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Short Communication

Bipolar Electrochemical Reactor Processes for Removal of Bacterial Spores: Effect of Ethanol and Potasium Iodide

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Electrochemical techniques have a great potential in removing organic matters and resistant microorganisms from water through water treatment processes. The present study was conducted to propose a standard solution for the removal of *Bacillus subtilis* as a model of *Cryptosporidium* from drinking water using a bipolar electrochemical system. The initial number of *Bacillus subtilis* spores used as a *Cryptosporidium* surrogate for water quality assessment was 10^2-10^4 spores/mL. The electrochemical reaction took 15 to 60 minutes to complete and occurred in the presence of 0.4 M of ethanol and 4 mg/L of KI and at a current density of 3-5 mA/cm^2, at ambient temperature and with normal pH water. The findings suggest that, under optimal conditions of *Bacillus subtilis* spore removal, the synergistic effects of bipolar electrochemical reactions in the presence of KI and ethanol lead to a 32% reduction in energy consumption compared to when only a single electrochemical process is used.

Keywords: Bipolar electrochemical reaction, ethanol, supporting electrolyte, *Bacillus subtilis* spores, water disinfection

1. INTRODUCTION

Cryptosporidium parvum is the most resistant water microorganisms to some disinctants, and thus plays a key role in the outbreak of gastroenteritis through the consumption of drinking water [1, 2]. *Bacillus subtilis* spores are suggested as an appropriate surrogate in the in-vitro study of

Cryptosporidium inactivation in water [3-6]. On the other hand, chlorination is a popular technique for water disinfection that able to removal various pathogen microorganisms. The chlorine and its by-products have some disadvantages, such as resistance of some microorganisms like *Cryptosporidium*, generation of unfavorable taste and odor and potentially for generation of toxic and mutagenic materials such as trihalomethanes and chloroform. Unfortunately, the conventional chlorination of drinking water performed during disinfection processes does not lead to the elimination of *Cryptosporidia* [7,8]. Various techniques have been proposed as alternative to chlorination such as I) utilization of chemical agent like ozone, hydrogen peroxide, potassium permanganate, II) physico-chemical process such as photocatalytic disinfection, III) electrochemical process, and IV) physical treatment such as sonication, microwave, and ultravioulet irradiation [6, 7]. Recently, various electrochemical process have been developed and proposed for water and wastewater treatments [9]. Researchers are thus growing more inclined to use electrooxidation as an appropriate method of removing resistant microorganisms [9-11]. To the best of our knowledge and based on the literature, there is no previous report on the synergistic effect of ethanol and KI supporting electrolyte in electrochemical processes. Hence, the aim of present study was to investigate effect of ethanol and potassium iodide in a bipolar electrochemical reactor processes for removal of *Bacillus subtilis* spores. To evaluate the process, the spores' removal was examined by current density of 3-5 mA/cm^2 for 15–60 min to *B. subtilis* spores (10^2–10^4 CFU/mL density), with stainless steel electrodes.

2. MATERIALS AND METHODS

2.1. Bacterial strains and culture media

Bacterial strains of *B. subtilis* (ATCC6633) grown on trypticase soy agar (TSA) culture media were supplied from Tehran University's Collection of Microorganisms and Cell Cultures. The spores were prepared by keeping vegetative bacteria at 35° C for seven days. The spore strains were kept at ambient temperature (22-25° C) and the entire process was carried out at this temperature too. The culture media were sterilized by autoclaving at 121° C for 15 min. The bacterial suspensions of *B. subtilis* spores were prepared according to McFarland standard of 0.5 [12]. To detect viable spores of *B. subtilis* after the electrochemical reaction, the electrolysis water was sampled at 15-minute intervals and immediately poured on 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. Microbial assay

The minimal inhibitory concentration (MIC) of ethanol and KI was measured both separately and in combination as per the National Committee for Clinical Laboratory Standards (NCCLS) guidelines [13]. The bacterial densities required were prepared based on the McFarland standard of 0.5. The optical absorbance of turbidities developed in McFarland tubes were measured at a wavelength of 625 nm using a spectrophotometer (0.08-0.1 range).

2.3. Bipolar electrochemical system

The experiments were carried out in a batch electrochemical reactor. As shown in Figure 1, an anode and a cathode electrode and two bipolar electrodes, all made of stainless steel, were placed 1 cm apart in a 250-cc glass beaker filled with 200-cc of tap water. The anode and cathode electrodes were connected to a DC power supply (PS-305D) to establish an electric current.

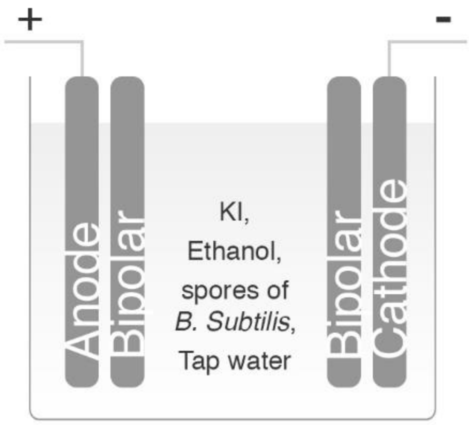

Figure 1. The electrochemical reactor used for the experiments

2.4. Energy consumption

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

$$P=IVt \qquad I$$

2.5. Synergistic effect

To investigate the synergistic effect of ethanol and KI in electrochemical systems based on the ability to reduce the duration of action of spore removal, the Hackerman equation [14] was used (Equation II), where S_1 is the synergism parameter, $I_{1+2}=I_1+I_2$ is the separate effectiveness of ethanol and KI and $\acute{I}_{1+2}$ is their combined effectiveness.

$$S_1 = \frac{1 - I_{1+2}}{1 - \acute{I}_{1+2}} \qquad II$$

$S_1=1$, $S_1>1$ and $S_1<1$ denote the compounds' lack of mutual effects, synergistic effects and antagonistic effects.

3. RESULTS & DISCUSSION

Vrious parameters can affect in the electrochemical disinfection process. In this study, the effects of ethanol and potasium iodide were investigated regarding the removal of *Bacillus subtilis* spores as an appropriate surrogate in the study of *Cryptosporidium* inactivation in water.

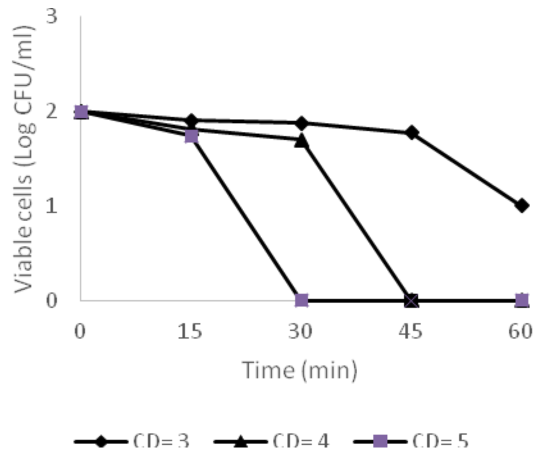

Figure 2a.

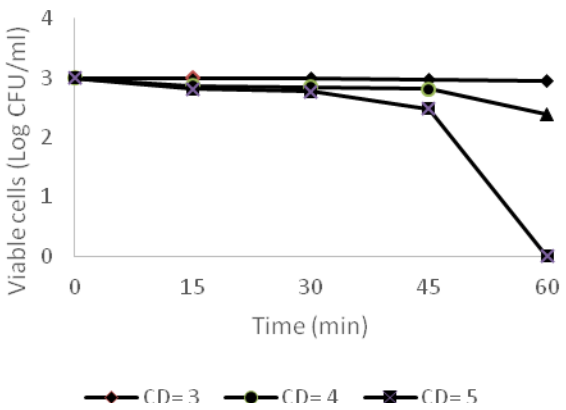

Figure 2b

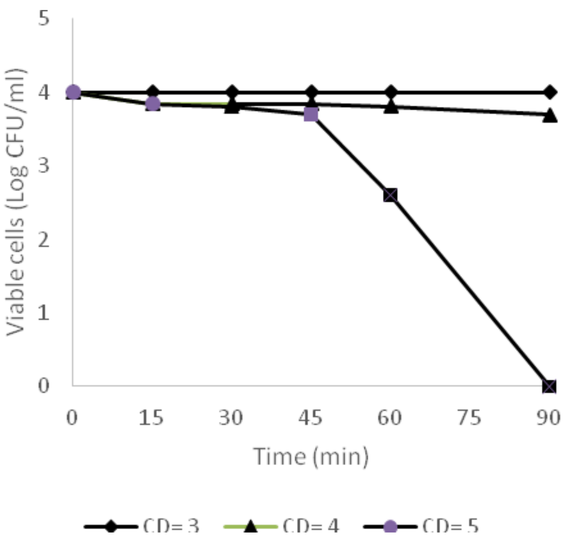

Figure 2C

Figure 2. The electrochemical elimination of *B. subtilis* spores from water at various bacterial concentrations (a: 10^2 ; b: 10^3; C: 10^4 CFU/ml)

The study of the MIC of ethanol (0.4 M) and KI (4 mg/L) in separation and in combination showed that neither affect the elimination of *B. subtilis* spores. The effect of the electrochemical system's current density on the elimination of *B. subtilis* spores (10^2-10^4 CFU/mL) at 30, 60 and 90 minutes indicates a reduction in the number of spores and ultimately their 100% removal (Figures 2).

Adding ethanol to the system enhances the electrochemical process capacity and reduces the duration of action of spore elimination (Figures 3).

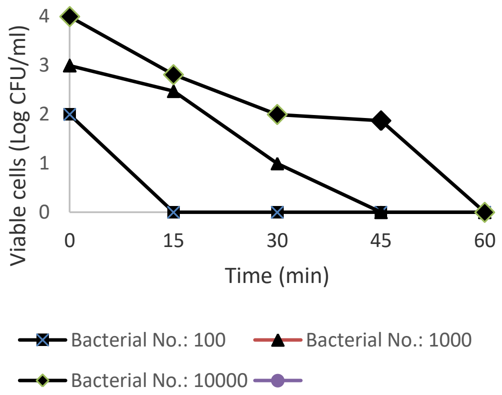

Figure 3. The antibacterial effects of ethanol on the electrochemical elimination of *B. subtilis* spores from water; Conditions of the experiment: CD=5 mA/cm^2; 0.4 mol/L of ethanol; T=25° C; pH=7.2

Adding KI supporting electrolyte was ineffective in the improvement of the spore elimination system. The combined application of ethanol and KI in the electrochemical system yielded 3.32, 2.66 and 3.04 S for bacterial densities of 10^2, 10^3 and 10^4 spore/mL and with a current density of 5 mA/cm^2, suggesting the synergistic effect of ethanol and KI (Table 1).

Table 1. The synergistic effects of the combined application of ethanol and KI in a bipolar electrochemical system; Conditions of the experiment: CD=5 mA/cm^2; pH=7.2; T=25° C; 0.4 M of ethanol, 4 mg/L of KI

Synergism parameter (S)	Microbial density (CFU/mL)
3.32	10^2
2.66	10^3
3.04	10^4

As shown in Figure 4, the highest electrical energy consumption of the system pertained to electrochemical conditions of 2.8 Wh and the absence of KI and ethanol. Adding ethanol to the system reduced the energy consumption to 2.1 Wh (a 25% saving), while KI had no effects on energy consumption and its addition kept the consumption at 2.8 Wh. The combined application of ethanol and KI reduced both the energy consumption to 1.9 Wh (a 32% saving) and the duration of action of *B. subtilis* spore elimination.

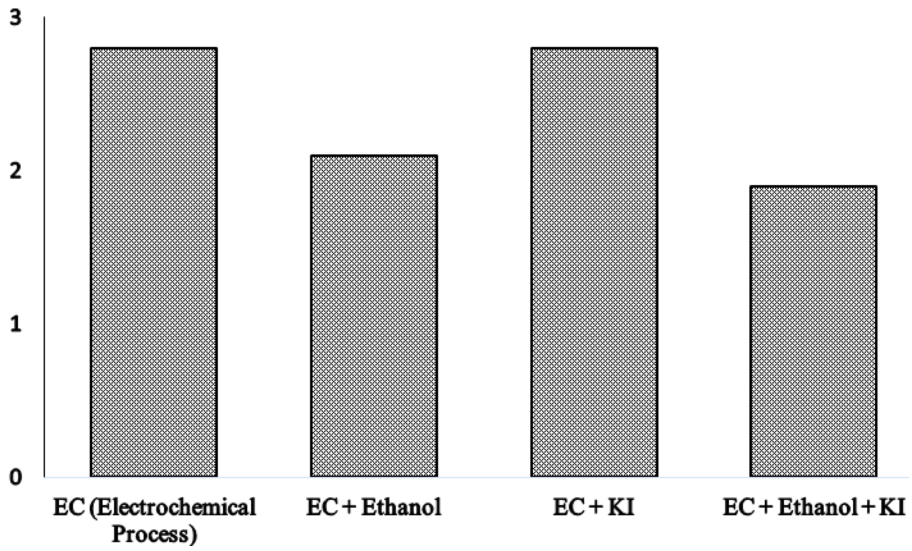

Figure 4. The energy consumption of an electrochemical system under optimal *B. subtilis* spore elimination conditions; Conditions of the experiment: Bacterial density=10^3 CFU/mL; CD=5 mA/cm^2; pH=7.2; T=25° C; 0.4 M of ethanol; 4 mg/L of KI

In addition to their direct effects on anodic oxidation and the electrical current in the electrooxidation process, electrochemical systems disrupt the permeability of bacterial cell membranes and facilitate the presence of antibacterial agents [15]. Their mechanism increases bacterial cell sensitivity to let ethanol into the cell and thus cause cellular protein denaturation. According to Priscila et al. (2009), although ethanol lacks the ability to eliminate spores outside an electrochemical cell, it contains a MIC of 87500 mg/L for eliminating *B. subtilis* spores [16]. Iodine-halogen has weaker antibacterial activities compared to chlorine. In order for this property to emerge, molecular iodine should penetrate the bacterial cell wall and subsequently interfere with protoplast metabolic reactions. The ineffectiveness of the electrochemical process in the presence of KI lies in the absence of proper conditions for the generation of enough molecular iodine (I_2) to be used in electrochemical bacterial inactivation (Equation III).

$$HOCl + 2I^- \longrightarrow Cl^- + OH^- + I_2 \qquad III$$

The increased concentration of KI, current density and reaction time for improving the efficiency of spore elimination have the effect of turning the water yellow. Measuring the synergism parameter for the combined application of ethanol and KI reveals the synergistic effect of these two compounds in electrochemical systems. The results of the pretests suggest that iodine-halogen has low oxidation properties and is therefore less affected by organic matters and also provides stronger antibacterial effects when combined with ethanol [17]. It has showed that the MIC of ethanol and iodine 10% combination used for eliminating *B. subtilis* spores is reduced by 50% compared to when only ethanol is used [16]. The synergistic effect of ethanol and KI on the reduction of the duration of action of electrochemical reactions under optimal conditions of *B. subtilis* spore elimination reduces the electrical energy consumption in electrochemical systems.

Various studies related to the disinfection have been reported using chemical and physic-chemical agents both in the laboratory and in the pilot scale (Table 2).

Table 2. The comparison between the electrochemical technique and other proposed methods for inactivation of *Cryptosporidium* and *Bacillus subtilis* spores

Disinfectant	Description	Referance
UV Irradiation	Inactivating *Cryptosporidium parvum* oocysts	18
Ozone	Pilot_Scale of *Cryptosporidium* inactivation and other microorganisms in natural water	19
Hypochlorite and chlorine dioxides	killing of *Bacillus Subtilis* Spores	20
Peroxynitrite	killing of spores of *Bacillus Subtilis*	21
a modificated Fenton reagent containing $CuCl_2$ and ascorbic acid	killing of *Bacillus Subtilis* spores	22
O_3/ H_2O_2 followed by Cl_2	Enhanced bactericidal effect	23
Ozonation	Inactivation of *Bacillus Subtilis* spors in water treatment plant	24
Electrochemical disinfection with chlorination	Inactivation of bacterial spores in drinking water	25
Sterilox®	killing of spores of *Bacillus subtilis*	26

4. CONCLUSION

The study of the synergistic effect of ethanol and KI supporting electrolyte in batch bipolar electrochemical processes revealed a 32% reduction in electrical energy consumption under optimal conditions of *B. subtilis* spore removal compared to when simple electrochemical processes are used.

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