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Short Communication

Removing *Bacillus subtilis* spores from drinking water using a bipolar electrochemical method

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Inactivation ability of persistent microorganisms, is great advantage of desirable disinfection method. As the main factor involved in the waterborne transmission of gastroenteritis, *Cryptosporidium* is highly resistant to conventional disinfection methods and is a major challenge to water supply systems across the world. In the assessment of water quality, the use of a bacterial spore model is recommended as a surrogate to the actual use of *Cryptosporidium*. In recent years, electrochemical processes have become more emphasized as eco-friendly and efficient technologies in water treatment and disinfection. The present study was conducted to present a suitable strategy for improving the quality of drinking water. The bipolar electrochemical system examined consisted of an anode electrode, a cathode electrode and two bipolar electrodes all made of stainless steel with dimensions of 4×8 cm and distanced 1 cm apart from each other in a glass reactor containing 200 cc of drinking water. The monopolar one, designe by the same figuration, only without the two bipolar electrodes. The variables examined included *Bacillus subtilis* spore counts of 10^2-10^4 CFU/mL, an electrochemical reaction time ranging from 15 to 60 min, a current density of 2-5 mA/cm^2 and normal-pH and normal-temperature water. The findings showed that, while improving the system's current and accelerating the electrochemical reactions, bipolar electrochemical systems are capable of the full removal of *Bacillus subtilis* spores with a current density of 5 mA/cm^2 and an electrochemical reaction time of 60 minutes.

Keywords: Bipolar electrochemical methods, water disinfection, *Bacillus subtilis* spores

1. INTRODUCTION

Many cases have recently been reported of the prevalence of waterborne diseases caused by protozoan cysts resistant to conventional disinfection. The main goal of the disinfection, is reduction of pathogens microbial of row water. As the main factor involved in the waterborne transmission of bacillus gastroenteritis, *Cryptosporidium* oocyst is a major challenge to the supply of safe water [1]. Since experiments for the direct investigation of pathogenic *Cryptosporidium* in water are costly, time-consuming and difficult, using non-pathogenic *Bacillus subtilis* spores is recommended as an alternative [2-9]. The conventional chlorination step in the disinfection of water is inefficient for the removal of resistant microorganisms and also produces toxic compounds including trihalomethanes and haloacetic acids in water [10]. In recent years, researchers have grown more interested in the use of electro-oxidation as an appropriate method for eliminating resistant microorganisms [11-14]. The present study investigates *Bacillus subtilis* spore removal from water at a bacterial density of 10^2-10^4 CFU/mL and with a current density of 2-5 mA/cm^2 and a reaction time ranging from 15 to 90 minutes in an intermittent-current bipolar and monopolar electrochemical process.

2. MATERIALS AND METHODS

2.1. Microbial Strains and Culture Media

Bacillus subtilis strain ATCC 6633 grown on trypticase soy agar (TSA) medium was procured from Tehran University’s Collection of Microorganisms and Cell Cultures. The spores were prepared by keeping the vegetative bacteria at 35 °C for seven days. The spore strains were kept at room temperature (22-25 °C) and the whole process was also carried out at this temperature. The culture media were sterilized by autoclaving at 121 °C for 15 minutes. The suspensions of *B. subtilis* spores were prepared according to the McFarland standard of 0.5 [15]. To detect viable spores of *B. subtilis* after the electrochemical reaction, the electrolysis water was sampled at 15-minute intervals and immediately poured onto TSA plates. The plates were kept at 25 °C for 48 hours. Viable spores were counted using the plate count technique and were reported in CFU/ml.

2.2. Bacterial Density

The bacterial densities required were prepared based on the McFarland standard of 0.5 [16]. The optical absorbance of turbidities developed in the McFarland tubes were measured at a wavelength of 625 nm using a spectrophotometer (0.08-0.1 range).

2.3. Electrochemical Systems

An anode and a cathode electrode and two bipolar electrodes made of stainless steel were placed 1 cm apart in a 200-cc glass reactor filled with tap water (Table 1 presents further details on the tap water used for the experiments) and the bipolar electrochemical process was thus carried out (Figure 1). A DC power supply (PS-305D) was used for supplying the voltage and the required induced current.

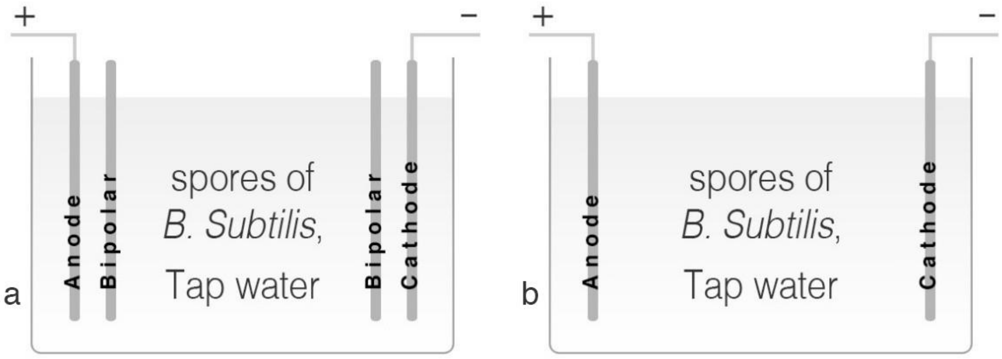

Figure 1. The electrochemical systems under study: a; bipolar, b; monopolar

Table 1. The results of the analysis of the tap water used for the experiments

Anion	mg/L	Cation	mg/L	Others	Value
Cl^-	53.5	Na^+	87.15	pH	7.4
F^-	0.24	K^+	1.264	Conductivity (µs/cm)	629
HCO_3^-	248.4	Ca^{+2}	56.1	(mg/L) TDS	364.8
SO_4^{-2}	28.6	Mg^{+2}	20.279		

2.4. Electrical Energy Consumption Estimation

The electrical energy used by the system was calculated using Equation I, where P is energy consumption (Wh), I electrical current (A), V voltage (V) and t time (h).

$$\mathbf{P = IVt} \qquad \text{(Equ. 1)}$$

3. RESULTS

Figures 2-4 present the effect of current density on the removal of *B. subtilis* spores in 15-minute intervals sampled at 30, 60 and 90 minutes for bacterial densities of 10^2-10^2 CFU/mL. With monopolar and bipolar apart systems. The applied current density values show that the current densities of 1 and 2 mA/cm^2 had no effects on the removal of spores. Increasing the current density to 2.5 mA/cm^2 at the electrochemical reaction time of 45 minutes enabled the 100% removal of the 10^2 CFU/mL suspension, at bipolar system (Fig. 2). Figure 3 shows the effect of the bipolar electrochemical process with a current density of 5 mA/cm^2 at the reaction time of 60 minutes on the full removal of the bacterial density of 10^3 CFU/mL.

The electrochemical process enabled the removal of bacterial densities of 10^4 CFU/mL at the current density of 5 mA/cm^2 and the reaction time of 90 minutes (Fig. 4). If the voltage and current density are fixed, increasing reaction time can improve the efficiency of the electrochemical removal of spores. Increasing the initial number of spores has an inverse relationship with the efficiency of their electrochemical removal. At the current density of 5 mA/cm^2, the spore density of 10^3 CFU/mL was completely removed within 60 minutes, while the spore density of 10^4 CFU/mL showed only a 45% removal during the same reaction time.

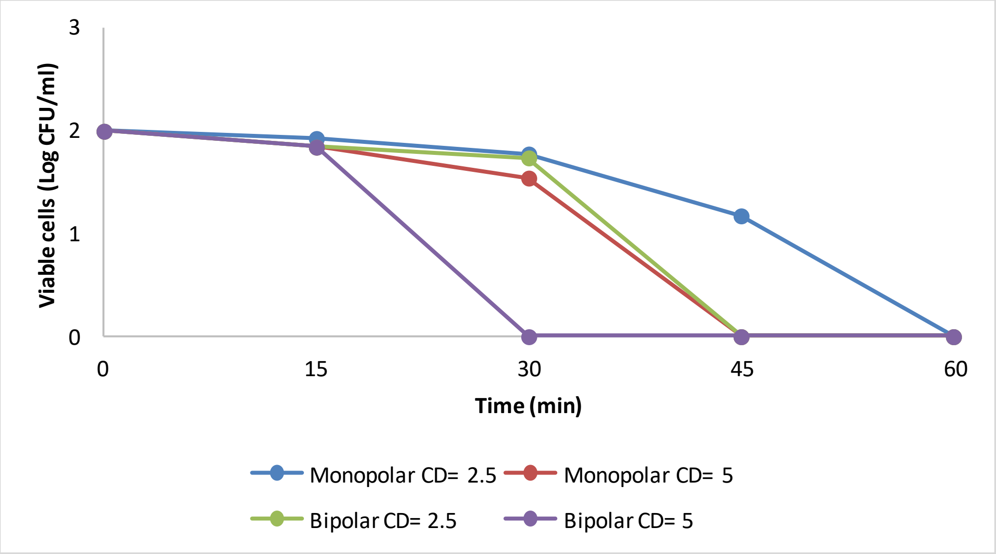

Figure 2. The electrochemical removal of *B. subtilis* spores from water; Test conditions: Bacterial density = 10^2 CFU/mL; Steel electrodes; T=25 °C; pH=7.2; Electrodes distanced 1 cm apart.

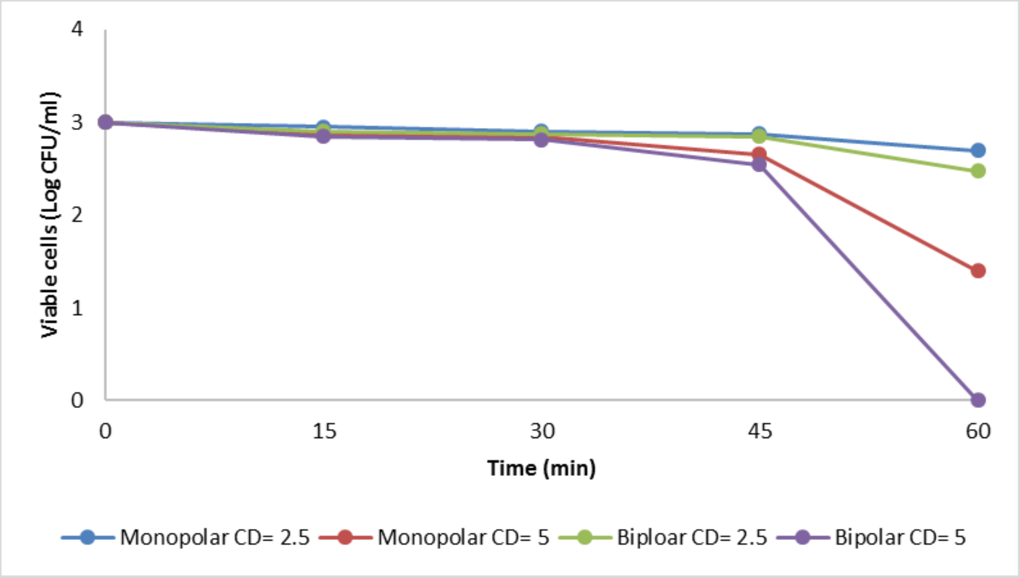

Figure 3. The electrochemical removal of *B. subtilis* spores from water; Test conditions: Bacterial density = 10^3 CFU/mL; Steel electrodes; T=25° C; pH=7.2; Electrodes distanced 1 cm apart.

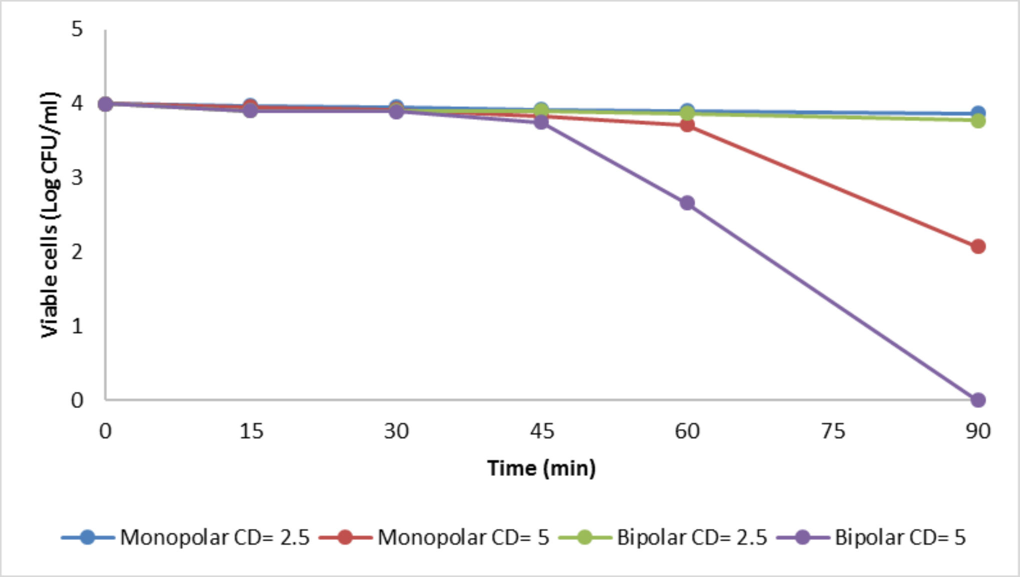

Figure 4. The electrochemical removal of *B. subtilis* spores from water; Test conditions: Bacterial density = 10^4 CFU/mL; Steel electrodes; T=25 °C; pH=7.2; Electrodes distanced 1 cm apart.

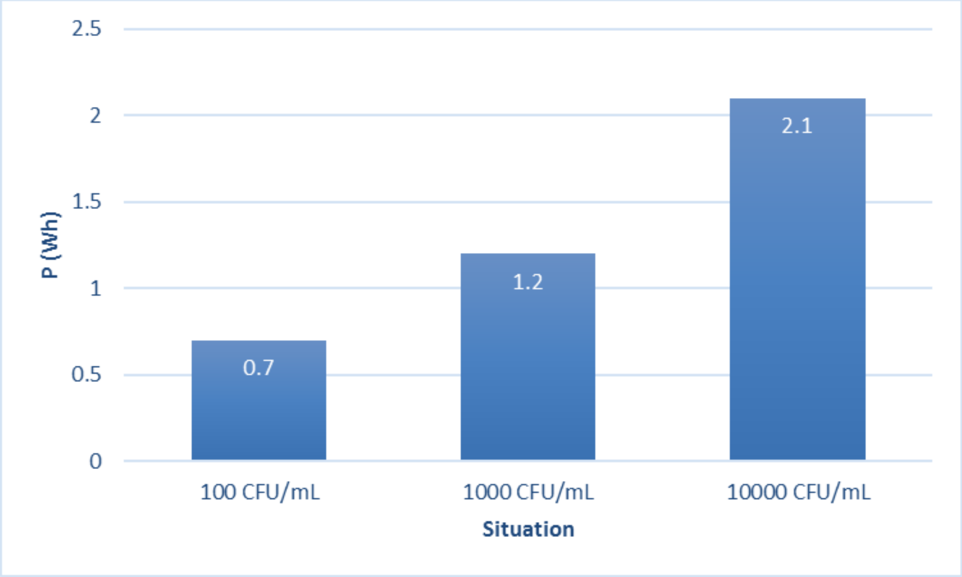

Figure 5. The energy consumption of the bipolar electrochemical system under optimal conditions of *B. subtilis* spore removal from drinking water

The system’s energy consumption also increased with the environment’s spore density. Figure 5 presents data on the energy consumption of a bipolar electrochemical system for the 100% removal of each bacterial suspension. The complete removal of spores thus requires 0.007, 0.012 and 0.021 KWh of energy per liter of tap water for each of the bacterial densities examined.

4. DISCUSSION

Microorganisms are destroyed through two major mechanisms involved in electrochemical oxidation processes. First, direct anodic oxidation, during which the bacteria move toward the anode surface due to the electrostatic force between the electrode and the charged amino and carboxyl groups in the cell wall and thus cause anodic cell adsorption[17-18]. As a result of the subsequent impaired permeability of the cell membranes and coenzyme A, cellular respiration stops and bacterial cell death occurs. Two, indirect oxidation, which causes the electrochemical production of chlorine on the anode surface and also leads to bacterial cell death by passing through the cell membranes and inhibiting the enzymes required for DNA synthesis [19].

$$2Cl^- \longrightarrow Cl_2 + 2e^- \qquad \text{II}$$

$$Cl_2 + H_2O \longrightarrow HOCl + H^+ + Cl^- \qquad \text{III}$$

$$HOCl \longrightarrow OCl^- + H^+ \qquad \text{IV}$$

The experiments showed that increased current density and reaction time increase the production of electrochemical antibacterial agents and the effectiveness of direct oxidation. Bergman et al. first presented the electrochlorination process in 2008 [20]. Mezule et al. (2014) then reported the use of chloride ion in water and the electrochemical production of hypochlorous acid and hypochlorite ion at the anode as important factors involved in electrochlorination [21]. The results obtained by Santana et al. in 2005 [22] and Young et al. in 2007 [23] confirmed the effectiveness of increased current density in improving the disinfection capacity of systems. Tsolaki et al. (2010) also found that the antibacterial effect of chlorine on the system and the direct anodic oxidation affect the electrochemical inactivation of *B. subtilis* spores [24].

5. CONCLUSION

The present study investigated the effectiveness of the intermittent-current bipolar electrochemical system in the 100% removal of *B. subtilis* spores from water and examined the effects of variables including current density, electrochemical reaction time and the initial number of spores in natural-pH water and ambient temperature. Using steel electrodes in a bipolar electrochemical system was determined as the optimum arrangement of electrodes to remove 100% of *B. subtilis* spores while also saving electrical energy. Increasing the initial number of spores was found to have an adverse effect on the reaction time of the electrochemical removal of spores and thus on electrical energy consumption too, as the amount of energy consumption tripled in the system containing 10^4 CFU/mL of *B. subtilis* spores (0.021 KWh) compared to the system with 10^2 CFU/mL spores (0.007 KWh). A current density lower than 2 mA/cm^2 and a reaction time of 60 minutes did not affect the removal of *B. subtilis* spores. Increasing the current density to 2.5 mA/cm^2 led to the full removal of the bacterial density of 10^2 CFU/mL at the reaction time of 60 minutes. A current density of 5 mA/cm^2 led to the full removal of 10^3 and 10^4 CFU/mL of *B. subtilis* spores at reaction times of 60 and 90 minutes, respectively. The bipolar electrochemical system, was promoted electrical conductivity, specially for waters that have poor conductivity. In this system bipolar electrodes have positive polarity on the one side, and negative polarity on the other side. The bipolar electrode (BPE) is a electrical conductive material which promotes electrochemical reactions even without ohmic contact.

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