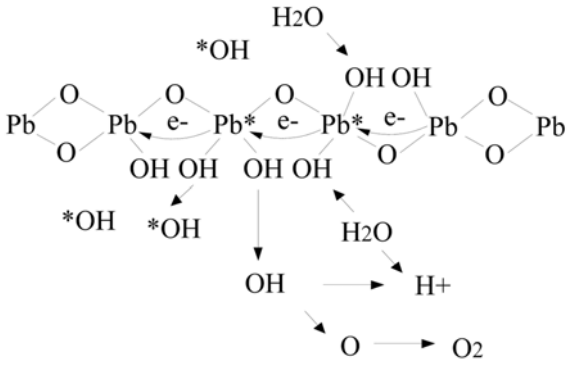

Fig. 3. The evolution reaction of hydroxyl radicals.

Electrons move along the hydrated layer and reach the crystal layer, as a result of which electric current passes through the electrode (Fig. 3). The active centers are charged positively. Their electric charge is neutralized through the chemical reaction as follows:

$$Pb^*O(OH)^+(OH)^o+H_2O \Rightarrow Pb^*O(OH)_2\ldots(OH)^o+H^+ \quad (3)$$

where "…" indicates a weak bond between the Pb center and the OH radical. H^+ ions migrate from the hydrated reaction zone into the electrolyte. Thus the active centers contain hydroxyl radicals. This phenomenon is in its essence absorption of OH radicals by the active centers. Some of the OH radicals would break away from the active centers and leave them. Thus OH radicals are formed and would make bacteria inactivated.

2. Effect of Chloride Ions

From the view of potential application, the absence of the ubiquitous chloride ions does not seem to be realistic. In natural water, the chloride ions are present with a diverse concentration between values as low as about 10 up to 250 mg L^{-1} or more [25]. In this work, the effect of chloride ions was examined with a concentration of 0-400 mg L^{-1}, which is two orders of magnitude lower than the concentration employed in conventional electro-chlorination. From Fig. 4, it can be seen that the presence of chloride ions could

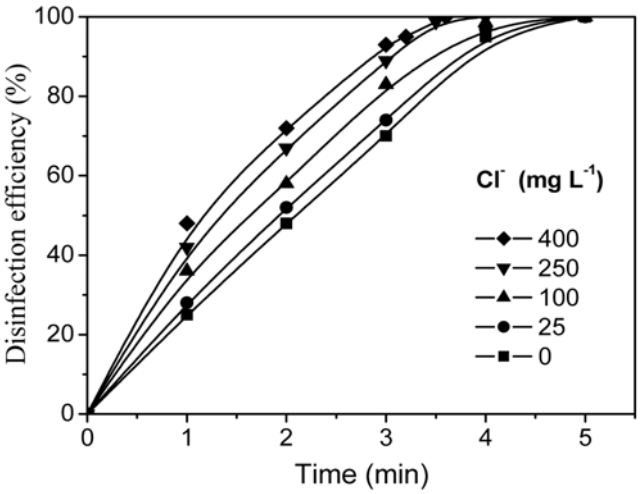

Fig. 4. Effect of chloride ions on electrochemical disinfection. Operation conditions: Coliforms density 8.56×10^5 cfu ml^{-1}; current density 7 mA cm^{-2}; T 25 °C; pH 6.78, volume 500 mL.

improve the electrochemical disinfection. The bactericidal efficiency increased with the augment of the concentration of chloride ions. However, the efficiencies for the concentration of chloride ions that ranges from 0 to 400 mg L^{-1} are actually quite similar. This indicated that the dilute chloride ions did not result in the significant enhancement of electrochemical disinfection. By GC/ECD analysis, the formation of DBPs (here, chloroform as a representative) was not found under the testing conditions. A possible explanation is that the low concentration of chloride ions results in the minor generation of chlorine. The other explanation is the competition action between active hydroxyl radicals and chlorine formation on account of the finite electrode area. As mentioned above, the most reactive hydroxyl radicals may provide more powerful killing capacity than electro-chlorination.

Fig. 5 shows the typical ESR spectra obtained during electrochemical disinfection with the presence of chloride ions. The DMPO-•OH spectrum characteristic of hydroxyl radicals was not observed. Instead, a seven-line spectrum was obtained and it might be 5,5-dimethyl-2-pyrrolidone-N-oxyl (DMPOX), which is similar with the DMPOX spectrum reported previously [26]. It was found that HOCl could oxidize DMPO to form DMPOX. Obviously, active chlorine was formed besides hydroxyl radical in electrochemical disinfection with the presence of chloride ions. Therefore, both electrogenerated hydroxyl radicals and electro-chlorination actions contribute to kill the coliforms, but active radicals are more green and efficient than chlorine.

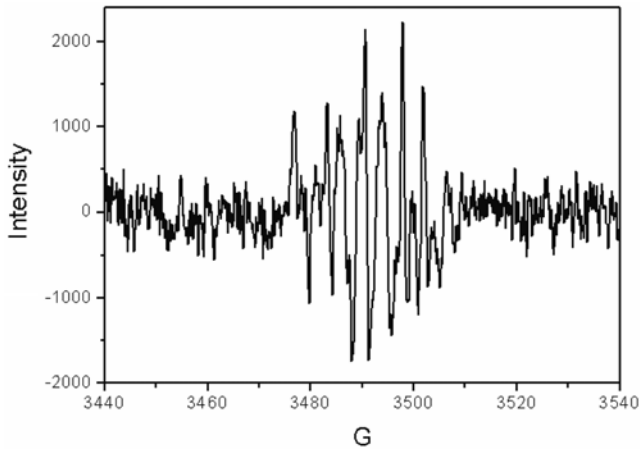

Fig. 5. Typical ESR spectra obtained during electrochemical disinfection with the presence of chloride ions. ESR parameters were as follows: Center field 3490.000 G; microwave frequency 9.805 GHz; power 19.970 mW; modulation amplitude 1.00 G; number of scans 5.

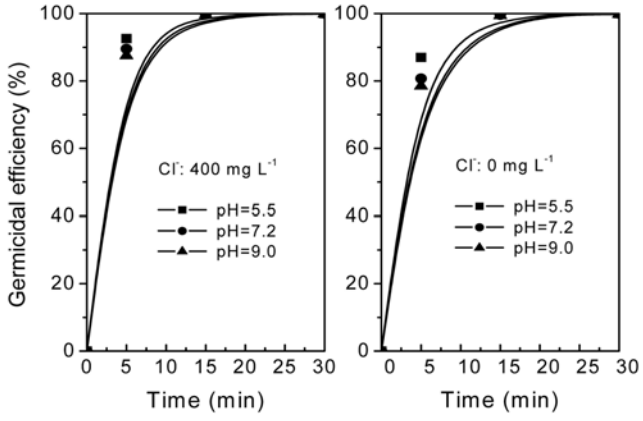

Fig. 6. Effect of pH. Operation conditions: Coliforms density 8.56 ×10^5 cfu ml^{-1}; current density 5 mA cm^{-2}; T 25 °C; volume 500 mL.

3. Effect of pH

The pH dependence of bacteria inactivation was investigated in the range of interest. Fig. 6 shows that pH in the range of 5-9 has no remarkable effect on the bacteria inactivation, no matter whether chloride ions were contained in the wastewater. One possible explanation was that the variation of pH did not improve the generation of hydroxyl radical disinfectants. Due to the low concentration of chloride ions employed, the effect of pH on generated chlorine could also be neglected. More detailed work is needed to find out the dependence relationship between radical generation and pH.

CONCLUSIONS

Electrochemical disinfection by in-situ generated radical species exhibited perfect bactericidal capability. Disinfection could be carried out in chloride-free or dilute chloride wastewater, thus avoiding to generate the poisonous DBPs. Electrogenerated powerful hydroxyl radicals played an important role in bacteria inactivation. Furthermore, in-situ generated radicals have more advantages to inactivate the bacteria that are hard to kill by chlorine. Green and efficient electrochemical disinfection by in-situ generated active radical species is feasible.

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